Thermotolerance classification of *Brassica carinata* genotypes using germination assay and vegetative growth parameters

By

Leelawattie Persaud

Approved by:

Bisoondat Macoon (Major Professor)
Kambam Raja Reddy (Co-Major Professor)
Brian S. Baldwin
Ramdeo Seepaul
Rebecca A. Melanson
Michael S. Cox (Graduate Coordinator)
George M. Hopper (Dean, College of Agriculture and Life Sciences)

A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Agronomy
in the Department of Plant and Soil Sciences

Mississippi State, Mississippi

May 2020

Name: Leelawattie Persaud

Date of Degree: May 1, 2020

Institution: Mississippi State University

Major Field: Agronomy

Major Professors: Bisoondat Macoon, Kambam Raja Reddy

Title of Study: Thermotolerance classification of *Brassica carinata* genotypes using germination assay and vegetative growth parameters

Pages in Study: 132

Candidate for Degree of Master of Science

Temperature is a major abiotic stress limiting plant growth. Thermotolerance evaluation during germination and early growth may help identify adaptable genotypes of new crops. Two studies were conducted to evaluate temperature effects on 12 *Brassica carinata* genotypes during germination and early growth. During germination, genotype AX17004 was both the most cold- and heat-tolerant. During early-season growth (35 d after seeding), there were temperature and genotype effects on shoot, root, and physiological components. Cumulative low- and high-temperature response indices, and cumulative root and shoot response indices were related, indicating the importance of these traits. Genotype AX17006 was identified as heat tolerant, and AX17009 as cold tolerant during early-season growth. When genotypes were grouped according to breed types, hybrids generally had better responses than the inbred lines, and double haploids and the check responses were intermediate. These studies provided rapid results that will reduce the number of genotypes assessed in field studies.

DEDICATION

I dedicate this thesis to my loving parents, friends, country, and all the people who believed in me and help made this dream a reality.

ACKNOWLEDGEMENTS

With a deep sense of gratitude, I am genuinely obliged to many people whose valuable support made it possible to complete this study. First, I will like to express sincere gratitude to the USDA National Institute of Food and Agriculture-Agriculture and Food Research Initiative Competitive Grant for providing the funding for this study [through the Southeast Partnership for Advanced Renewables from Carinata (SPARC) project: Project no. USDA-NIFA 2016-11231. I am grateful to my principal advisor Dr. Bisoondat Macoon, for his guidance, continuous encouragement, constructive critiques, and motivation, and for providing me with an excellent opportunity to complete my Master's research. Special thanks to my thesis committee: Dr. K. Raja. Reddy, for his belief and confidence in me, his supervision, and for creating an excellent atmosphere for the successful completion of this study; Dr. Brian S. Baldwin, Dr. Ramdeo Seepaul, and Dr. Rebecca A. Melanson, for their time, insight, invaluable criticism, guidance, and support throughout my research and academic works. I am also appreciative of Mr. Richard Nigel Cumberbatch for having confidence in me and recommending me to pursue my graduate studies.

Special thanks to all the faculty and staff members of the Department of Plant and Soil Sciences, Mississippi State University (MSU), for the tremendous support when needed. I am thankful to David Brand of the MSU Environmental Plant Physiology Laboratory for his hard work and assistance during my research. Also, great gratitude goes out to my present and past colleagues, graduate students and undergraduate students of the Environmental Plant Physiology

Laboratory, whom I had the pleasure of working with during my research, especially Mr. Hunt Walne, Dr. Chathurika Wijewardana, Dr. Salah H. Jumaa, Dr. Firas Alsajri, Dr. Naqeebullah, and Ms. Grace Adegoye. Thank you for all your assistance during my data collection and for also adopting me into your families and filling my life with joy during the time spent together.

I wish to acknowledge my supporting colleagues and friends, Abiola Bruce Smith, Sapna Pokrel, and Zia Khan, for all the emotional support and help in the time of need. Special thanks to Mrs. Macoon and Mrs. Reddy for all your support and for making me feel at home. Above all, I will like to express my gratitude to my family, my dear and loving parents, sisters, and brother, for their unconditional love and encouragement. Finally, I glorify the almighty God for blessings during this journey.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

GENERAL INTRODUCTION

Global greenhouse gas emissions associated with fossil fuel usage. Escalating petroleum prices and a greater demand for energy, food, and fiber by the earth's population has forced the pursuit of alternative sources of energy that are renewable and less damaging to the environment (Schneider, 2006; Atabani et al., 2013; EPA, 2015). In recent years, the use of biofuels globally has increased due to declining oil reserves and climate change concerns (Balat and Balat, 2009; Ozturk, 2014). Biofuels refer to fuels that are liquids, solids, or gases derived from plant biomass, food crops, urban wastes, and by-products from forestry and agricultural cultivation (Qin et al., 2010; Altun and Yasar, 2013; Aburas and Demirbas, 2015).

Currently, fossil fuels are the leading source of energy, although they have adverse effects on the global climate and are non-renewable (Selvakumar et al., 2016). Fossil fuels, such as gasoline and diesel, account for approximately 40 to 80% of the total energy consumption in the world (Tan et al., 2008; Escobar et al., 2009). Of this total consumption, the transportation sector uses 58% for fuel. The transportation sector ranks third after the industrial and building industry at the global level for its high energy consumption. The world's carbon dioxide (CO_2) emission is predicted to increase by 60% by 2030 (Bhuiya et al., 2016), and the transportation sector accounts for 23% of the CO_2 emissions related to fossil fuels at a global level (Du Ploy and Nel, 2012). Increased petroleum usage gives rise to net CO_2 increase into the atmosphere, hence a rise in global surface and ocean temperatures (Schneider, 2006). The earth's average

surface temperature has increased at a rate of 0.2ºC per decade between 1950 and 1993 and may reach a threshold of 2 to 4.5ºC by 2100 (Meehl et al., 2007). Projected changes associated with increased temperatures include a rise in sea level, an increase in precipitation frequency and intensity, and decreased seasonal perennial snow and ice (Chiotti and Johnson, 1995). These factors will force us to revolutionize our current food production systems to mitigate and adapt to climate change. Drought conditions likely will be more frequent due to the combined effects of elevated temperatures and a reduction of available crop water, which will affect global crop productivity and food security (Chiotti and Johnson, 1995). The adaptation of renewable sources for energy production, such as biofuels, maybe a solution for the reduction of environmental problems associated with the use of fossil fuels (Cherubini et al., 2009; Appels et al., 2011; González-Garcia et al., 2012a). Biofuels are alternative renewable sources of energy capable of reducing greenhouse gases (Nelson et al., 2014) and, at the same time, mitigate present-day and imminent global power and rural economic disasters (Alagumalai, 2014).

In recent years, several alternative biofuel energies have been explored to decrease the dependency on petroleum-based fuels. In response to increased energy costs in the 1970s, the ethanol industry diversified and started using corn (*Zea mays* L.) as a feedstock for biofuels production (EIA, 2017). This industry is unable to satisfy the demands, however, due to regulations by the USEPA (2011) that allow only 10 to 15% of ethanol mixtures in gasoline (Knoll et al., 2009). Also, there are concerns that competition will exist between long term nutrition supply and biofuel feedstock (Chhetri et al., 2008) and land allocation for food and biofuel crop cultivation (FAO, 2008). Success was achieved, converting oils from algae into biofuel, but the sourcing of biomass, and the cost and efficiency of processing into the final product remains challenging (NAABB, 2014).

Additionally, the aviation industry is seeking to invest in the development of biofuel alternatives for jet fuel as the prices for jet fuel and petroleum increase (Biello, 2008). The aviation sector's goal is to increase its carbon-neutral growth by 2020 and reduce the use of petroleum jet fuel by 50% by 2050 (Gesch et al., 2015; Chu et al., 2017). Commercial airlines in the United States utilized approximately 18 billion gallons of jet fuel in 2016 (EIA, 2017). Further, the U.S. transportation sector has expressed interest in renewable biofuels to reduce the usage of petroleum as its dependency on foreign sources for crude oil increases (Schnepf and Yacobucci, 2013). From 2007 to 2016, the production of biofuels increased by an average of one billion liters annually (EIA, 2017). The Energy Independence and Security Act of 2007 expanded its target to produce and use 136 billion liters of renewable biofuels by the year 2022, mandating that at least 58% of that production must be second-generation biofuels (meaning that they come from non-food crops) that will help reduce greenhouse gas emissions by 50% (Schnepf and Yacobucci, 2012).

Bioethanol produced from sugar beet (*Beta vulgaris* L.), corn, and sugarcane (*Saccharum officinarum* L.) is the most common biofuels used, generally in mixtures with gasoline (Perlack et al., 2005). The hydrocarbons chains are shorter in biodiesel produced from oilseed rape (*B. napus* subsp *oleifera* L.) and soybean [*Glycine max* (L.) Merr], requiring more significant energy consumption processes to create the longer hydrocarbon chains necessary to produce high-energy fuels (Bona et al., 1999; Perlack et al., 2005). Rising oil prices and increasing environmental pollution are the main reasons for the exploration of new feedstock to produce alternative fuels to petroleum (Cahoon et al., 2007). One oilseed crop that spurred great interest in research for biofuel production over the last few years is *Brassica carinata* (A. Braun),

commonly called Abyssinian mustard, Ethiopian mustard, or simply carinata (Cardone et al., 2003).

For the biofuel industry to be sustainable, the feedstock must have the following characteristics: (i) the source of energy that it provides must be equivalent in quality to that produced by fossil-based fuels, (ii) it must be able to be grown in large quantities, and (iii) it must not compete for land required to provide food and fiber crops (Wilkes et al., 2013). Carinata is highly preferred for biofuel production because of its high concentration of erucic acid, a long-chain fatty acid essential in producing high-energy fuels (Cardone et al., 2003; Warwick, 2011; Enjalbert et al., 2013). This crop belongs to the mustard family, *Brassicaceae,* and originates from the Ethiopian highlands, where its cultivation has started since around 5000 BP (Alemayehu and Becker, 2002). Because of its place of origin, carinata is better adapted to semi-arid regions across the world (Barthet, 2008; Marillia et al., 2014). Carinata likely emerged from interspecific hybridization between black mustard (*B. nigra* L.) and wild cabbage (*B. oleraceae*) (Prakash and Hinata, 1980; Gomez-Campo and Prakash, 1999; Alemayehu and Becker, 2002). Even though other related oilseed species such as oilseed rape and canola (*B. napus* L.) are commonly grown for oil production in North America (Bona et al., 1999; Perlack et al., 2005), there is a rise in interest to produce carinata as a winter crop for biofuel production in subtropical regions.

Carinata has very-long-chain fatty acids that are favorable for the production of biofuel, lacquers, bio-plastics, and paints (Carlsson, 2009; Impallomeni et al., 2010, Newson et al., 2013). Carinata is not a food crop because of high glucosinolate levels that may be harmful to human health (Rosenthal et al., 2017). Breeding and refinement can reduce the concentration of glucosinolates; however, allowing its use as a meal for animals after the oil extraction process is

completed (Rosenthal et al., 2017). Oil concentration and chemical composition can vary. Mulvaney et al. (2019) in Jay and Quincy, Florida, reported that oil concentration averaged 400 g kg^{-1}, and more than a third were erucic acid (C22:1), while protein concentration was 310 g kg^{-1}. Similar ranges, from 250 to 500 g kg^{-1} oil concentration and 250 to 410 g kg^{-1} for protein concentration, were reported (Alemayehu and Becker, 2002; Zanetti et al., 2009; Ban et al., 2017). Gesch et al. (2015) reported that carinata seed yield was 20% greater than commercial canola varieties grown in Minnesota.

Compared to oilseed rape and canola, carinata is more tolerant of warmer environments and has relatively low seed shattering potential (Seepaul et al., 2016), has better drought tolerance (Kumar et al., 1984), and resistance to diseases (Shivpuri et al., 1997). Carinata also has low cold tolerance (Monti et al., 2009). Carinata has several agronomic characteristics that contribute to its ability to adapt to an environment where other oilseed crops cannot. It an excellent rotational crop, can grow off-season, and is resistant to flea beetles, aphids, and blackleg disease (Marillia et al., 2014; Zhao et al.,2016; Basili and Rossi, 2018). There is limited availability of commercial varieties, however (Gugel et al., 1990).

The interest of growing carinata as feedstock for biofuels has increased because of the advantages it has over other conventional crops grown for the same purpose. Current production regions include the United States (Great Plains and Pacific Northwest), Canada, and Italy (Cardone et al., 2003; Drenth et al., 2015; Zhu et al., 2016). Carinata is a relatively new winter oilseed crop in the southeastern United States (de Koff et al., 2017), where studies are currently ongoing to identify lines best suited for commercial cultivation. The University of Florida leads this initiative in collaboration with the Southern Partnership for Advanced Renewables from Carinata (SPARC) consortium. In studies conducted during the last three years, yield ranged

from 1960 kg ha^{-1} to 2580 kg ha^{-1}. These yields are greater than those reported in northern U.S. states and Canada (Seepaul et al., 2019). Evaluations in Florida since 2012 helped identify promising genotypes with good tolerance to cold weather, better shatter resistance, and potential for a higher yield than the popular commercial variety (Seepaul et al., 2019). These results led to the expansion of evaluation of carinata across states in the Southeast, including Mississippi, to select entries best suited for these environments (Seepaul et al., 2015).

Most carinata genotypes can be utilized as a biofuel feedstock, but differences in genetic characteristics among genotypes may contribute to variation in the ability of a particular genotype to grow in one specific region or agroecosystem (Gesch et al., 2015). There is an opportunity for row crop growers in the U.S. Southeast to invest in the cultivation of carinata as a winter crop and diversify their existing systems to increase profitability. Furthermore, the biofuel industry is working towards promoting carinata feedstock production as a winter crop, which will complement summer production in temperate regions (Mulvaney et al., 2019). There is little information; however, on carinata growth and performance and production requirements in the U.S. Southeast, where the introduction of this crop for commercial cultivation is ongoing (Agrisoma Biosciences Inc., 2017).

Climate change is occurring rapidly, resulting in variation among abiotic stresses, frequent flooding, and prolonged drought periods (Jagadish et al., 2012). Due to this, plant breeders and agronomists are working to develop new crop cultivars that are more tolerant to these conditions to combat the adverse effects of climate change (Seepaul et al., 2011). The successful establishment of a new feedstock species to a new environment depends on its ability to grow and produce high yields under a wide range of environments. The new cultivar should be able to establish uniformly and rapidly, and combat weed competition and drought

(Hacisalihoglu, 2008). The yield of *Brassica* species depends on changing environmental conditions during growth and developmental stages (Saha and Khan, 2008). Therefore, identifying suitable genotypes and management practices to have sustainable production under a changing climate is crucial (Aggarwal and Kalra, 1994).

High germination rate, emergence, and uniform stand establishment are prerequisites to establish advanced biofuel feedstocks successfully for optimum yields (Dawadi et al., 2019). Both internal factors (seed viability maturation, genotype, and dormancy) and external factors (water, light, temperature, and oxygen) affect seed germination (Durr et al., 2016; Wang et al., 2016). Among the external abiotic stress factors, temperature plays a dominant role in seed germination and emergence (Milbau et al., 2009; Reddy et al., 2017). High temperatures may result in a limited supply of photosynthetic assimilates during the development of seeds (Spears et al., 1997; Shinohara et al., 2006), causing physiological damage resulting in the loss of seeds' ability to germinate (Hampton et al., 2013). All plant species have a range of temperatures at which germination takes place, called the cardinal temperatures (Bewley, 1997; Finch-Savage and Leubner-Metzger, 2006). In this range, there is minimum, optimum, and maximum temperatures that are useful in constructing models to predict germination and development processes (Kebreab et al., 2000; Ghaderi et al., 2008). Several mathematical functions define the relationship between germination rate and temperature (Shafii et al., 2001; Soltani et al., 2006). Using mathematical functions to determine the effect of temperature on seed germination may be useful in evaluating germination characteristics and the adaptability potential among crop species or genotypes (Jordan and Haferkamp, 1989). Cardinal temperatures are critical in identifying plant species that are tolerant of low and high temperatures and environmental conditions under which some crops can successfully germinate and establish (Ghaderi et al., 2008). Knowledge of

many parameters is essential when seeding fields, including moisture conditions and optimum temperature for rapid germination and establishment of the crop (Fulbright, 1988; Hanson and Johnson, 2005).

Germination studies can determine the adaptability range of a particular species to different environmental stresses, which can be useful in decision making regarding the planting period (Dawadi et al., 2019). Seed weight and germination are useful indicators when establishing a new crop (Jordan et al., 1989; Hampton et al., 2000). It is easy to determine seed germination characteristics using in vitro seed germination assay. This method has been widely used to assess plant tolerance to environmental stress in cotton (*Gossypium hirsutum* L) (Reddy et al.; 2017), rice (*Oryza sativa* L.) (Singh et al., 2017), pepper (*Capsicum annuum* L.) (Gajanayake et al., 2011), switchgrass (*Panicum virgatum* L.) (Seepaul et al., 2011), soybean (*Glycine max* L.) (Alsajri et al., 2019), oilseed species (*Brassica sp*.) (Dawadi et al.; 2019), and big bluestem (*Andropogon gerardii*) (Singh et al., 2019). Dawadi et al. (2019) showed that carinata germinated rapidly at a constant temperature of 25ºC, reaching maximum germination after approximately 60 hours of incubation. There is limited information available on the cardinal temperatures, germination rates, and germination capacity for carinata genotypes.

Temperature is an important abiotic stress factor, which plays a dominant role in the control of plant growth rates and developmental processes under optimum nutrient and water conditions. Plant species differ in their response to temperature, and even hybrids or cultivars can vary in their sensitivity to temperature (Wijewardana et al., 2015; Reddy et al., 2017). There is a spatial and temporal variation of temperature throughout a crop-growing season. Each developmental aspect or crop event has its specific temperature optimum, above which plant growth processes will decline (Alsajri et al., 2019). A rise in temperature may lead to an altered

geographical distribution and growing season by modifying the threshold temperature for the start season and crop maturity period (Porter, 2005). High temperatures accelerate the rate of plant development and reduce the length of the growing period and the yield potential (Entz and Fowler, 1991). Elevated temperatures beyond the maximum tolerance of the plant have a negative effect on plant growth and survival and hence, crop yield (Boyer, 1982). Lobell and Asner (2003) reported that each degree centigrade increase in average growing season temperature might reduce crop yields as much as 17%. High-temperature stress directly or indirectly affects plant photosynthetic functions by changing the structural organization and chemical properties of thylakoid membranes and other components of the photosynthetic apparatus (Lichtenthaler et al., 2005). The rate of photorespiration increases with increasing temperature, which reduces net photosynthesis (Sage and Sharky, 1987), and probably the seed yield of the crop. Seed yield potential in *Brassica* crops depends on the events occurring before and during the flowering stage (Mendham and Salisbury, 1995).

As greenhouse gas emission increases, extreme weather events, such as prolonged high temperatures, will intensify (Singh et al., 2008). There will be frequent changes in the weather pattern, which includes fluctuation in low and high temperature in the future (Meehl and Tebaldi, 2004). Several researchers have shown that these projections in climate change can reduce crop production when they occur at the same time plants are in their reproductive stage (Hall, 1992; Reddy et al., 1992, 1997). For example, a yield reduction occurred in canola grown in both winter and spring (Reddy et al., 2005). Additionally, canola produced less mature seeds under low temperatures (JinLing, 1997). Angadi et al. (2000) reported a yield drop in *Brassica* species (exposed to a high day/night temperature of 35/15ºC for seven days during the flowering stage). Usually, the damage caused by temperature all depends on the plant growth stage at a specific

time and the extent to which the stress lasted (Li et al., 1981). Studies with sorghum [*Sorghum bicolor* (L.) Moench] indicated that genetic differences play a role in response to abiotic stresses under field conditions (Igartua et al., 1995; Cosentino, 1996; Yu et al., 2004).

Root development, along with seedling vigor, are critical aspects for plant growth and plays a vital role in canopy growth, plant development, and crop productivity (Wijewardana et al., 2016a, 2016b; Reddy et al., 2017). Soil temperature plays a vital role in how the plant root system penetrates soil structure. Root development has a direct relation to plant growth and development and is temperature-dependent (Kaspar and Bland, 1992). Plant root system architecture and its components are essential when selecting lines with high environmental stress tolerance characteristics (Lynch, 1995). During plant growth and establishment, poor root development may lead to reduced shoot and canopy growth at later growth stages of plants (Gajanayake et al., 2014; Wijewardana et al., 2017). Studies on root systems of rice, corn, and cotton helped to identify stress tolerance at the seedling growth stage (Wijewardana et al., 2015; Singh et al., 2017a, 2017b; Singh et al., 2018). The selection of crops for tolerance to abiotic stress based on root system characteristics is deficient due to the tedious work associated with the massively structured belowground distribution, dynamic interaction with the environment, and lack of phenotyping methods for root systems (Brand et al., 2016; Khan et al., 2016). Many studies conducted in the past mainly screened genotypes for tolerance to abiotic stresses based on aboveground traits, like plant height, number of nodes, and leaf area (Salmeron et al., 2014, 2015). Due to technological advancement, today's scientist can now evaluate root systems, minimizing their destruction using various tools such as gels, hydroponics systems, WinRHIZO root scanner, and wax-petroleum layers (Brand et al., 2016; Singh et al., 2017; Reddy et al., 2017). Recent studies have successfully determined the relationship that exists between

temperature stress tolerance and root traits for different crops using the technologies mentioned above (Wijewardana et al., 2015; Brand et al., 2016; Singh et al., 2018). Differences in the correlation between shoot and root traits to various abiotic stresses were found at the early seedling stage in cotton (Singh et al., 2018), hence the importance of studying above- and below-ground growth and developmental traits in the identification of tolerant genotypes to abiotic stress.

The growing interest in carinata as a feedstock for biofuel production has led to the development and selection of various carinata genotypes that must undergo tolerance testing to various abiotic stresses before releasing for commercial cultivation. Evaluating crop species for dominant traits that make it resilient to extreme weather conditions is the critical goal for most breeding programs globally (Singh et al., 2007). More suitable and relatively faster methods of selection can speed up the breeding process. Commonly used screening methods are limited to visual observations and field performance that may mask a genotype tolerance level or the real potential to varying temperature ranges and moisture content in the field. Besides, screening in the field for temperature tolerance is tedious, seasonally limited, and inconsistent; therefore, there is a need for a more rapid, consistent and straightforward methods to identify tolerant genotypes to facilitate the observation of a large number of genotypes under a controlled environment (Setimela et al., 2005). There is a need for experimental facilities that mimic environmental field conditions that also include solar radiation (Reddy et al., 2001). Thermotolerance selection is possible through the evaluation of plant physiological processes such as photosynthesis and chlorophyll fluorescence on a whole-plant basis (Hall, 1992; Fracheboud et al., 1999). Other proposed traits used to determine temperature tolerance in plants include productivity or growth rate, germination rate (Hotchkiss et al., 1997; Revilla et al.,

2003), and various root traits (Hund et al., 2008). Several physiological and morphological parameters have been used to categorize multiple crop genotypes to temperature tolerance (Singh et al., 2007). There are limited studies on carinata screening for thermotolerance using plant morpho-physiological traits.

Determining the temperature tolerance capacity of various genotypes is done by identifying indices based on relative ranking using single value indices, cumulative indices, percentiles, and quartiles relative to control studies and groupings based on the statistical separation of means under single or multiple stresses were developed (Emerson and Minor, 1979). These are known as total temperature response indices (TRI) and represent the multigenic nature of stress in crops (Emerson and Minor, 1979; Koti et al., 2004; Salem et al., 2007). Another proposed method is using quantitative relationships determined by principal component analysis (PCA) (Singh et al., 2008). This multivariate technique helps to reduce a large number of traits observed into smaller groups that contribute to separating genotypes, but the contribution of each trait is based on ranking. The TRI technique takes into account all the traits of interest that may have contributed considerably to a particular stress event, sensitivity, or tolerance, with each trait having an equal contribution (Wijewardana et al., 2015). Hence, using either of these methods to classify carinata genotypes in response to temperature stresses will help select suitable genotypes for Mississippi.

We tested the hypothesis that carinata genotypes exhibit varied levels of thermotolerance at seed and seedling growth stages, and seed germination and early-season vigor traits could be used to classify carinata genotypes into various thermotolerance groups. Also, we tested the hypothesis that seed and seedling thermotolerance behave similarly. To test these hypotheses, studies were designed to (a) quantify the effect of temperature on carinata seed germination and

rate, (b) determine the cardinal temperatures for carinata seed germination, and rate, (c) determine the temperature effect on early vegetative growth of carinata, and (d) classify carinata genotypes for temperature tolerance. The functional algorithms developed for seed germination and temperature-dependent responses from these studies are a prerequisite for modeling the germination of various carinata genotypes adapted to different climatic zones.

CHAPTER II

LITERATURE REVIEW

Biofuels

Global greenhouse gas emissions associated with fossil fuels usage, escalating petroleum prices and a greater demand for energy, food and fiber by the earth's population has forced the pursuit for alternative sources of energy that are renewable and less damaging to the environment (Schneider, 2006; Atabani et al., 2013; Ho, 2014; EPA, 2015). Over the years, the use of biofuels globally has increased due to fewer oil reserves and climate change concerns (Balat and Balat, 2009; Ozturk, 2014). The earth's average surface temperature has increased at a rate of 0.2ºC per decade between 1950 and 1993 and is expected to reach a threshold of 2 to 4.5ºC by 2100 (Meehl et al., 2007).

Other changes associated with increased temperature are projected, such as a rise in sea level, increase in precipitation frequency and intensity, and decreased seasonal and perennial snow and ice. These are all factors that will force us to revolutionize our current food production system to mitigate and adapt to climate change. Drought conditions will be more frequent due to a combined effect of elevated temperatures and a reduction of available crop water, which will affect agriculture production globally, affecting crop productivity and food security (Chiotti and Johnson, 1995). Increased petroleum usage gives rise to net carbon dioxide increase into the atmosphere, hence causing an upsurge in global surface and ocean temperature (Schneider, 2006).

Biofuels are alternative renewable sources of energy capable of reducing the release of greenhouse gases (Nelson et al., 2014) and, at the same time, abate present-day and imminent global power and rural economic disasters (Alagumalai, 2014). Relative to fossil fuels, biofuels are "cleaner" and significantly reduces greenhouse gas emissions, particulates, carcinogens, sulfur, and hydrocarbons (Goldemberg et al., 2008). Wilkes et al. (2013) concluded that for the biofuel industry to be sustainable, the feedstock produced must be equivalent in quality to that produced by fossil-based fuels, it must have potential to be grown in large quantities, and must not compete for land required to provide food and fiber crops. Replacing fossil fuel with fuel from bioenergy crops and cultivating these by proper management can help sequester large quantities of carbon into the soil and abate greenhouse gas emission caused by fossil fuel combustion (Ma et al., 2001).

Brassica carinata

Brassica carinata (A. Braun), commonly called Abyssinian mustard, Ethiopian mustard, or simply carinata, originated from the highlands of Ethiopia. Carinata is an oilseed crop that has gained much attention due to its usage in non-food products, such as lubricants, soaps, and, most importantly, biofuels (Cardone et al., 2003). Carinata cultivation started from around 5000 BP (Alemayehu and Becker, 2002). Because of its place of origin, carinata is better adapted to semi-arid regions across the world (Barthet, 2008; Marillia et al., 2014). Globally, carinata has been recognized to have a high potential as an oilseed crop in several countries, including Canada and Spain (Rakow, 2004), India (Singh, 2003), Italy (Cardone et al., 2003), and the USA (Cardone et al., 2003), mainly because of its tolerance to moisture stress and high temperatures (Singh, 2003). During the last several years, carinata has been cultivated commercially as a summer

crop in the Canadian prairie and the U.S. northern plains and as a winter crop in the southeastern United States and Uruguay (Seepaul et al., 2016).

Even though other related oilseed species such as oilseed rape (*Brassica napus* L.) and canola are commonly grown for oil production in North America (Bona et al., 1999; Perlack et al., 2005), there is increased interest to produce carinata as a winter crop for biofuel production in subtropical regions. Carinata oilseeds are highly preferred for biofuel production because they have a high concentration of erucic acid (Cardone et al., 2003; Warwick, 2011; Enjalbert et al., 2013). Gesch et al. (2015) reported that carinata seed yield was 20% greater than commercial canola varieties grown in Minnesota, USA. Across three years in a Florida study, seed yields ranged from 1960 kg ha^{-1} to 2580 kg ha^{-1} (Seepaul et al., 2019). The advanced commercial cultivar, Avanza 641, yielded approximately 2580 kg ha^{-1}. These yields are greater than those reported in northern US states and Canada.

To date, field tests of carinata have been successful in South Dakota, Minnesota, North Dakota, Montana, and Florida (Gesch et al., 2015; Zhao et al., 2016; Alberti, 2017). Similarly, studies conducted from 2011 to 2014 at 12 study sites across U.S. northern states and various regions across Saskatchewan, Canada, on two cultivars of carinata released in Canada, AAC A110 and AAC A100, and reported that AAC A110 had greater yields. Yields recorded during the four years ranged from 2134 to 3421 kg ha^{-1} for AAC A110, and 2121 to 3176 kg ha^{-1} for AAC A100 (Resonance Carinata, 2015). Carinata is well adapted to its native habitat in the highlands of Ethiopia. This area has annual average rainfall of 600 to 1000 mm, temperatures ranging from 14 to 18ºC, an elevation of 2200 to 2800 m above sea level, and a long growing season of 180 days (Asamenew et al., 1993; Alemayehu and Becker, 2002).

Among the various *Brassica* oilseed crops, carinata has a unique growth habit, producing plants with more branches than other oilseed species. Compared to the current commercial oilseed crop, rapeseed (*B. napus*), carinata produced two times greater aboveground biomass per unit area (Gesch et al., 2015). The estimated carbon concentration of carinata is between 45 to 47% of the dry weight of its biomass, accounting for a significant sink of carbon accumulated during its growing season (Gasol et al., 2007; Duca et al., 2015). Carinata has a deep and extensive taproot system that extends as far as 60 to 90 cm below ground, with more than 50% of its root mass in the first 30 cm of the soil (Seepaul et al., 2016). The taproots penetrate through compacted soil layers, thus improving soil structure. Carinata roots comprise as much as 20 to 25% of the plants' total biomass, which returns to the soil after harvest as an additional sink of carbon (Gan et al., 2009a).

Probable effects of employing renewable resources

A Life Cycle Assessment (LCA) was conducted for carinata to evaluate its energetic balance, environmental impact and economic performance as a biomass crop with regards to cultivation, collection, transportation, and the conversion from biomass to energy fuel or electricity (Gasol et al., 2007; Butnar et al., 2010). The LCA was carried out in Spain (Butnar et al., 2010), southern Europe (Gasol et al., 2007), and Italy (Cardone et al., 2003) for carinata as a lignocellulosic biomass crop for biofuel and energy use. Butnar et al. (2010) performed analysis impacts for a native crop species and carinata on six categories: global warming, human toxicity, acidification, abiotic depletion, ozone layer depletion, and photochemical oxidation. Compared to traditional electricity-producing systems, biomass crops used to generate power were found to be more harmful to the environment, according to these LCA analyses. With an increase in biomass production, however, it was observed that the negative impact decreases (Butnar et al.,

2010). Environmental benefits and energy increase with varying management practices of carinata production (Gasol et al., 2009). Fertilizer used for carinata production had the most significant impact, but to reduce this, substituting with another alternative such as livestock manure was recommended (Gasol et al., 2009).

In Italy, the analysis carried out regarding the use of carinata for biofuel based on agronomic performance and energy balance results were favorable (Cardone et al., 2003). Compared to rapeseed, carinata required less input and management in terms of tillage, fertilizer, and weed control. Carinata grown in coastal regions outperformed rapeseed in production, due to its tolerance to warmer environmental conditions. Because of this crop's ability to withstand drought, heat, and diseases, carinata does not compete for land designated for food production (AAFC, 2015; Seepaul et al., 2016) and is easily incorporated in present crop rotation systems with other crops or cultivated on fallow land, when food crops cultivation is absent (Marois et al., 2015). Furthermore, the biofuel derived from carinata showed similar characteristics to that of biodiesel produced commercially, and the cost of production is potentially less (Cardone et al., 2003).

Current interest to utilize more renewable resources for energy security, climate change mitigation, and sustainability are the driving forces for evaluating various feedstocks as prospective biofuel sources (Seepaul et al., 2016). Also, the utilization of carinata in the southeastern USA supports the Rural Energy for America Program (REAP) agenda, established by the United States Department of Agriculture (USDA, 2013), through renewable bioenergy crops grown domestically using sustainable resources. Overall, cultivating carinata as a winter crop in the southeastern U.S. states can increase ecosystem services and revenue for farmers (Seepaul et al., 2019).

Carinata taxonomy and genetics

Carinata is a member of the *Brassicaceae (Cruciferae)* family, which currently includes 338 genera and 3709 species, with *Brassica* containing 39 of these species (Warwick et al., 2006b). Brussels sprouts (*B. oleraceae*), Chinese cabbage (*B. rapa*), black mustard (*B. nigra*), canola, Indian mustard (*B. juncea*), and carinata are the most economically important species of *Brassica* grown in commercial production for food and industrial uses. These species are closely related genetically (Nagaharu, 1935). Carinata is thought to have emerged from interspecific hybridization between black mustard and wild cabbage. This crop is an amphidiploid and possesses a complete diploid set of chromosomes from each parent, thereby acquiring valuable traits inherent to each parent (Prakash and Hinata, 1980; Gomez-Campo and Prakash, 1999; Alemayehu and Becker, 2002). Due to this characteristic, carinata has an advantage in that it can use traits from both genomes for survival and adaptability.

Carinata reproduces sexually, by either self- or cross-pollination or seed set, but does not show potential for vegetative reproduction (Mnzava and Schippers, 2007; Warwick et al., 2009). The temperature affects the seed set, and once flowering occurs before the hottest days of summer, higher yields are achieved (Gan et al., 2004). Seeds produced from manipulated carinata lines for industrial use are not suitable for human consumption. Due to its limited use globally and its ability to adapt to drought-like and heat conditions, carinata is a suitable candidate for the biofuel industry, at the same time allowing food crops to be grown in more fertile soils (Kumar et al., 1984; Malik, 1990; Getinet et al., 1996; Schreiner et al., 2009).

Botanical description

Carinata is an annual crop with a determinate growth habit (Zanetti et al., 2013). Plants are erect with a defined taproot and a comprehensive rooting system. The plant has abundant

branches on the main stem with lateral buds (Barro and Martin, 1999). The inflorescence is an extended raceme that is loose, highly branched (Seegeler, 1983), and attached terminally to the main stem and branches and is composed of perfect and actinomorphic flowers that are bright yellow, cream or white (Mnzava and Schnippers, 2007). Carinata fruit is a long narrow pod called a silique. Siliques are highly shattering resistant and usually contain up to 20 seeds (Barro and Martin, 1999; Banga et al., 2011). The leaves of carinata plants are alternate, glabrous to somewhat hairy, and usually waxy (Seegeler, 1983). The seeds of brassica are globose, 1 to 1.5 mm in diameter, finely reticulated, and vary from yellow-brown to brown (Getinet, 1986; Rahman and Tahir, 2010).

Oil and protein content

Carinata seeds have a high concentration of long-chain fatty acids, such as erucic acid, which permits high-energy biofuel production at a low energy requirement during the refinement process (Prakash and Chopra, 1988; Choudhary et al., 2000; Seepaul et al., 2016). An agronomic assessment conducted on carinata indicated that oil concentration and composition could vary among carinata lines. Studies conducted by Mulvaney et al. (2019) in Jay and Quincy, Florida, reported that oil concentration in carinata seeds averaged 400 g kg^{-1}, where more than a third were erucic acid (C22:1), while protein concentration was 310 g kg^{-1}. Similar ranges were reported, ranging from 250 to500 g kg^{-1} oil concentration and 250 to 410 g kg^{-1} for protein concentration (Alemayehu and Becker, 2002; Warwick et al., 2006, Zanetti et al., 2009; Ban et al., 2017). Carinata lines that contain seed oil concentration greater than 400 g kg^{-1} have been identified, which is comparable to canola (Ripley et al., 2006).

Oil concentration may also vary with environmental factors (Canvin, 1965) and the sowing date (Matile, 1975). Biofuel produced from carinata oilseed is considered superior

because of its slow oxidation rate and stability during long-term storage (Bouaid et al., 2009). Cardone et al. (2003) reported that biodiesel produced from carinata oil exhibited similar physical and chemical properties as traditional diesel fuel, with the lower release of impure carbon particles and reduced levels of particulate matter during engine performance. Commercial airplanes have begun testing jet biofuel derived from carinata through collaboration between the National Research Council, Agrisoma, Honeywell UOP Inc., and Saskatoon's Genome Prairie-led Prairie Gold project, in the hope of producing an environmentally friendly fuel source (Larson and Pilieci, 2012).

Temperature effects on seed germination

Germination is the biological process that begins with seed imbibition and ends with the protrusion of the seedling root (radicle) (Bewley, 1997; Finch-Savage and Leubner-Metzger, 2006). Both internal factors (seed viability maturation, genotype, and dormancy) and external factors (water, light, temperature, and oxygen) affect seed germination (Durr et al., 2016; Wang et al., 2016). Among the external abiotic stress factors, temperature plays a dominant role in seed germination and emergence (Milbau et al., 2009; Reddy et al., 2017). Seed germination percentage (MSG) and germination rate (SGR) for most crop species are determined by temperature (Ellis et al., 1986; Kebreab and Murdoch, 2000). Also, germination events are controlled by maternal and nuclear genetics, as well as maternal and ambient environments (Meyer and Pendleton, 2000; Baskin and Baskin, 2001). Plants' ability to adapt to local habitats is generally increased by genotypic inheritance, enabling seeds to germinate at the right time and place, while the diversity of seed germination in time is increased by phenotypic variation (Bradford, 1990; Gutterman, 2000; Baskin and Baskin, 2001).

Temperatures lower than 10ºC have been reported to cause poor germination of canola (Christensen et al., 1985). The germination rate of canola seeds decreased when temperature decreased from 25 to 5ºC (Witcombe and Whittington, 1971). Andrucci et al. (2016) used linear and nonlinear models to estimate cardinal temperatures for germination of brassicas and reported base (T_b), optimum (T_{opt}), and maximum (T_{max}) temperatures for canola germination of 0 to 3ºC T_b, 29 to 33ºC T_{opt}, and 35ºC T_{max}, respectively. Luo et al. (2018) tested eight rapeseed cultivars at constant temperature treatments of 3, 5, 8, 10, 13, 18, and 23ºC and reported that the germination rate of seeds decreased as temperature decreased from 23 to 3ºC.

High temperature may result in a limited supply of photosynthetic assimilates during the development of seeds (Spears et al., 1997; Shinohara et al., 2006), causing physiological damage resulting in the loss of seeds' ability to germinate (Hampton et al., 2013). Several methods were employed to screen crops for tolerance to abiotic stress conditions, and these include the use of biochemical and physiological constants at the emergence, germination, vegetative, and reproductive stages. The genotypic-environmental adaptability range and stress tolerance capacity of crop genotypes can also be determined using in vitro germination assays (Gajanayake et al., 2011). These methods have been widely used to assess plant tolerance to environmental stress in various crops, including cotton (*Gossypium hirsutum* L) (Reddy et al., 2017), rice (*Oryza sativa* L.) (Singh et al., 2017), pepper (*Capsicum annuum* L.) (Gajanayake et al., 2011), switchgrass (*Panicum virgatum* L.) (Seepaul et al., 2011), soybean (*Glycine max* L.) (Alsajri et al., 2019), oilseed species (*Brassica sp*.) (Dawadi et al., 2019) and big bluestem (*Andropogon gerardii*) (Singh et al., 2019). Dawadi et al. (2019) showed that carinata germinated rapidly at a constant temperature of 25ºC, reaching maximum germination after approximately 60 hours of incubation. The use of in vitro germination assay helps to understand better how a genotype may

react to set abiotic conditions even before field-testing. Significant progress was made in determining environmental stress tolerance for these crops, and such studies are needed for carinata since there is limited information on carinata temperature tolerance based on germination parameters.

Increasing interest in carinata production as a feedstock for biofuel production has led to breeding and field screening of a wide selection of genotypes. These genotypes must be screened for abiotic stress resistance before releasing for commercial production. Several studies have used single value indices that summarizes germination time course using coefficients, percentiles, and quartiles related to control trials, and cumulative index based on statistical grouping and mean separation (Emerson et al., 1979; Koti et al., 2004; Salem et al., 2007). Other researchers used quantitative relationships that were determined by principal component analysis (Kakani et al., 2002, 2005; Singh et al., 2008) to quantify crop response to temperature. Single values indices include germination rate index (GRI), heat tolerance index, and corrected germination rate index (CGRI). Germination rate, which is a reciprocal of time to 50% germination, is used to express the speed of germination and usually increases with rising temperature (Hsu et al., 1985). Various studies have reported that heat enhances seed germination based on results showing GRI, CGRI, and germination rate to be positively correlated with temperature (Hsu et al., 1985). These indices can also be used to screen crop germplasm and determine the potential of genotypes to respond to temperature (Hardegree et al., 2008). Using indices can have its limitations. Among these limitations are: (a) ambiguousness, insensitive, and incompleteness of germination; (b) does not determine the location; (c) rate and extent of germination and dispersion time; (d) assume a normal distribution for germination frequency; and (e) mostly represents germination processes rather than describe them (Shafi et

al., 1991). Even though single value indices allow the relative ranking of seed lots based on inter-lot variations, these indices may not be robust statistically and do not support substantial biological evidence that inter-seed lot dynamics are directly related to seed germination capacity and rate (Shafii et al., 1991).

Growth models are other alternative approaches that use index numbers to describe the germination process. Growth models can help generate a vast amount of data that gives results to parameter constants with credible and applicable biological data once the correct mathematical equation accompanied by the appropriate statistical procedures are applied (Shafii et al., 1991). Seed performances are classified by three factors: (1) time of germination onset; (2) germination rate; and (3) germination capacity (cumulative germination percentage at the end of the evaluation period). These parameters are used to help determine suitable conditions to produce seed at a commercial level (El-Kassaby et al., 2008). To date, several mathematical functions that define the relationship between germination rate and temperature (Shafii et al., 2001; Soltani et al., 2006) have been used. The use of mathematical functions to determine the effect of temperature on seed germination may be useful in evaluating germination characteristics and the adaptability potential among crop species or genotypes (Jordan and Haferkamp, 1989). Several authors have used linear and non-linear regression models to quantify germination response and crop seed germination against temperature stress (Mwale et al., 1994; Hardegree, 2006; Gajanayake et al., 2011; Seepaul et al., 2011). Several studies have reported the effects of low and high-temperature stress on germination capacity and rate for various crop species along with their cardinal temperatures, but no such research has been conducted for carinata.

Temperature effects on plant growth

Carinata thrives well in semi-arid environments and is reported to be a cool-season crop (Marillia et al., 2014). This oilseed crop is also tolerant of heat and drought-like conditions (Getinet et al., 1996; Schreiner et al., 2009). Field tests for several carinata cultivars were successful across Canada and various regions in the United States (Marillia et al., 2014), indicating that USDA Plant Hardiness Zones 4 to 9 are suitable for carinata cultivation (Magarey et al., 2008). Frost tolerance in carinata cultivars has also been reported (Seepaul et al., 2015), but these temperature limits and exposure time are yet to be published.

Abiotic stress is a crucial factor that compromises crop development and production globally (Boyer, 1982; Gao et al., 2007). Temperature is an important abiotic stress factor that plays a dominant role in the control of plant growth and developmental processes under optimum nutrient and water conditions. Plant species, and even cultivars within species, vary in their sensitivity to temperature (Wijewardana et al., 2015; Reddy et al., 2017). A rise in temperature may lead to altered geographical distribution and growing seasons of crops by modifying the temperature ceiling for the start of the season and crop maturity period (Porter, 2005). Crop yield can be reduced substantially due to heat stress. A study in soybean showed that temperature increase caused a reduction in aboveground biomass, pod and seed number, and seed size (Tacarindua et al., 2013). These authors suggested that the increased temperature possibly contributed to delayed pod setting and reduced seed growth rate.

Each developmental aspect or crop event has its specific optimum temperature, above which plant growth processes will decline (Alsajri et al., 2019). Elevated temperatures beyond the maximum tolerance of the plant can negatively affect plant growth and survival and hence, reduce crop yield (Boyer, 1982). Lobell and Asner (2003) reported that each degree centigrade

increase in average growing season temperature might reduce crop yields as much as 17%. As greenhouse gas emissions increase, extreme weather events, such as prolonged high temperature, will intensify (Singh et al., 2008). There will be frequent variations in the weather pattern, including fluctuations in low and high temperatures (Meehl and Tebaldi, 2004). Several researchers have shown that these projections in climate change can reduce crop productivity when these perturbations occur during the reproductive stage (Hall, 1992; Reddy et al., 1992, 1997). Yield reductions occurred when canola was grown in both winter and spring (Reddy et al., 2005). Indian mustard plants exposed to high temperatures during early season growth showed a decline in growth (Shamsul et al., 2009). High-temperature stress also reduced the number of pods on the main stem, the number of seeds per pod, and the seed weight in several *Brassica* species (Gan et al., 2003). Also, Alsajri et al. (2019) reported that both low and high temperatures had significant effects on soybean cultivars during the seedling growth stage. Usually, the damage caused by temperature stress all depends on the plant growth stage at a specific time and the extent to which that stress persisted (Li et al., 1981).

Plants have a specific response mechanism to cope with abiotic stresses that are activated when exposed to these different stresses. Studies have shown that plants may require an exceptional response mechanism to handle several abiotic stresses at the same time (Zhao et al., 2009; Prasch and Sonnewald, 2014; Rivero et al., 2014). To understand plant tolerance mechanisms to a combined number of abiotic stresses, however, it is necessary to have a comprehension of plant responses to each environmental factor (Mittler, 2006). Several studies have used a suite of parameters including morphological, physiological and reproductive factors to evaluate temperature and drought stress tolerance in multiple crops, including peppers (Reddy and Kakani, 2007; Gajanayake et al., 2011), soybean (Salem et al., 2007), maize (*Zea mays* L.)

(Wijewardana et al., 2016a, 2017), cotton (Kakani et al., 2005; Reddy et al., 2017), peanut (*Arachis hypogaea* L.) (Kakani et al., 2002), and canola (Singh et al., 2008). All of these studies reported that variability existed among the cultivars for the evaluated physiological factors in all species examined. There is limited information available on carinata's response to different temperature ranges at different growth stages. Therefore, information on temperature and carinata oilseed crop will be valuable to develop models for field applications.

Cardinal temperatures

The seed germination process of all crops generally occurs in a specific temperature range called the cardinal temperatures (Bewley, 1997). Cardinal temperatures are critical in identifying plant species that are tolerant of minimum and maximum temperatures and environmental conditions under which some crops can successfully germinate and establish (Ghaderi et al., 2008). All crop species have a minimum (T_{min}), optimum (T_{opt}), and a maximum (T_{max}) or ceiling temperature at which seed germination occurs. The minimum thermal level at which seed germination occurs is called the minimum temperature, the optimum temperature is the point where germination rate is highest, and the maximum temperature is the temperature above which seed germination does not occur (Ramin, 1997; Alvarado et al., 2002). Knowledge of these parameters is essential when seeding fields (Hanson and Johnson, 2005). The germination adaptability range for carinata seeds, as defined by cardinal temperatures, is still not established for any production regions.

Temperature thresholds at which seed germination reaches its maximum can vary depending on plant species and seed quality (Ellis et al., 1981). A complete description of temperature stress response requires at least five cardinal temperatures thresholds, including the minimum or base, optimum and maximum temperatures, and the limits for the optimum range

(Garcia-Huidobro et al., 1982). For most plants, the growth rate tends to increase when the temperature rises from the minimum to the optimum temperatures and decreases between the optimum and the maximum temperature (Soltani et al., 2006). Also, similar occurrences were reported for seed germination rate and percentage regarding temperature (Iannucci et al., 2000; Al-Ahmadi and Kafi, 2007). Typically, an S-shaped curve represents the cumulative seed germination of seed lots when germination is occurring (Roche et al., 1996). In this curve, a lag phase represents the delay in the onset at initial water uptake, a rising or approximately linear stage characterizes the rate of physiological processes leading to radical emergence, and the curve terminates with a straight line or an upper asymptote at the maximum percentage of germination. A perfect description of seed germination must be complete, neat, definite, and susceptible to statistical assessment and produce data on the three phases of the curve (Brown and Mayer, 1988). The use of cardinal temperatures is crucial in agronomic and management decision making, and being able to determine the specific cardinal temperature for a given genotype can be beneficial for deciding optimum sowing windows and potential growing regions for carinata.

Using thermal units to model seed germination requires a precise determination of base temperature (Madakadze et al., 2001). Gajanayake et al. (2011) found that cardinal temperatures derived from the germination time series for 12 ornamental pepper differed among all the cultivars. Similarly, Seepaul et al. (2011) reported that cardinal temperatures differed among 14 genotypes of switchgrass evaluated and concluded that differences among genotypes in cardinal temperatures might be attributed to different areas of adaptation or origin. The thermal time model was successfully used in other studies to determine cardinal temperatures (T_{min}, T_{opt}, and T_{max}) for MSG and SGR. Several authors used quadratic functions (Robocker et al., 1953; Hsu

et al., 1984; Hanson and Johnson, 2005) in their studies to estimate cardinal temperatures for different crops, such as switchgrass (Seepaul et al., 2011), big bluestem (Singh et al., 2019), pepper (Gajanayake et al., 2011) and common ragweed (*Ambrosia artemisiifolia* L.) (Leiblein-Wild and Tackenberg, 2014). Using both pollen and physiological parameters, these thermal constants were used to classify different genotypes into thermotolerance groups (Peet et al., 1998; Kakani et al., 2005; Singh et al., 2008). Similar studies were also conducted for pepper (Aloni et al., 2001; Reddy and Kakani, 2007; Gajanayake et al., 2011). Along with genotypic variability among switchgrass genotypes and their cardinal temperatures, there was variation in MSG and SGR (Seepaul et al., 2011). Cardinal temperatures can be genotype-specific as well as process-specific, as suggested by Kiniry et al. (2005).

Thermotolerance screening

Thermotolerance is defined by the plants' ability to avoid damages caused to heat-sensitive structures and organic compounds. The ability of plants to survive and yield in temperatures above its optimal growth temperature is referred to as basal thermotolerance, while acquired thermotolerance is when plants are exposed to a short period of acclimation under high temperatures, after which plants are then able to withstand greater than optimum temperature conditions (Larkindale et al., 2005). Evaluating crop species for primary traits that make it resilient to extreme weather conditions is the primary goal for most breeding programs (Singh et al., 2007). To determine the temperature tolerance among genotypes, screening can be conducted at either low or high or both high and low temperatures (Potaczek and Kozik, 2000). The long-term goal of the temperature tolerance breeding program should be the development of germplasm with improved field-level tolerance under variable temperature conditions (Porch, 2006). Any parameter that changes with temperature can be used to screen genotypes; however,

Srinivasan et al. (1996) contended that screening for tolerance must have the following requirements: (a) must be performed with a suitable physiological parameter sensitive enough to respond to induced temperature stresses and also be able to stratify genetic differences at early stages; (b) must be precise, rapid and reproducible in the detection of selected parameter changes under variable field conditions; and (c) must allow for performing a large number of measurements with many breeding lines and cultivars.

Temperature variation response among genotypes can be used as a method of analysis for screening seed populations based on the assumptions that: (a) there are positive and negative linear relationships between the rate of germination and temperature at sub- and supra-optimal temperatures, respectively; (b) there is no variation of minimum temperature within one seed population, but there is a normal distribution of thermal times at sub-optimal temperatures; and (c) within a seed population, there is no difference in thermal time, but there is a normal distribution of maximum temperatures at supra-optimal temperatures (Ellis et al., 1987). The growing interest in carinata as a feedstock for biofuel production has led to research, development, and selection of various carinata genotypes that must undergo tolerance testing to various abiotic stresses before released for commercial cultivation since information on response to these abiotic stresses is limited. Multiple studies were conducted on other crops to identify cold and heat tolerant cultivars at the early season growth stage (Wijewardana et al., 2015; Alsajri et al., 2019). Knowing how plants respond to temperature stress is essential in breeding cultivars to be cultivated in hostile environments. Besides, the effect of high temperatures will aid in the prediction of the agronomic penalty due to climate change and rising greenhouse gases to assure agriculture sustainability (Paulsen, 1994; Reddy and Hodges, 2000). Knowledge of

carinata genotypes response to both low and high temperatures is essential for identifying genotypes that are best suited for cultivation under Mississippi's environmental conditions.

Selecting superior genotypes from populations has been aid by stress indices based on physiological parameters associated with a desirable trait. Some indices reported to screen genotypes includes geometric mean, stress tolerance index, and stress susceptibility index (Porch, 2006). Temperature tolerance screening can be achieved using physiological and biochemical parameters at the vegetative and reproductive stage (Singh et al., 2007). These traits were successfully used for temperature tolerance screening among common bean (*Phaseolus vulgaris* L.) genotypes (Petkova et al.,2007), cotton (Singh et al., 2018) and legume species, including chickpea (*Cicer arietinum* L.), groundnut, pigeon pea [*Cajanus cajan* (L.) Millsp.], and soybean (Srinivasan et al., 1996). Small plants, narrow leaves, dense tillers, excessive root growth, and more significant root to shoot ratios could be used for selecting low- and high-temperature tolerant cultivars. Basu and Minhas (1991) and Nagarajan and Minhas (1995) stated that several vegetative parameters such as internode elongation could be useful in screening potato (*Solanum tuberosum* L.) genotypes. Physiological and morphological traits have been used to screen corn hybrids for cold tolerance (Wijewardana et al., 2015, 2016) and cotton for heat and drought tolerance (Singh et al., 2018).

Crop modeling

Crop simulation models are developed and used for numerous purposes, such as forecasting plant growth and development, yield prediction, hypothesis testing, and making management decisions (Vandendriessche and Van Ittersum, 1995). To better understand the complexity of biological systems, robust approaches to using mathematical models have been explored (Meng et al., 2004), allowing the testing and development of models that use functional

algorithms between crop growth and the environment. Quantifying the effects of environmental factors on several distinct phenological and physiological processes of particular crop species from sowing to maturity can be achieved by modeling crop growth and development (Reddy et al., 1997; Reddy, 2008). Robust and mechanistic-field tested models will be of great value for on-farm resource management and policy decisions (Reddy et al., 2002).

One type of model that is widely used for crop yield prediction is regression models that use environmental factors as independent variables. Nonetheless, researchers found that process-based crop simulation models based on crop, soil, and weather variables were deemed more effective as a tool in research, land use planning, cropping, and water management strategic plans (Jordan, 1983). Using crop growth simulation models has made it easier for producers and lawmakers to review and resolve the inconsistency in crop requirements and improve management practices (Singh et al., 2002). Models are used to establish appropriate cropping systems, crops to be planted, and genotypes best suited for distinct agro-climatic regions and diverse soil types (Singh et al., 2001). A carinata simulation model can be a vital component for feedstock production systems. There are limited studies and reports available on comprehensive simulation models for oilseed *Brassicas* that measures growth and yield except for several trials where the attempt was made to develop various simulation models for few *Brassica* species, including Indian mustard and rapeseed (Rao, 1992).

Crop simulation models help predict changes in crop status over time as a function of exogenous factors (Whisler et al., 1986). Early attempts were made to model dry matter production of *Brassica* species (Hellstorm and Kjellstorm (1989) using the grassland model developed by Torssell and Kornher (1983), which was based on soil water balance, relative growth rate, a development term, and weather index. Rao (1992) developed a process-oriented

dynamic simulation model BRASSICA retaining some features from the PNUTGRO model and tested it under non-restrictive nutrient and moisture environment to forecast potential production under varying radiation and thermal regimes. The BRASSICA model was tested for Indian mustard, where result showed that biomass production and phenological development was within acceptable limits to the prediction made, however, there was an overestimation of leaf area index and an underestimation seed yield compared to the observed data collected (Singh et al., 2001). There was no application of this model for most varieties and fields where oilseed *Brassicas* are cultivated, however.

Additionally, to evaluate the growth and yield of *Brassica* species, Aggarwal et al. (1994) developed the InfoCrop model. Adak et al. (2009) concluded that the InfoCrop model needs further refinement since it overestimated the parameters like leaf area index and biomass for all the seasons tested and showed differences between the simulated and observed yield. Various models have been tested on horticultural seeds to determine the effect of temperature on SGR, mostly carried out under in vitro conditions. Quantitative models were based mainly on population statistics for studying seed behavior following earlier studies conducted (Ellis and Roberts, 1981). Several researchers have used those methods to develop a physiologically based population model, which describes seed behavior relationship with germination time, thermal relations, seed dormancy, and other factors (Bradford et al., 1993; Bradford and Somasco, 1994; Bradford, 1995).

The growing demand for agricultural goods increases pressure on water, land, and other resources and requires prompt decision making at all levels, requiring a vast amount of information to do so. Generating data through research using traditional agronomic methods and publication is not adequate to meet these increasing requests. Agronomic experiments

traditionally are carried out at a specific time and place, causing results to be season and site-specific, time-consuming, and expensive (Jones et al., 2003). Employing crop simulation models will be beneficial to determine crop management alternatives, to predict yields and gaps, and also assess environmental impacts on crop growth and yield (Pathak and Wassmann, 2009). Crops simulation models can be a tool for yield forecasting before harvesting to aid in the extrapolation of the outcome from one location or season to the other (Anapalli et al., 2005). Another advantage of crop growth models is that they can measure the variability of crop yield or performance caused by varying weather patterns and long-term projection impacts of land use and climate change (Timsina and Humphreys, 2006; Liu et al., 2010).

Modeling germination response to temperature

Seed germination should be absolute, precise, unambiguous, amenable to statistical analysis, and easy to understand. As early as 1926, (Kotowski, 1926) to recently developed models, seed germination thermal responses have been processed down into single value indices that try to describe the process of germination. Their effectiveness in describing the germination process has been debatable. The individual value index cannot combine all of these properties of germination (delay, lag, speed, and extent) into a single value. Several assessments on the validity of various single value indices (Maguire's rate of germination, Timson's cumulative germination, Czabator's germination value, Diavanshir, and Pourbiek's germination value, Kotowski's coefficient of velocity, Lehle's function index, and Putnam Richards function index, Smith and Millett's sprouting index, and Tucker and Wright's regression index) were carried out and concluded that all of the index except for Timson's cumulative germination method were incapable in simulating field-level germination data or ranking seed germination responses (Brown and Mayer, 1988a). Also, there were some potentially severe statistical problems when

using germination indices. These only make use of limited data collected from an experiment (McNair et al., 2012). Knowing the variation in temporal patterns of seed germination is essential, and verifying these patterns requires several observation days. Besides, using germination indices results in wastage of data (Brown and Mayer, 1988a). Furthermore, because they failed to summarize the process of seed germination and comparing treatment effects satisfactorily, none of the single value index methods evaluated were recommended (Scott et al.,1984; Brown and Mayer 1988a).

Nevertheless, finding alternatives to using single value germination indices has led to the application of maximum seed germination (MSG), statistical analysis, or using curve-fitting methods (Bonner and Dell, 1976; Brown and Mayer, 1988b; Carneiro, 1994). Fitted curves could summarize germination time course information precisely, providing that it matches the observed data closely and adequately enough, and at the same time, preserve essential details on the commencement, rate, and extent of seed germination (Brown and Mayer, 1988b). Various curve fitting methods were proposed to delineate the process of germination. Brown and Mayer (1988b) applied the Weibull function among others to data of several cumulative germinations for non-dormant seed. They concluded this function consistently produced the best fit with its four factors (maximum germination, seed germination rate, the lag in the onset of germination, and the shape of the cumulative distribution). These cumulative germination curves are typically sigmoid curves that can be quantified by the standardized normal distribution (Janssen, 1973) or by logistic curve procedure, as suggested by Hsu et al. (1984).

Effect of temperature on germination rate and maximum seed germination percentage

Seed germination duration, consistency, and the rate at which this process occurs are descriptions of a seedlot. Current environmental conditions can easily alter these attributes.

Maximum seed germination percentage (MSG) and seed germination rate (SGR; defined as the reciprocal of time taken for half the population to germinate) are two seedlot quantification and descriptive parameters that respond to temperature differently, and quantifying these responses is necessary for germination modeling using thermal parameters. Garcia-Huidobro et al. (1982) reported that SGR has a sharply defined optimum, while MSG is achieved across a range of temperatures. Schimpf et al. (1977) reported that MSG is positively correlated with SGR, suggesting that SGR is more sensitive to temperature than MSG in yellow foxtail *(Setaria lutescens)* and redroot amaranth *(Amaranthus retroflexus* L.). For several crops including pearl millet (Garcia-Huidobro et al., 1982), chickpea, lentil (*Lens culinaris* Medik), soybean, cowpea (*Vigna unguiculata*) (Covell et al., 1986), carrot (*Daucus carota* L.) (Hegarty, 1973), and 31 vegetable species (Bierhuizen and Wagenvoort, 1974), SGR is mostly bilinear in response to constant sub- and supra-optimal temperature.

Garcia-Huidobro e al. (1982) and Ellis et al. (1986) reported that SGR increased linearly with temperature from a base temperature to a sharply defined optimum, beyond which germination rate decreases linearly and reached zero at maximum temperature. The linearity between SGR and temperature over a specified range, for example, between the minimum temperature and the optimum temperature, means that the thermal time required for germination is a constant and can, therefore, be used to compare germination in different species, climates, and locations. This relationship between rate and temperature is observed in many other physiological and phenological processes, including the rate of pollen germination and tube length growth (Kakani and Reddy, 2007), radicle and plumule emergence, and early growth (Arndt, 1945; Blacklow, 1972).

Covell et al. (1986) and Ellis et al. (1987) suggest that the thermal time approach can be modified to provide equations that describe the variation in germination time within a seed population at sub-optimal temperatures, Eq. [2.1], and the variation at supra-optimal temperatures, Eq. [2.2].

$$1/t(G) = [T-Tb]/([probit(G)-K]\sigma) \quad (2.1)$$

Where:

t/(G) is the time taken for cumulative germination to reach the percentile G at temperature T

T_b is the base temperature (at which temperature $t(G) = \infty$)

K is a constant,

σ is the standard deviation of the distribution of thermal times for germination within the seed population.

$$1/t(G) = (([K_s-probit(G)]\ \sigma)-T)/(\theta_2) \quad (2.2)$$

Where:

K_s is a constant

σ is the standard deviation of the distribution of the ceiling temperature within the population [$T_c(G)$, at which temperature $t(G) = \infty$],

θ_2 is the thermal time for germination at supra-optimal temperatures.

Covell et al. (1986) found that T_b does not vary for different fractions within a seed population. Also, they reported that thermal time across the sub-optimal range varies within each seed population.

Temperature tolerance screening tools

Evaluation of temperature adaptability ranges (TAR) of carinata genotypes has been limited to field performance, screening nurseries, and visual assessment based on survival, which can be protracted and resource-intensive. It is challenging to separate heat stress, water stress, and biotic factors from germination potential because of uncontrollable interactions that are occurring. Therefore, screening of crop genotypes before conducting field trials requires a controlled environment where moisture and temperature are monitored. For that reason, a rapid, reliable, and simple method for screening are required to screen a large number of genotypes for thermotolerance under controlled conditions (Setimela et al., 2005).

Also, field evaluations are usually confounded with several co-variables, which include fluctuating temperatures and unpredictable moisture conditions, which can mask the true germination potential of a genotype. Seed germination under controlled temperatures is a reasonably inexpensive and straightforward method to screen large numbers of genotypes. De La Soujeole (1984) suggested that sorghum should be evaluated at germination, emergence, and seedling growth for chilling tolerance, contending that these three stages are independently sensitive to cold temperature. Furthermore, the rate of germination better separates the thermal genotypic response than MSG and early seedling growth rate (Tiryaki and Andrews, 2001). These parameters were successfully used to classify several crops, including lentil (Mohammad and Haghnazari, 2008), cowpea (Murillo-Amador et al., 2000), soybean (Hou and Thseng, 1992), canola (Acharya et al., 1983) and sorghum (Tiryaki and Andrews, 2001), in response to various abiotic stress conditions. There are limited data in the literature on studies screening carinata for temperature tolerance using seed-based parameters.

Evaluating crop species for primary traits that make them resilient to extreme weather conditions is the critical goal for most breeding programs globally (Singh et al., 2007). More suitable and relatively faster methods of selection can be used to speed up the breeding process. Therefore, there is a need for experimental facilities that mimic environmental field conditions that also include solar radiation (Reddy et al., 2001). Thermotolerance selection is possible through the evaluation of plant physiological processes such as photosynthesis and chlorophyll fluorescence on a whole-plant basis (Hall, 1992; Fracheboud et al., 1999). Other proposed traits used to determine temperature tolerance in plants includes the ranking of plants based on productivity or growth rate, germination rate (Hotchkiss et al., 1997; Revilla et al., 2003), and root traits (Hund et al., 2008). Several crop physio-morphological parameters have been used to screen and categorize genotypes to temperature tolerance (Singh et al., 2007). As noted for seed-based parameters, there are limited studies on carinata screening for thermotolerance using plant morpho-physiological traits at different growth stages.

Genotype classification methods

Techniques for genotype classification ranged from simple to statistically rigorous procedures, including single value indices (Brown and Mayer, 1988a), percentiles and quartiles relative to control studies, cumulative index, and principal component analysis (PCA). Emerson and Minor (1979) classified soybean genotypes for high-temperature tolerance using a confidence interval about the mean germination. Similar classification approaches have been used by Kakani and Reddy (2007) and Salem et al. (2007) to classify pepper and soybean genotypes, respectively, using pollen-based parameters and a temperature response index (TRI). The TRI relates the value of a genotype to the maximum or minimum value of all genotypes. The summation of individual TRI results in a cumulative TRI that is then separated by standard

deviation based on the number of classes of interest. Cumulative TRI has been used to screen genotypic variability under multiple environmental conditions in soybean (Koti et al., 2004), to screen switchgrass genotypes for heat tolerance (Seepaul et al., 2011), and to screen corn hybrids for cold tolerance (Wijewardana et al., 2015, 2016).

Genotypic classification can also be achieved by PCA, which is a multivariate technique that examines the relationships among a large number of quantitative traits. Kakani et al. (2002) and Singh et al. (2008) demonstrated the utility of this method by classifying peanut, cotton, and canola genotypes based on eigenvectors and eigenvalues. The TRI method uses all traits of interest that may potentially contribute to a given stress condition tolerance or sensitivity, and each trait will have an equal contribution. The PCA analysis, on the other hand, will take into account only one to three traits that have a maximum contribution in separating the genotypes.

CHAPTER III

THERMOTOLERANCE CLASSIFICATION OF *Brassica carinata* GENOTYPES USING IN VITRO SEED GERMINATION ASSAY

Abstract

Temperature plays a crucial role in seed germination processes. Understanding the response of carinata to thermal stress and developing a reliable and straightforward thermotolerance screening method will be beneficial for breeding programs and model applications. A study was conducted to evaluate 12 carinata genotypes response to eight temperatures during germination; 8.2, 12.73, 15.65, 19.93, 23.8, 29.28, 34.22, and 36.96ºC. Effect on maximum seed germination (MSG) and seed germination rate (SGR) were measured, and their cardinal temperatures and thermotolerance groups were determined. The mean minimum, optimum, and maximum temperatures for MSG across carinata genotypes were -0.14, 20.28, and 40.69ºC, respectively. For the SGR, the mean minimum temperature was 5.01ºC, optimum was 24.85ºC, and the maximum was 44.69ºC. Among genotypes, there was variation in MSG, SGR, and their cardinal temperatures. Of the 12 genotypes evaluated, 8.3% were identified as cold-tolerant, 25% as moderately cold-tolerant, 33.3% as moderately cold-sensitive, and 33.3% as cold-sensitive. Also, 8.3% were identified as heat-sensitive, 58.3% as moderately heat-sensitive, 25% as moderately heat-tolerant, and 8.3% as heat-tolerant. The genotype AX17004 was identified as both the most cold- and heat-tolerant line. Based on breed types, double haploid and hybrid groups had a stable thermotolerance response at both germination and

early growth stages. In contrast, the inbred group had a wider cluster in responses to a minimum and maximum temperatures. The *in vitro* assay method is an inexpensive technique for thermotolerance screening.

Introduction

For biofuel feedstock crops to be successful, high germination rate, emergence, and stand establishment are required (Dawadi et al., 2019). The successful establishment of a new feedstock species in a new environment depends on its ability to emerge, and to establish uniformly and rapidly (Hacisalihoglu, 2008) and yield under varying environmental conditions. Since climate change is occurring rapidly, causing variation among abiotic stresses, frequent flooding, and prolonged drought periods (Jagadish et al., 2012). This makes it crucial to identify adaptable genotypes and suitable management practices to optimize yield (Aggarwal and Kalra, 1994). Stand establishment for any crop is affected by both internal factors (seed viability, maturation, genotype, and dormancy) and external factors (water, light, temperature, and oxygen) (Durr et al., 2016; Wang et al., 2016).

Brassica carinata (A. Braun), commonly called Ethiopian mustard, or carinata, is an oilseed crop with potential for biofuel production (Cardone et al., 2003) due to its high concentration of erucic acid (Cardone et al., 2003; Warwick, 2011). Because of its area of origin, carinata is better adapted to semi-arid regions (Barthet, 2008; Marillia et al., 2014), with a greater tolerance to moisture stress, high temperatures (Singh, 2003), seed shattering (Seepaul et al., 2016), drought (Kumar et al., 1984), and diseases (Shivpuri et al., 1997). Carinata resulted from interspecific hybridization between black mustard (*B. nigra* L.) and wild cabbage (*B. oleraceae*), which are *Brassica* species that are closely related genetically (Nagaharu, 1935). This crop is an amphidiploid, having a complete diploid set of chromosomes from each parent,

with high survival and adaptability (Prakash and Hinata, 1980; Gomez-Campo and Prakash, 1999; Alemayehu and Becker, 2002) but low cold tolerance (Gugel et al., 1990). There is limited availability of commercial varieties (Monti et al., 2009). Differences in genetic characteristics among genotypes may contribute to variation in the adaptability range of a genotype to a particular region (Gesch et al., 2015). Carinata is a relatively new winter oilseed crop in the southeastern USA (de Koff et al., 2017), where studies are currently ongoing to identify genotypes best suited for commercial feedstock production. This initiative is led by the University of Florida (UF) in collaboration with the Southern Partnership for Advanced Renewables from Carinata (SPARC) consortium.

Temperature plays a dominant role in seed germination, emergence, growth, and development (Milbau et al., 2009; Reddy et al., 2017). For most crop species, both seed germination percentage and rate are determined by temperature (Garcia-Huidobro et al., 1982; Ellis et al., 1986; Kebreab and Murdoch, 2000). High temperature may result in a limited supply of photosynthetic assimilates during the development of seeds (Spears et al., 1997; Shinohara et al., 2006), causing physiological damage resulting in reduced or no germination (Hampton et al., 2013). Knowledge of moisture conditions and optimum temperature for rapid germination and establishment of feedstock plots are required (Fulbright, 1988; Hanson and Johnson, 2005). The yield of *Brassica* species is highly dependent on environmental conditions during growth and developmental stages (Saha and Khan, 2008).

Germination studies can help determine the adaptability range of a particular plant species to different environmental conditions (Dawadi et al., 2019). Seed germination processes generally occur in a specific temperature range called the cardinal temperatures (Bewley, 1997; Finch-Savage and Leubner-Metzger, 2006). In this range, there are minimum (T_{min}), optimum

(T_{opt}), and maximum (T_{max}) temperatures, which are useful in constructing models to predict germination and developmental processes (Kebreab et al., 2000; Ghaderi et al., 2008). To date, several mathematical functions have been used to define the relationship between germination rate and temperature (Shafii et al., 2001; Soltani et al., 2006). Also, quadratic, linear, and non-linear regression models to quantify the seed germination response to temperature stress were used (Gajanayake et al., 2011; Seepaul et al., 2011; Andrucci et al., 2016; Singh et al., 2019). Although several studies have reported the germination capacity and rate for various crop species along with their cardinal temperatures, no such research has been conducted for carinata. Cardinal temperatures are critical in identifying plant species that are tolerant of minimum and maximum temperatures (Ghaderi et al., 2008).

Evaluating temperature adaptability of genotypes have been limited to field performance, screening nurseries, and visual evaluation based on survival (Setimela et al., 2005). It is challenging to separate heat stress, water stress, and biotic factors from germination potential because of uncontrollable interactions that are occurring. For that reason, a fast, consistent, and simple method for screening is required to screen a large number of genotypes for thermotolerance under controlled conditions (Setimela et al., 2005). Several researchers have used seed-based parameters such as germination rate and capacity to evaluate crop genotypes tolerance to abiotic stresses in various crops (Gajanayake et al., 2011; Seepaul et al., 2011; Reddy et al., 2017; Singh et al., 2017; Dawadi et al., 2019; Singh et al., 2019), including both vegetative and reproductive or physiological and biochemical factors at the germination stage (Singh et al., 2007). Several studies used the cumulative temperature response index (CTRI) (Emerson and Minor, 1979; Koti et al., 2004; Salem et al., 2007; Seepaul et al., 2011) to group various crop genotypes into tolerance groups for temperature stresses.

The objectives of this study were to (a) quantify the effect of temperature on carinata MSG and SGR, (b) determine the cardinal temperatures for MSG and SGR, and (c) classify carinata genotypes for temperature tolerance.

Materials and Methods

Seed materials

For this study, seed material of 11 advanced carinata genotypes (meaning that they are close to commercial deployment) of three breed types (inbred, double haploid, and hybrid) and one commercial check entry were evaluated (Table 3.1). Seeds were collected from eight genotypes grown in Florida and four grown in Canada during the 2017 growing season. All seeds were provided by Agrisoma Biosciences Inc. Canada (now Nuseed Australia). After harvesting, seeds were treated with Helix Vibrance, which contains four fungicides (difenoconazole, metalaxyl-M, fludioxonil, sedaxane), and one insecticide (thiamethoxam), to minimize fungal infections and insects.

Table 3.1 Type and origin of *Brassica carinata* genotypes sourced from Agrisoma Biosciences, 2019 (now Nuseed Australia).

Genotype	Type†	Justification
AX17001	I	Selection from SE16-17 AYT (Avanza family selection) Florida
AX17002	I	Selection from SE16-17 AYT (Avanza family selection) Florida
AX17004	I	High shatter tolerance family, good potential in a winter environment
AX17005	I	High shatter tolerance family, good potential in a winter environment
AX17006	I	High shatter tolerance family, good potential in a winter environment
AX17007	DH	Among the highest Sclerotinia incidence, Jay and Quincy, FL
AX17008	DH	Selection from SE16-17 PYTB Florida
AX17009	DH	Selection from SE16-17 PYTA Florida
AX17010	DH	Selection from SE16-17 PYTB Florida
AX17014	H	Top 2016-17 Quincy test hybrid Florida
AX17015	H	Promising test hybrid from 2017, frost tolerant female
Avanza 641	Check	Commercial check

†Genotypes are classified into three types (I = inbred, DH = double haploid, and H = hybrid). Seed trials (SE - Southeast, AYT - advanced yield trial, PYT - preliminary yield trial).

All seeds were stored in a refrigerator at 4ºC to maintain optimum quality until time for further use. Seeds for each genotype were counted manually, placed into small paper pockets, and stored in an airtight container at room temperature for 24 h before being put into the germination chamber.

Seed germination and temperature treatments

Carinata seed germination testing at different temperature treatments was carried out from May to September 2019. This research was performed in an *in vitro* environment at the Environmental Plant Physiology Laboratory, Mississippi State University, MS, USA. The germination study was conducted according to guidelines described by the Association of Official Seed Analyst (AOSA; Baalbaki et al., 2009) with no humidity control. This experiment was a two-factor factorial (12 genotypes × 8 temperatures) arranged in a completely randomized design with each treatment replicated four times using 100 seeds for each replicate. The eight levels of germination temperatures tested were intended to range from 8 to 38 in 5ºC increments

but actual experimental temperatures 8.2, 12.73, 15.65, 19.93, 23.8, 29.28, 34.22, and 36.96ºC. For each experimental unit, 100 seeds were counted and weighed then arranged uniformly onto sterilized plastic trays lined with double layers of sterilized paper towels (Scott Shop towels, Kimberly-Clark, USA). Paper towels were moistened with sterile distilled water, and trays were covered to minimized moisture loss and stacked vertically in a germination chamber (Fisher Scientific, Inc., Suwanee, GA, USA), set at the specific treatment temperature. The internal temperature in the chamber was recorded using data loggers (WatchDog Model 100, Spectrum Technologies, Inc., Aurora, IL, USA) that were placed uniformly in the top, middle, and bottom shelves. Following incubation, trays were examined at two-hour intervals. Seeds were considered germinated if the radicle was at least half the length of the seed. The number of germinated seeds was counted, recorded, and discarded. The seed germination experiment was terminated when there was no germination for five consecutive days or eight days after incubation.

Germination-time course curve fitting procedure

Temperature and seed germination time course data were fitted using a 3-parameter sigmoidal function (Equation 3.1) with the use of the Sigma Plot 13 (Systat Software, Inc., San Jose, CA, USA; Shafii and Price, 2001; Seepaul et al., 2011).

$$Y = Gmax / \{1 + \exp[-(t - t50) / Grate]\} \quad (3.1)$$

This function estimates the total seed germination percentage (Y) based on the maximum cumulative seed germination percentage (G_{max}) at a given time (t), the shape and steepness of the curve (G_{rate}), and the time to reach 50% of the MSG (t_{50}). The reciprocal of time to 50% of the cumulative MSG (t_{50}) was used as the rate of development or the SGR.

Determination of cardinal temperatures

The MSG and SGR responses to temperature treatments were analyzed using the best-suited linear and nonlinear regression models to determine the cardinal temperatures for all genotypes tested. Based on the overall highest coefficient of determination (r^2) value, the best curve-fitting model was selected. A quadratic model was found to be the best and most realistic model that described MSG and SGR responses to temperature (mean $r^2 = 0.85$) because the modified bilinear model tested overestimated the T_{max} and underestimated the T_{min} for SGR. The quadratic model was used to estimate MSG (%; Equation 3.2) and SGR (d^{-1}; Equation 3.3). Their respective cardinal temperatures (T_{min}, T_{opt}, and T_{max}) were calculated using Equations 3.4, 3.5, and 3.6. Temperature adaptability range (TAR) for each genotype was calculated using Equation 3.7.

$$MSG = a + bT - cT^2 \quad (3.2)$$

$$SGR = a + bT - cT^2 \quad (3.3)$$

$$T_{opt} = -b/(2c) \quad (3.4)$$

$$T_{min} = -b + (\sqrt{b^2 - 4ac}) / 2c \quad (3.5)$$

$$T_{max} = -b + (\sqrt{b^2 - 4ac}) /2c \quad (3.6)$$

Where:

T_{min}, T_{opt}, and T_{max} are the minimum, optimum, and maximum cardinal temperatures for seed germination.

a, *b*, and *c* are genotype-specific regression constants.

T is the treatment temperature at which MSG was determined.

$$TAR = T_{max} - T_{min} \quad (3.7)$$

Cumulative temperature response index (CTRI) for heat tolerance classification

The 12 carinata genotypes evaluated in this study were classified into heat tolerant groups using a similar protocol used by Salem et al. (2002), Reddy and Kakani (2007), and Gajanayake et al. (2011). The individual temperature response index (ITRI) of each of the six parameters (P) for high-temperature tolerance was determined as the value for each genotype (P_t) divided by the maximum value observed among all of the studied genotypes (P_h; Equation 3.8), where t and h refer to genotype-specific and maximum values, respectively. The heat CTRI (CHTRI; Equation 3.9) for all genotypes were determined by summing the six ITRI derived from MSG, SGR, and T_{opt}, and T_{max} of MSG and SGR. Based on the CHTRI derived, genotypes were classified as heat-tolerant (> minimum CHTRI + 4 standard deviations [SD]), moderately heat-tolerant (> minimum + 3 SD), moderately heat-sensitive [> minimum CHTRI + 2 SD] or heat-sensitive [between minimum CHTRI and minimum CHTRI + 1 SD].

$$ITRI = P_t / P_h \text{ (heat)} \quad (3.8)$$

$$CHTRI = \left[\frac{MSG_t}{MSG_h} + \frac{MSG\ T_{opt_t}}{MSG\ T_{opt_h}} + \frac{MSG\ T_{max_t}}{MSG\ T_{max_h}} + \frac{SGR_t}{SGR_h} + \frac{SGR\ T_{opt_t}}{SGR\ T_{opt_h}} + \frac{SGR\ T_{max_t}}{SGR\ T_{max_h}}\right] \quad (3.9)$$

Cumulative temperature response index (CTRI) for cold tolerance classification

Carinata cold tolerance classification was derived from the summation of six individual temperature responses index (ITRI) factors following a similar method used by Gajanayake et al. (2011). The ITRI for cold tolerance was derived by dividing the values for each genotype (P_t) by

the least value observed overall for all the genotypes (P_l; Equation 3.10) for cold-tolerant parameters (T_{min} and T_{opt} temperatures for MSG and SGR). In addition, the ITRI for other cold tolerance parameters of MSG and SGR was obtained by dividing the value observed of a given genotypes (P_t) by the maximum value observed overall for all the genotypes (P_h; [Equation 3.11], where t and h refers to genotype-specific, minimum, and maximum values). By summing all six ITRI, the cold CTRI (CLTRI) were determined (Equation 3.12). From the CLTRI calculated, the genotypes were classified into four groups, cold-sensitive (between minimum CLTRI and minimum CLTRI + 1 SD), moderately cold-sensitive (> minimum CLTRI + 2 SD), moderately cold-tolerant (> minimum CLTRI + 3 SD), and cold tolerant (> minimum CLTRI + 4 SD).

$$\text{ITRI} = P_t / P_l \text{ (cold)} \tag{3.10}$$

$$\text{ITRI} = P_t / P_h \text{ (cold)} \tag{3.11}$$

$$\text{CLTRI} = \left[\frac{\text{MSG}_t}{\text{MSG}_h} + \frac{\text{MSG } T_{min_t}}{\text{MSG } T_{min_l}} + \frac{\text{MSG } T_{opt_t}}{\text{MSG } T_{opt_l}} + \frac{\text{SGR}_t}{\text{SGR}_h} + \frac{\text{SGR } T_{min_t}}{\text{SGR } T_{min_l}} + \frac{\text{SGR } T_{opt_t}}{\text{SGR } T_{opt_l}}\right] \tag{3.12}$$

Data analyses

Regression procedures in Sigma Plot 13 were used for estimating MSG with time and for fitting sigmoidal and polynomial functions for cumulative time series and germination rate data. The data for MSG, SGR, $T_{min,}$ T_{opt}, T_{max}, and TAR were analyzed using the PROC GLM (one-way AVOVA) procedure in SAS to determine the effect of the germination temperature treatments on MSG and SGR together with their respective cardinal temperatures. Means were separated using Fisher's protected least significant difference (LSD) at $p < 0.05$. Temperature

and time to germination were treated as independent variables and germination factors (MSG and SGR) as dependent variables.

Results and Discussion

Temperature plays a vital role in regulating all facets of crop growth and development, and this is the first study to address temperature effects on seed germination traits on several *B. carinata* genotypes. The seed germination and temperature functional algorithms provided in this study will be valuable for developing models for carinata for field applications. Also, the thermotolerance capacity among carinata genotypes will be useful for breeders to use in developing new germplasm for low- and high-temperature during seed germination.

Germination time course

A 3-parameter sigmoidal function fitted well in all carinata genotypes tested (Table 3.2; mean $r^2 = 0.85$). The effect of temperature on the cumulative MSG and SGR, as well as variation in germination time among genotypes, are shown in Figure 3.1. All genotypes varied in their response to the different temperature treatments. At the highest temperature tested, 36.96ºC, less than 10% germination was observed for more than 50% of the genotypes. Cumulative MSG for all genotypes decreased below 23.80ºC. The MSG was greatest (99%; AX17009) at 23.80ºC and least (0%; AX17002) at 37ºC (Figure 3.2). Dawadi et al. (2019) reported that carinata seed MSG was greater than 80% at a constant temperature of 25ºC and took approximately 60 h to reach that MSG, requiring 12 h for the onset of germination. In contrast, the time required for the onset of germination (≤ 24 h) varied among carinata genotypes and treatments in our study (Figure 3.1).

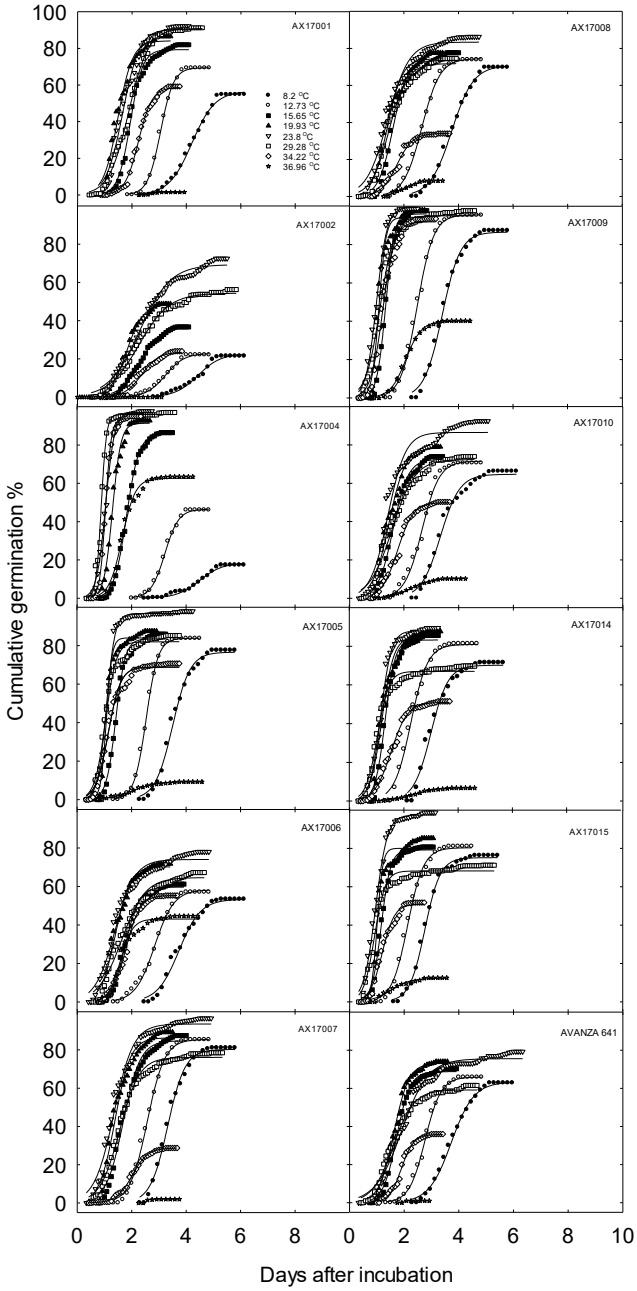

Figure 3.1 Seed germination time courses of 12 *Brassica carinata* genotypes germinated at eight temperatures (8.2, 12.73, 15.65, 19.93, 23.8, 29.28, 34.22, and 36.96ºC).

In Fig. 3.1, the symbols indicate the observed cumulative germination data, and the lines indicate the germination time courses fitted using a 3-parameter sigmoidal function. Data are means of four replications.

Table 3.2 Quadratic equation constants (a, b, and c), coefficients of determination (r^2), cardinal temperatures (T_{min}, T_{opt}, and T_{max}), maximum seed germination (MSG), temperature adaptability range (TAR) for MSG, and mean individual seed weight (SWGT) of 12 *Brassica carinata* genotypes evaluated under eight temperature treatments.

Genotype	Equation constants				r^2	Cardinal temperatures (°C)			TAR (°C)	SWGT (mg seed^{-1})
	MSG (%)	a	b	c		T_{min}	T_{opt}	T_{max}		
AX17001	95.2	-36.46	12.50	-0.2968	0.83	3.15	21.06	38.97	35.82	0.426
AX17002	57.0	-74.54	11.53	-0.2528	0.73	7.64	22.75	37.86	30.02	0.372
AX17004	100.0	-91.50	15.13	-0.2923	0.88	6.93	25.91	44.90	37.80	0.343
AX17005	96.9	12.88	8.60	-0.2207	0.87	-1.58	19.45	40.47	41.90	0.506
AX17006	71.1	-22.62	8.32	-0.1850	0.81	-0.25	22.31	44.87	39.21	0.378
AX17007	96.4	16.47	8.89	-0.2476	0.85	-1.91	17.91	37.73	39.45	0.500
AX17008	85.4	13.37	7.76	-0.2091	0.91	-1.72	18.53	38.78	40.42	0.408
AX17009	100.0	41.70	6.23	-0.1564	0.84	-7.10	19.81	46.72	51.55	0.514
AX17010	85.5	2.86	8.38	-0.2129	0.87	-0.36	19.69	39.74	40.08	0.384
AX17014	92.8	11.30	8.64	-0.2295	0.87	-1.49	18.74	38.96	40.19	0.493
AX17015	93.0	17.77	8.02	-0.2141	0.91	-2.36	18.64	39.64	41.68	0.470
AVANZA641	76.5	14.31	6.69	-0.1804	0.77	-2.58	18.54	39.66	41.17	0.428
Mean	88.2	-	-	-	0.85	-0.14	20.28	40.69	39.94	0.4355
LSD	4.7**	-	-	-	-	4.43***	0.89***	3.74***	7.97*	0.0203*

*Significant at the 0.05 probability level. *** Significant at the 0.001 probability level.

The temperature has a large effect on seed germination (Milbau et al., 2009; Wijewardana et al., 2015; Reddy et al., 2017). Information relating to carinata germination capacity and rate under a wide range of temperatures is limited. Hence, this study provided not only the functional database for temperature and seed germination traits but also the thermo-tolerance classification for carinata genotypes.

Maximum seed germination response to temperature

There was a genotype × temperature interaction ($p < 0.0001$) on MSG. The MSG response to temperature was fitted to a quadratic regression model (mean $r^2 = 0.85$). For MSG, carinata genotypes varied in their response to temperature, ranging from 57.01 (AX17002) to 100% (AX17004 and AX17009), with a mean of 88% among genotypes (Table 3.2; Figure 3.2). The differences in the pattern of responses contributed to the interaction effect.

Based on parameters calculated from the germination-time course data, T_{min}, T_{opt}, and T_{max} for MSG were calculated. These cardinal temperatures were different among genotypes ($p < 0.0001$; Table 3.2). The estimated T_{min} values ranged from -7.10 (AX17009) to 7.64ºC (AX17002) with a mean of -0.14ºC. The mean T_{opt} was 20.28ºC. Genotype AX17004 had the greatest T_{opt} value (25.91ºC), while genotype AX17007 had the least (17.91ºC). Genotype AX17007 recorded the least T_{max} value (37.73ºC), while genotype AX17009 had the greatest value (46.72ºC). The mean T_{max} was 40.69ºC (Table 3.2).

The TAR (T_{max}-T_{min}) provides genotype-specific germination capacities under a wide range of temperatures. Carinata genotypes varied for TAR ($p < 0.05$; Table 3.2) with a mean value of 39.94ºC and ranged from 30.02 (AX17002) to 51.55ºC (AX17009).

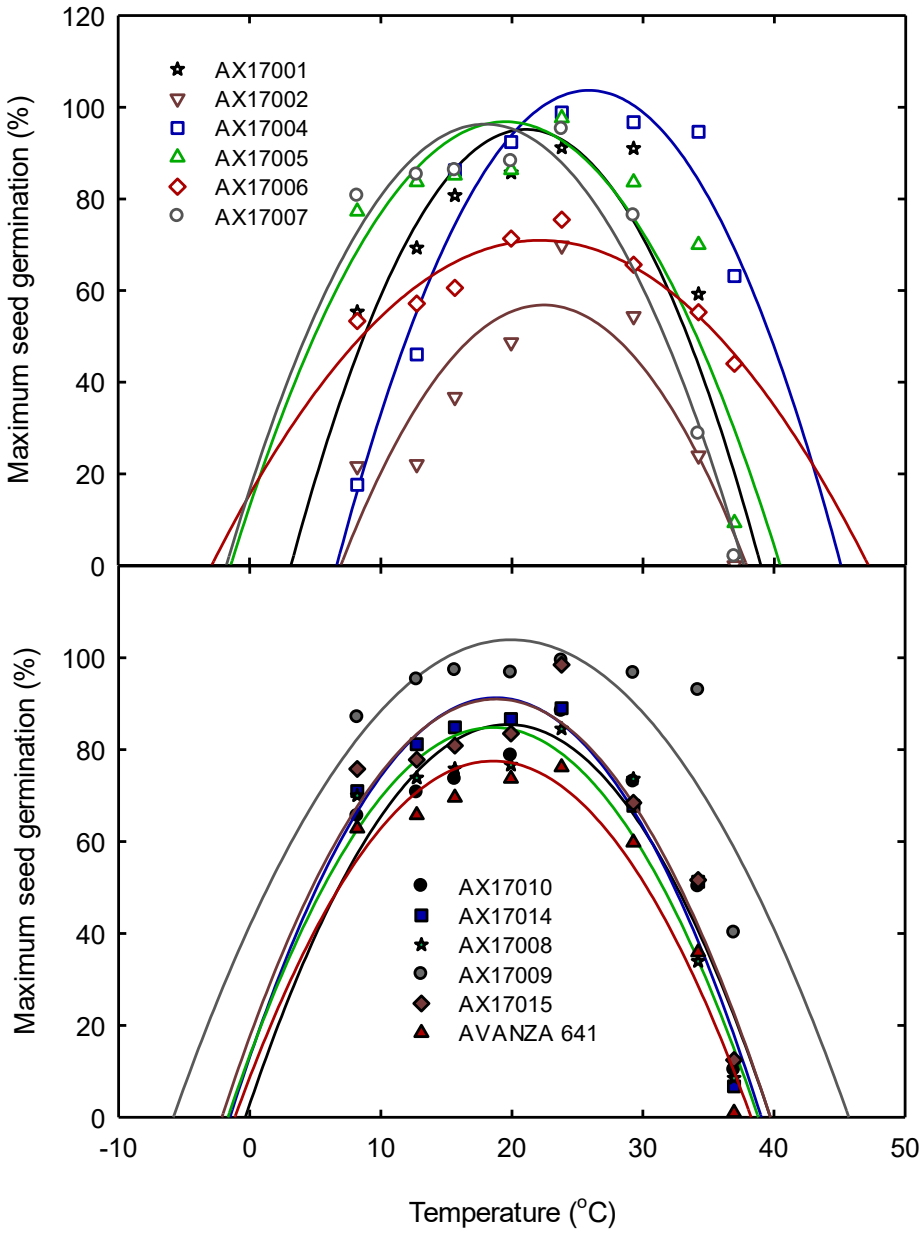

Figure 3.2 Temperature effects on maximum seed germination (MSG) of 12 *Brassica carinata* genotypes.

In Fig. 3.2, lines are fitted quadratic equations. Data are means of four replications. The symbols are observed maximum seed germination percentage.

With respect to MSG, studies conducted on germination response to temperature in other plant species concurred that although there is variation in seed germination, it may not be practical to use this parameter for screening of genotypes because MSG depends on various variables (Gajanayake et al., 2011) such as seed traits (Ellis et al., 1987), storage time between reaping and planting (Jensen and Boe, 1991), and the maternal seed production environment (Fenner, 2008). In addition, the variation in TAR among genotypes can be an indication that varying genetic traits among these genotypes have an effect on how these adapt or preform under different temperatures (Gesch et al., 2015). The optimal temperatures for MSG varied across genotypes, occurring over a range of temperatures, where all genotypes responded differently with varying MSG. Genotype AX17002 recorded the least at 57% MSG. The cardinal temperatures reported for canola using a similar model reported a T_{min} range of 0 to 3ºC, T_{opt} of 29 to 33ºC, and T_{max} of 35ºC (Andrucci et al., 2016). In our study, the values for T_{min} and T_{opt} ranges were less while T_{max} was greater. In Canada and the northern tier states of the United States, carinata and canola are grown in similar areas. Defining the cardinal temperatures for carinata genotypes is beneficial to plant breeders and producers since it helps better to understand crop species and their required conditions for establishment.

Seed germination rate response to temperature

There was a genotype × temperature interaction ($p < 0.0001$) on SGR. Similar to MSG, a quadratic regression model best described the relationship between SGR and temperature (mean $r^2 = 0.85$; Table 3.3). Among genotypes, SGR increased when the temperature increased from 8.2 to 23.8ºC and gradually decreased as temperature increased to 38ºC. The estimated SGR was greatest (0.96 d^{-1}) at 19.93ºC and least (0 d^{-1}) at 36.96ºC (Figure 3.3). These findings concurred

with similar results obtained from eight rapeseed cultivars evaluated under a temperature range of 3 to 23ºC (Luo et al., 2018).

The cardinal temperatures for SGR differed among the genotypes ($p < 0.0001$; Table 3.3). The T_{min} ranged from 2.78 (AVANZA 641) to 7.05ºC (AX17005) with a mean of 5.01ºC. For $T_{opt,}$ the estimated mean was 24.85ºC ranging from 22.57 (AX17002) to 27.81 (AX17004). These two genotypes also recorded the least T_{max} (39.28 and 48.78ºC), respectively, with an estimated mean of 44.69ºC (Table 3.3).

The TAR for SGR ranged from 33.42 (AX17002) to 45.64ºC (AVANZA 641) with a mean of 39.67ºC (Table 3.3). In contrast to reports on other crop species, the SGR for carinata occurred across a range of temperatures but did not portray a sharply defined optimum (Singh et al., 2019; Seepaul et al., 2011) where the rate declined in a linear and rapid manner with increased temperature. Soltani et al. (2006) also reported that in most plant species, the growth rate increased from minimum to optimum temperatures and declined between optimum and maximum temperature, as we observed with the quadratic model prediction and actual data for the carinata genotypes studied. The SGR cardinal temperatures were greater than that of MSG in our study, which agreed with reports on ornamental peppers (Gajanayake et al., 2011) and switchgrass (Seepaul et al., 2011). This further concurred with Schimpf et al. (1977), who reported that MSG is less temperature sensitive than SGR. Although seed germination is said to be a temperature-dependent process (Reddy et al., 2017), there was variation in cardinal temperatures among the genotypes, which is due to intra-specific differences based on genetic diversity, area of origin or adaptation of these entries. Similar findings were reported for switchgrass (Seepaul et al., 2011). Kiniry et al. (2005) also suggested that cardinal temperatures could be genotype-specific as well as process-specific.

Table 3.3 Quadratic equation constants (a, b, and c), coefficients of determination (r^2), cardinal temperatures (T_{min}, T_{opt}, and T_{max}), seed germination rate (SGR), and temperature adaptability range (TAR) for SGR, of 12 *Brassica carinata* genotypes evaluated under eight temperature treatments.

Genotypes		Equation constants				Cardinal temperatures (˚C)			
	SGR; d^{-1}	a	b	c	r^2	Tmin	Topt	Tmax	TAR
AX17001	0.63	-0.4299	0.0920	-0.0020	0.83	5.10	23.19	41.28	36.17
AX17002	0.52	-0.4601	0.0861	-0.0019	0.73	5.86	22.57	39.28	33.42
AX17004	0.91	-0.6929	0.1156	-0.0021	0.88	6.83	27.81	48.78	41.96
AX17005	0.94	-0.8829	0.1433	-0.0028	0.87	7.05	25.32	43.60	36.55
AX17006	0.68	-0.4876	0.0940	-0.0019	0.81	4.95	25.74	46.53	41.58
AX17007	0.71	-0.3470	0.0886	-0.0019	0.85	4.27	23.94	43.61	39.34
AX17008	0.72	-0.3130	0.0807	-0.0016	0.91	4.19	25.61	47.03	42.84
AX17009	0.94	-0.6728	0.1301	-0.0026	0.84	5.85	24.77	43.69	37.84
AX17010	0.70	-0.2764	0.0795	-0.0016	0.87	3.71	24.54	45.37	41.66
AX17014	0.88	-0.5669	0.1209	-0.0025	0.87	5.27	23.94	42.61	37.35
AX17015	0.98	-0.4591	0.1145	-0.0023	0.91	4.32	25.17	46.03	41.71
AVANZA 641	0.62	-0.1933	0.0655	-0.0013	0.77	2.78	25.60	48.42	45.64
Mean	0.77	-	-	-	0.85	5.01	24.85	44.69	39.67
LSD	0.04***	-	-	-	-	2.03*	1.73***	4.58*	6.58*

*Significant at the 0.05 probability level. *** Significant at the 0.001 probability level.

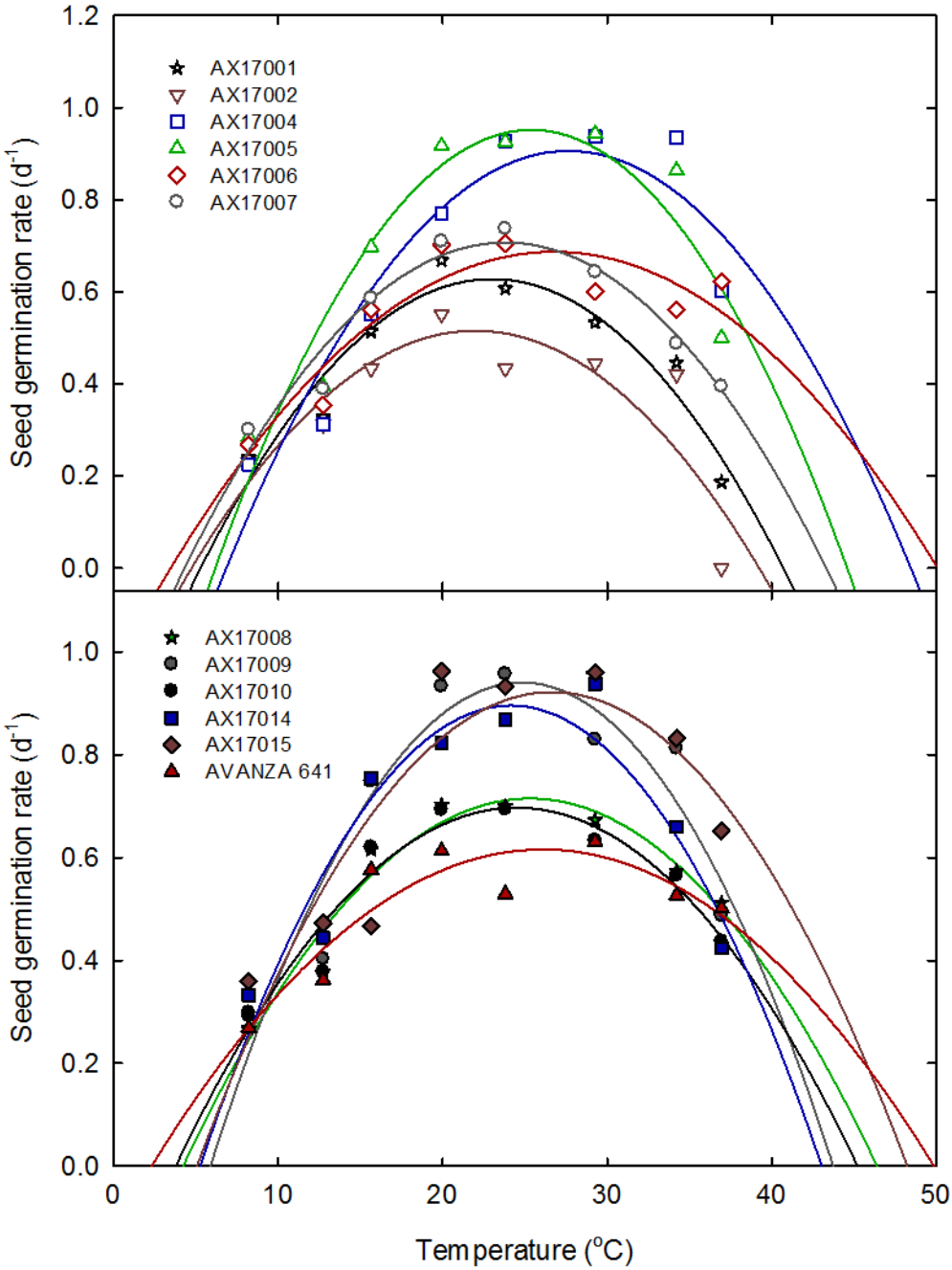

Figure 3.3 Temperature effects on seed germination rate (SGR) of 12 *Brassica carinata* genotypes.

In Fig. 3.3, data are means of four replications. The symbols are observed seed germination rate, and the curves are fitted lines derived from quadratic functions.

Cumulative high-temperature response index (CHTRI) for heat tolerance classification

Six parameters (MSG, SGR, and the $T_{opt,}$ and T_{max} of both MSG and SGR) were used to classify carinata genotypes into heat-tolerant groups. With this method, each individual indices contributed differently based on their relationship to the maximum value of that specific parameter among the genotypes evaluated. The CHTRI derived from the summation of all individual temperature response indices for all seed-based parameters for each carinata genotype varied among the entries (Table 3.4), indicating a hereditary variation for high thermal tolerance in the carinata genotypes studied. The CHTRI classification was done using one standard deviation, where the individual scores ranged from 4.41 to 5.89, and was classified into four tolerant groups (heat-sensitive [4.41 - 4.78]; moderately heat-sensitive [4.79 - 5.15]; moderately heat-tolerant [5.16 - 5.52]; and heat-tolerant [5.53 - 5.89]). The genotype AX17002 was the only entry classified as heat sensitive. AVANZA 641, AX17001, AX17007, AX17010, AX17008, AX17006, AX17014, and AX17009 were classified as moderately heat-sensitive; AX17005, AX17015, and AX17009 were identified as moderately heat-tolerant; and AX17004 was classified as heat tolerant (Table 3.4).

Table 3.4 Classification of 12 *Brassica carinata* genotypes into heat-tolerant groups based on cumulative high temperature stress response index (CHTRI; individual CHTRI values in parenthesis).

Carinata genotypes heat-tolerant classification based on CHTRI			
Heat-sensitive (CHTRI = 4.41- 4.78)	Moderately heat-sensitive (CHTRI = 4.79 - 5.15)	Moderately heat-tolerant (CHTRI = 5.16 - 5.52)	Heat-tolerant (CHTRI = 5.53 - 5.89)
AX17002 (4.41)	AVANZA 641 (4.88)	AX17005 (5.35)	AX17004 (5.89)
	AX17001 (4.92)	AX17015 (5.35)	
	AX17007 (4.94)	AX17009 (5.51)	
	AX17010 (4.99)		
	AX17008 (5.02)		
	AX17006 (5.11)		
	AX17014 (5.14)		

Cumulative low-temperature response index (CLTRI) for cold tolerance classification

As with the CHTRI, genotypes were categorized into cold-tolerant groups by adding six factors derived from seed germination assay study (MSG, SGR, and the $T_{min,}$ and T_{opt} for both MSG and SGR), where each parameter varied in their input based on its relation to the maximum or minimum constant for that particular factor measured across all the genotypes. Similar to the heat-tolerance classification, one standard deviation was also used for the classification of CLTRI values into cold-tolerant groups. The CLTRI ranged from 4.70 to 8.56 and permitted carinata genotypes to be categorized into four tolerant groups (cold-tolerant [7.61 - 8.56]; moderately cold-tolerant [6.64 - 7.60], moderately cold-sensitive [5.68 - 6.63]; and cold-sensitive [4.70 - 5.67]) based on calculations of the minimum and standard deviation of the CLTRI across all genotypes (Table 3.5).

Table 3.5 Classification of 12 *Brassica carinata* genotypes into cold-tolerant groups based on cumulative low-temperature stress response indices (CLTRI; individual CLTRI values in parenthesis).

Carinata genotypes cold-tolerant classification based on CLTRI			
Cold-sensitive (CLTRI = 4.70 - 5.67)	Moderately cold-sensitive (CLTRI = 5.68 - 6.63)	Moderately cold-tolerant (CLTRI = 6.64 - 7.60)	Cold-tolerant (CLTRI = 7.61 - 8.56)
AVANZA 641 (4.70)	AX17006 (5.58)	AX17002 (6.64)	AX17004 (8.02)
AX17010 (5.09)	AX17001 (5.83)	AX17005 (6.72)	
AX17008 (5.33)	AX17015 (5.75)	AX17009 (7.27)	
AX17007 (5.36)	AX17014 (5.87)		

A crucial goal for most plant breeding programs can identify genotypes with essential traits that make it resilient to extreme thermal conditions (Singh et al., 2007). It is also crucial to develop new genotypes with high field stress tolerance that can thrive under variable weather conditions (Porch, 2006). Several studies have used identical parameters used in this study to screen crops for temperature tolerance (Singh et al., 2007; Gajanayake et al., 2011; Seepaul et al., 2011), but the classification of carinata genotypes into heat and cold-tolerance groups has not been reported. Evaluating a crop genotype's thermal adaptability range is typically limited to visual field observation, field performance, and nursery screening. In these conditions, it is challenging to separate different abiotic and biotic stress factors, hence the need for an environment where these stress factors are controlled. Setimela et al. (2005) suggested that evaluation of thermal adaptability range has paved the way for the development of faster, reliable, and low-cost methods that can screen a large batch of plant materials for thermotolerance characteristics. In our study, genotype AX17004 was grouped as most cold and heat-tolerant, indicating that this genotype has both heat and cold tolerance potentials (Agrisoma Biosciences, 2019). Also, genotypes AX17005 and AX17006 appear to have good germination at the low-temperature treatment. Data are limited in the literature concerning thermotolerance

screening for carinata genotypes and intraspecific variation in the establishment under different regions and thermal conditions; however, so there is a need for further testing.

Parameter relationships

A weak positive linear relationship was observed ($r^2 = 0.35$) between cumulative low-temperature response index (CLTRI) and cumulative high response index (CHTRI) for 12 carinata genotypes evaluated (Figure 3.4). This relation indicates that cold and heat tolerance responses among these genotypes are divergent because these are separate traits, and it is not easy to identify genotypes that have both cold- and heat-tolerance attributes; therefore when developing tolerant genotypes for both low and high-temperature, selection has to be carried out separately.

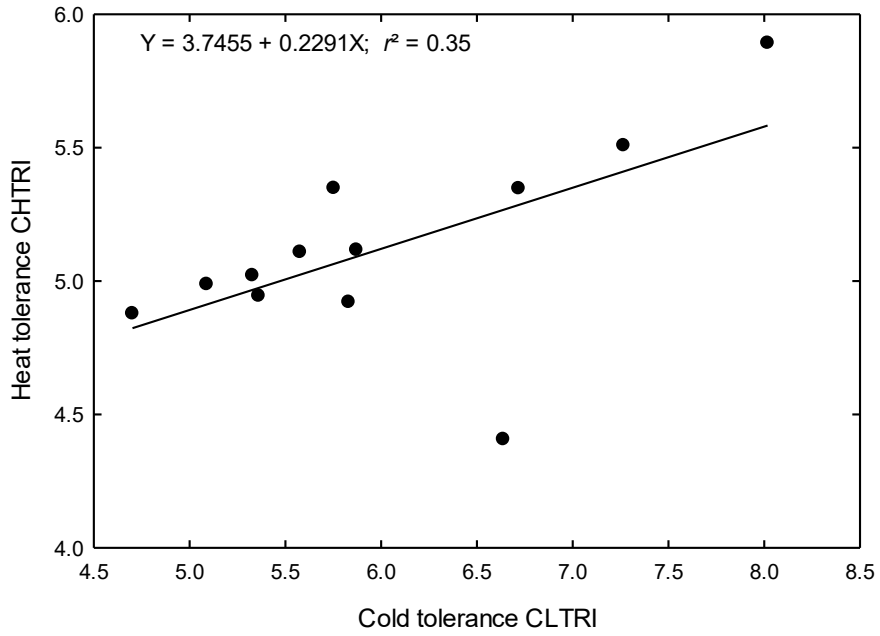

Figure 3.4 The relationship between cumulative low- and high-temperature response index (CLTRI; CHTRI) of 12 *Brassica carinata* genotypes.

Brassica carinata seeds were germinated under eight temperatures 8.2, 12.73, 15.65, 19.93, 23.8, 29.28, 34.22, and 36.96ºC.

A weak linear relationship was found between MSG T_{opt} and T_{max} ($r^2 = 0.23$), while a poor inverse relationship was found between MSG T_{min} and T_{max} ($r^2 = 0.031$; Figure 3.5A-B). As MSG T_{min} for carinata genotypes increased, the T_{opt} also increased ($r^2 = 0.59$; Figure 3.5C). Also, SGR T_{max} increased as T_{opt} increased ($r^2 = 0.81$; Figure 3.6A). Additionally, a poor inverse relationship between SGR T_{min} and T_{max} ($r^2 = 0.098$) and a poor linear relationship between T_{min} and T_{opt} ($r^2 = 0.0169$) were found (Figure 3.6B-C).

The results of this study demonstrated that as the optimum temperature increased, the maximum temperature varied among the genotypes but was not strongly related to T_{opt}, indicating that all genotypes differed in the T_{max} required for reaching MSG. Similarly, there was a weak relationship between T_{min} and T_{opt}. As the minimum temperature increased for

MSG, so did the optimum temperature. With SGR, as optimum temperature increased linearly with maximum temperature, but T_{min}, T_{max}, and T_{opt} were more genotype-specific for SGR due to poor relationships observed.

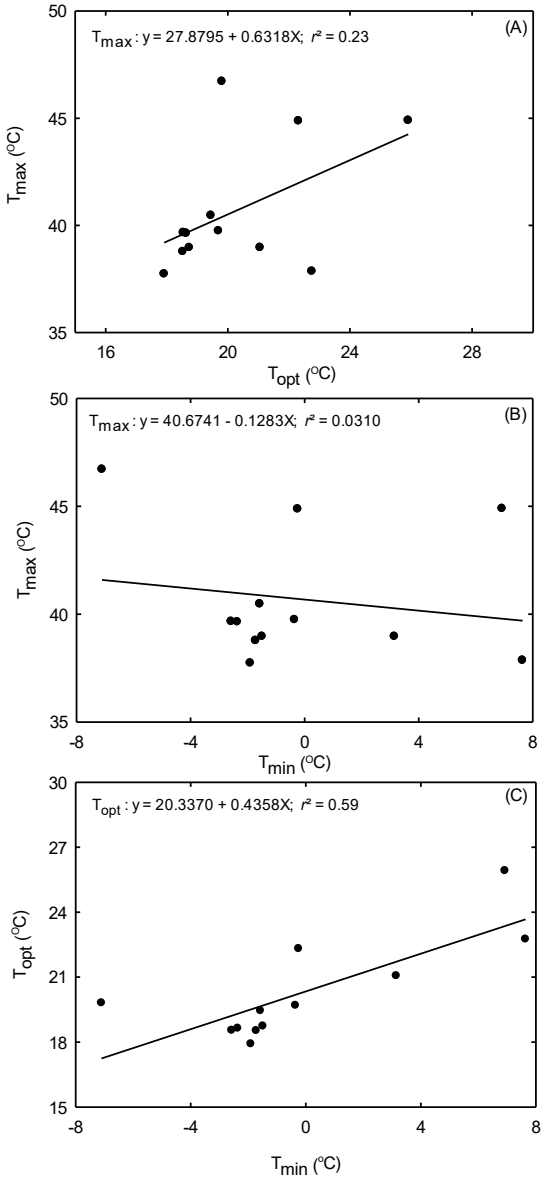

Figure 3.5 Relationship between the cardinal temperatures for maximum seed germination (MSG) of 12 *Brassica carinata* genotypes.

In Fig. 3.5, relationship between (A) optimum (T_{opt}) and maximum (T_{max}) temperatures, (B) minimum (T_{min}) and maximum (T_{max}) temperatures, and (C) minimum (T_{min}) and optimum (T_{opt}) temperatures for maximum seed germination of 12 *Brassica carinata* genotypes.

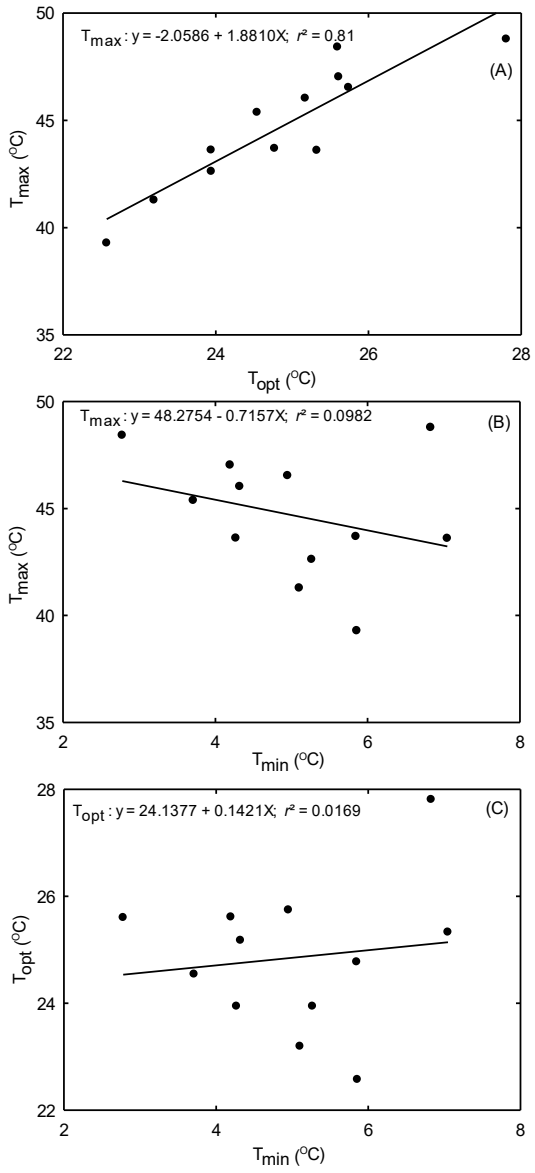

Figure 3.6 The relationship between the cardinal temperatures for seed germination rate (SGR) of 12 *Brassica carinata* genotypes.

In the Fig. 3.6, relationship between (A) optimum (T_{opt}) and maximum (T_{max}) temperatures, (B) minimum (T_{min}) and maximum (T_{max}) temperatures, and (C) minimum (T_{min}) and optimum (T_{opt}) temperatures for seed germination rate of 12 *Brassica carinata* genotypes.

In addition, a weak positive linear relationship was observed between MSG and seed weight (r^2 = 0.28; Figure 3.7) and between SGR and seed weight (r^2 = 0.25; Figure 3.8).

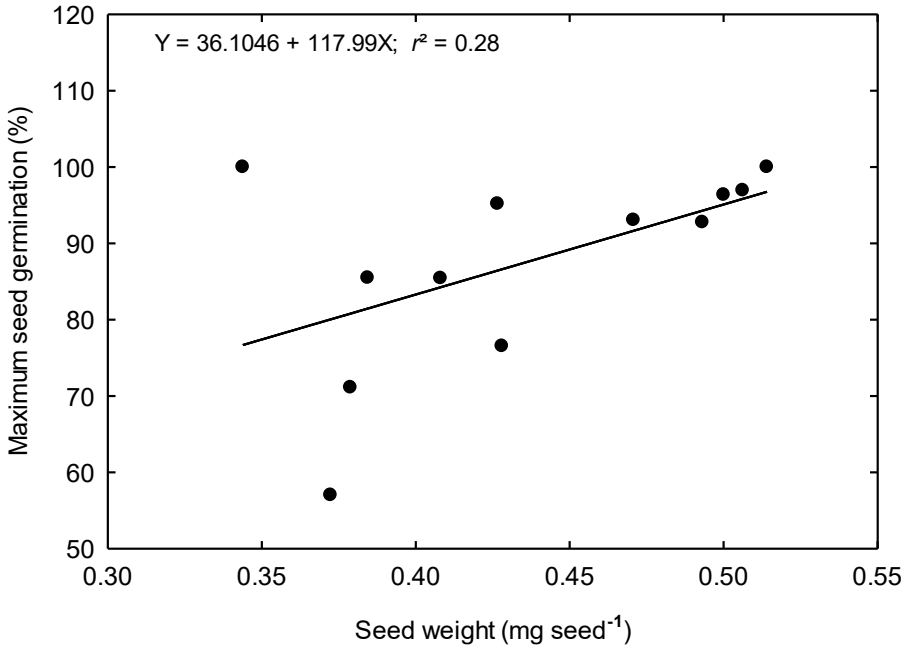

Figure 3.7 The relationship between maximum seed germination (MSG) and seed weight of 12 *Brassica carinata* genotypes.

Brassica carinata seed weight was measured, and seeds were germinated under eight temperatures: 8.2, 12.73, 15.65, 19.93, 23.8, 29.28, 34.22, and 36.96ºC.

Individual seed weight was different among genotypes ($p < 0.05$; Table 3.2), and also showed a poor relationship with MSG and SGR (Figures 3.7; 3.8), indicating that seed weight did not have any effects on both MSG and SGR. Studies have reported that seed weight was not a parameter used to classified crop species into thermotolerance groups due to variation in place of origin or effects of the parental environment (Singh et al., 2019).

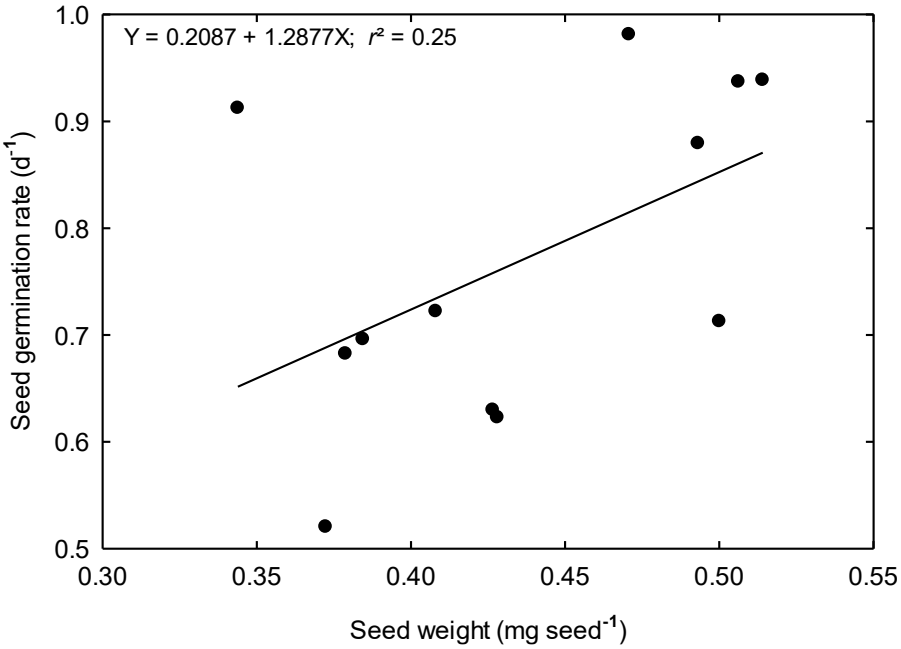

Figure 3.8 The relationship between seed germination rate (SGR) and seed weight for 12 *Brassica carinata* genotypes.

Brassica carinata seed weight was measured, and seeds were germinated under eight temperatures: 8.2, 12.73, 15.65, 19.93, 23.8, 29.28, 34.22, and 36.96ºC.

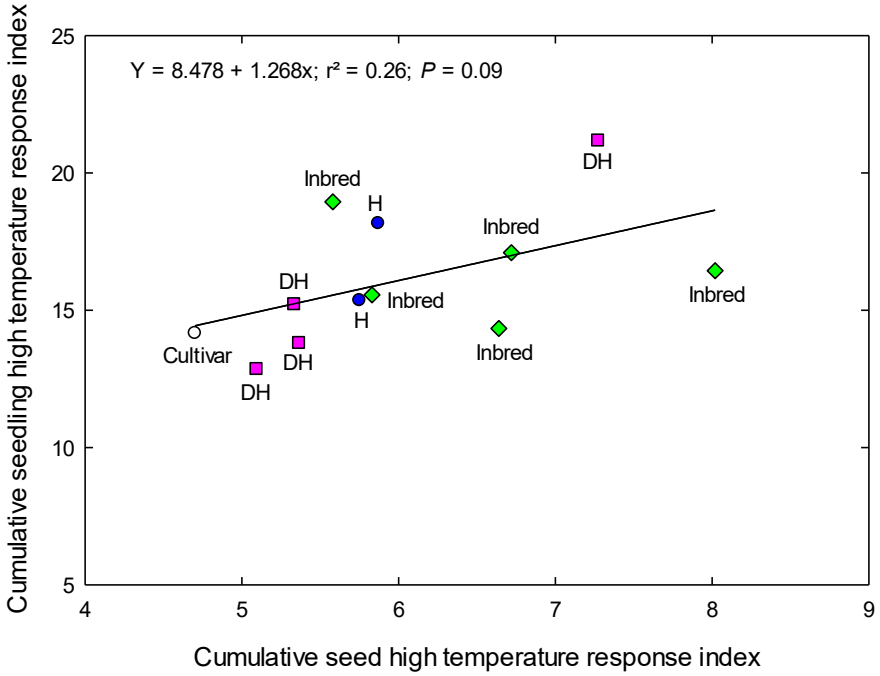

Figure 3.9 The relationship between cumulative seed and seedling high-temperature response indices (CHTRI) of 12 *Brassica carinata* genotypes.

Colored symbols represent *Brassica carinata* breed types; green-diamond = inbred; pink-square = double haploid (DH); blue-circle = hybrid (H); and white-circle = check cultivar. Carinata seeds were germinated under eight temperatures; 8.2, 12.73, 15.65, 19.93, 23.8,29.28, 34.22, and 36.96ºC, and grown under three day/night temperatures; low (17/09ºC), optimum (22/14ºC), and high (27/19ºC).

The relationship between the different carinata types, hybrid (H), inbred, double haploid (DH), and the check (cultivar) was examined at the seed germination and early seedling growth at high temperature. It was observed that the groups had defined clusters, and a poor relationship ($r^2 = 0.26$; Figure 3.9), indicating a difference in heat tolerance at the two growth stages among the types. A similar trend was observed across the carinata types at low temperature ($r^2 = 0.02$; Figure 3.10), except for the one DH genotype that seems like an outlier. The inbred lines had a more extensive variation across the two stages, with higher CLTRI at the germination stage. This general trend observed suggests that the thermotolerance of DH and H genotypes is much more stable, meaning tolerance at the germination and seedling stages than that of inbred lines. This is excepted since inbred lines may still be undergoing genetic differentiation.

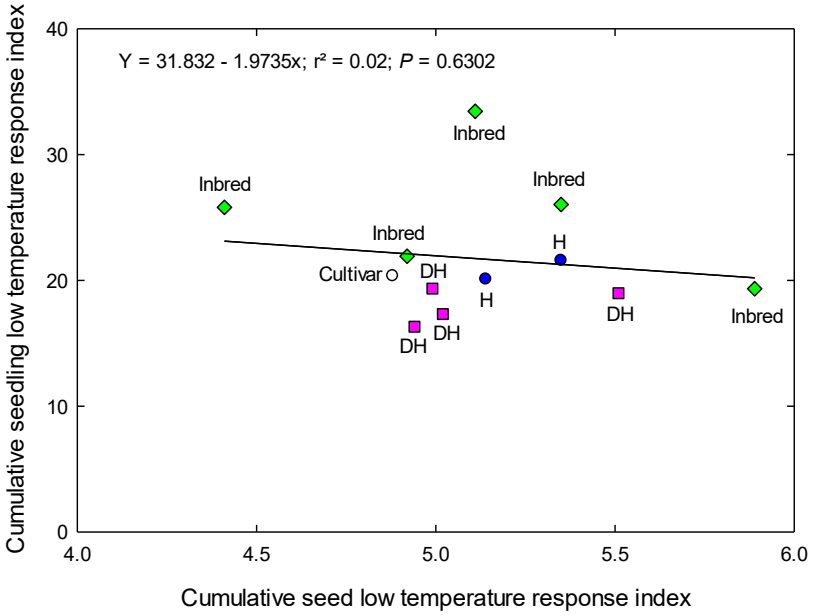

Figure 3.10 The relationship between cumulative seed and seedling low-temperature response index (CLTRI) of 12 *Brassica carinata* genotypes.

Colored symbols represent *Brassica carinata* breed types; green-diamond = inbred; pink-square = double haploid (DH); blue-circle = hybrid (H); and white-circle = check cultivar. Carinata seeds were germinated under eight temperatures; 8.2, 12.73, 15.65, 19.93, 23.8,29.28, 34.22, and 36.96ºC, and grown under three day/night temperatures; low (17/09ºC), optimum (22/14ºC), and high (27/19ºC).

Summary and Conclusions

The current study quantified the effects temperature on the MSG capacity and SGR of 12 advanced carinata genotypes using the *in vitro* method. Based on best-fit regression models, genotype-specific cardinal temperatures and TAR were estimated. The mean minimum, optimum, and maximum temperatures for MSG across carinata genotypes were -0.14, 20.28, and 40.69ºC, respectively. For SGR, the mean minimum temperature was 5.01ºC, the mean optimum was 24.85ºC, and the mean maximum was 44.69ºC. The mean TAR across genotypes was 39.94 and 39.67ºC for MSG and SGR, respectively. Variability existed among the genotypes for MSG, SGR, TAR, and cardinal temperatures. Based on these seed germination vitality traits, carinata genotypes were grouped into various cold and heat tolerant groups using the minimum plus standard deviations of the mean. The genotype AX17004 was identified as the most cold- and heat-tolerant line. Of the 12 genotypes evaluated, 25% was classified as moderately cold-tolerant, 33.3% was moderately cold-sensitive, and 33.3% cold-sensitive. Also, 8.3% was heat-sensitive, 58.3% was identified as moderately heat-sensitive, while 25% was moderately heat-tolerant, and 8.3% was heat-tolerant.

The double haploid and hybrid carinata breeding groups had a stable thermotolerance response at both stages, while the inbred group had a more extensive cluster at both stages in response to a minimum and maximum temperatures. From the data gathered in this study, the cardinal temperatures determined will be beneficial for carinata crop model development and application in field production systems. Besides, the identification of cold- and heat-tolerance genotypes will help to define regions for cultivation further. There is also a need for future evaluation under field conditions of these genotypes, however, in order to identify and quantify tolerant genotypes for both cold and heat tolerance.

CHAPTER IV

THERMOTOLERANCE CLASSIFICATION AND TEMPERATURE EFFECTS ON *Brassica carinata* EARLY-SEASON GROWTH AND DEVELOPMENT

Abstract

Temperature is a major abiotic stress factor limiting plant growth. Information on carinata thermal response, which is necessary for selecting genotypes suited for specific ecoregions, is limiting. Twelve carinata (*Brassica carinata* A. Braun) genotypes were evaluated under low (17/09ºC), optimum (22/14ºC), and high (27/19ºC) day/night temperatures. Growth and developmental traits were recorded 24 d after treatment imposition. There were main effects of temperature and genotype on several shoot, root, and physiological traits measured. A poor relationship (r^2 = 0.09) was found between low- and high-temperature indices, indicating differences in tolerance mechanisms. There was a strong relationship between low temperature and cumulative shoot and root indices, as well as between high temperature and cumulative shoot and root indices, which shows the importance of both traits in tolerance selection. Genotype AX17006 was identified as heat-tolerant and AX17009 as cold-tolerant among the 12 genotypes tested. The heat-tolerant genotype, AX17006, performed 78, 54, and 29% better than the heat susceptible, moderately heat-susceptible, and moderately heat tolerant-groups, respectively. Similarly, the most cold-tolerant genotype, AX17009, exhibited 50, 31, and 14% more cold-tolerance than the cold-sensitive, moderately cold-sensitive, and moderately cold-tolerant groups, respectively. When genotypes were grouped according to breed types, hybrids

generally had better responses to temperature than the inbred lines, and double haploids and the check responses were intermediate in early season growth response traits measured. The inbred group showed greater cold tolerance at the germination stage, while the hybrid group was more cold-tolerant at the early growth stage. The variation in thermotolerance could be a valuable source for identifying tolerant genotypes.

Introduction

Globally, carinata (*Brassica carinata* A. Braun) is an important oilseed crop in several countries, including Canada and Spain (Rakow, 2004), India (Singh, 2003), Italy (Cardone et al., 2003), and the USA (Cardone et al., 2003), for use in lubricants, soaps, and, most importantly, biofuels (Cardone et al., 2003). Carinata is a relatively new winter oilseed crop in the southeastern USA (de Koff et al., 2017), where studies are currently ongoing to identify lines best suited for commercial production. This initiative is led by the University of Florida (UF) in collaboration with the Southern Partnership for Advanced Renewables from Carinata (SPARC) consortium. Even though other related oilseed species such as oilseed rape and canola are grown commercially in North America (Bona et al., 1999; Perlack et al., 2005), developing a winter crop such as carinata for biofuel production in subtropical regions has picked up interest in recent years. This is because it has a high concentration of erucic acid (Cardone et al., 2003; Warwick, 2011; Enjalbert et al., 2013). Carinata has been cultivated on a commercial scale as a summer crop in the Canadian prairie and the US northern plains and as a winter crop in the US Southeast (Seepaul et al., 2016). Currently, there is an opportunity for row crop growers in the US Southeast to invest in the cultivation of carinata as a winter crop and diversify their existing systems, thus increasing their profitability (Seepaul et al., 2019). This initiative can also complement summer production in temperate regions (Mulvaney et al., 2019).

Temperature is an important abiotic stress factor that plays a dominant role in the control of plant growth and developmental processes under optimum nutrient and water conditions. Plant species, and even cultivars within species, vary in their sensitivity to temperature (Wijewardana et al., 2015; Reddy et al., 2017). Carinata can thrive well in semi-arid environments and has been reported to be a cool-season crop (Marillia et al., 2014). The crop is also tolerant of heat and drought-like conditions (Kumar et al., 1984; Malik, 1990; Getinet et al., 1996; Schreiner et al., 2009). Although most carinata genotypes can be utilized as a biofuel feedstock, differences in genetic characteristics among cultivars may contribute to variation in the ability of a particular genotype to grow in a specific region (Gesch et al., 2015). To date, however, there is limited information available on carinata response to different temperature ranges at different growth stages; hence, the evaluation of this oilseed crop response to temperature stress will be of importance in research and commercial production.

The yield of *Brassica* species is highly dependent on environmental conditions during its growth and developmental stages (Saha and Khan, 2008). Therefore, identifying suitable genotypes and management practices to have sustainable production under a changing climate is crucial (Aggarwal and Kalra, 1994). Field tests for several carinata cultivars were successful across Canada and various regions in the United States (Marillia et al., 2014), indicating that USDA Plant Hardiness Zones 4 to 9 are suitable for carinata cultivation (Magarey et al., 2008). Frost tolerance in carinata cultivars has also been reported (Seepaul et al., 2015), but these temperature limits and exposure time are yet to be established. The origin of this oilseed crop makes it well adapted to its native habitat, the highlands of Ethiopia in the cold temperature of 14 to 18ºC at elevations of 2200 to 2800 m above sea level (Asamenew et al., 1993). It has a long growing season of 180 days (Alemayehu and Becker, 2002).

Studies have shown that projections in climate change can reduce crop production when they occur at the same time plants are in their reproductive stage (Hall, 1992; Reddy et al., 1992, 1997). It was reported that yield reduction occurred in canola grown in both winter and spring (Reddy et al., 2005). When Indian mustard plants were exposed to high temperatures during the early season growth stage, a decline in growth was seen (Shamsul et al., 2009). High-temperature stress also reduced the number of pods on the main stem, the number of seeds per pod, and the seed weight in *Brassica* species (Gan et al., 2003). Angadi et al. (2000) also reported a yield drop in *Brassica* species when exposed to a high day/night temperature of 35/15°C for seven days during the flowering stage. Usually, the damage caused by temperature stress all depends on the plant growth stage at a specific time and the extent to which that stress persisted (Li et al., 1981). It was also reported that each developmental aspect or crop event has its specific temperature optimum, above which plant growth processes will decline (Alsajri et al., 2019).

To understand plant tolerance mechanisms to a combined number of abiotic stresses, however, it is necessary to have a proper comprehension of plant responses to each environmental factor individually (Mittler, 2006). Several studies have used various parameters such as morphological, physiological and reproductive factors to evaluate temperature and drought stress tolerance in multiple crops, including pepper (Reddy and Kakani, 2007; Gajanayake et al., 2011), soybean (Salem et al., 2007), maize (*Zea mays* L.) (Wijewardana et al., 2016a, 2017, 2018), cotton (Kakani et al., 2005; Reddy et al., 2017), peanut (*Arachis hypogaea* L.) (Kakani et al., 2002), and canola (Singh et al., 2008). Additionally, plant root system architecture and its components are essential when selecting lines with high environmental stress tolerance characteristics (Lynch, 1995), since poor root development may lead to reduced shoot

and canopy growth at later growth stages of plants (Gajanayake et al., 2014; Wijewardana et al., 2017). Studies on root systems of rice, corn, sweet potato, and cotton helped identify stress tolerance at the seedling growth stage (Wijewardana et al., 2015, 2018; Singh et al., 2017a, 2017b; Singh et al., 2018). Recent studies have determined the relationship between temperature stress tolerance and root traits for different crops using the WinRHIZO root scanning technology (Wijewardana et al., 2015, 2018; Brand et al., 2016; Singh et al., 2018).

Selecting superior genotypes from populations has been aided by stress indices based on physiological parameters associated with a desirable trait. Such classification approaches have been used by Kakani and Reddy (2007) and Salem et al. (2007) to classify pepper and soybean genotypes, respectively, using pollen-based parameters and a temperature response index (TRI). The TRI relates the value of a genotype to the maximum or minimum value of all genotypes. The summation of individual TRI results in a cumulative TRI that is then separated using its standard deviation based on the number of interested groups. Cumulative TRI has been used to screen soybean for genotypic variability under multiple environmental conditions (Koti et al., 2004), switchgrass genotypes for heat tolerance (Seepaul et al., 2011), sweet potato and corn hybrids for cold and heat tolerance (Wijewardana et al., 2015, 2016, 2018). There are limited studies on carinata screening for thermotolerance using plant morpho-physiological traits, however. Before this study, there have been no other studies that classified carinata genotypes for temperature stress responses at these growth stages. Therefore, the objectives of this study were to (a) determine temperature effect on early vegetative growth of carinata (for this study defined as 35 days after seeding or 24 d after temperature treatment imposition), and (b) classify carinata genotypes for temperature tolerance.

Materials and Methods

Experimental conditions

This study was conducted at the Rodney Foil Plant Science Research facility of Mississippi State University, Mississippi State, MS (33˚20′ N, 88˚47 ′ W), from November to December 2018. Carinata genotypes were planted in three sunlit, controlled environment units called Soil-Plant-Atmosphere-Research (SPAR) chambers. Each of these chambers consists of a built-in soil bin made from steel (1-m depth × 2-m length × 0.5-m width) to accommodate belowground plant parts and transparent chamber made of 1.27-cm thick Plexiglas (2.5-m height × 2-m length × 1.5-m width) as room for aboveground plant growth. The Plexiglas on each unit allows 97% of visible incoming solar radiation to go by without spectral variation in absorption, with wavelength 400 - 700 nm (Zhao et al., 2003). These SPAR chambers are equipped to monitor and control air temperature accurately and maintain the atmospheric CO_2 concentration at a fixed calibrated point. The chambers are kept close to ambient levels of photosynthetically active radiation. The SPAR chambers are also equipped with a cooling and heating system, which are connected to air ducts that carry conditioned air through the crop canopy to cause leaf flutter. Further details on this SPAR unit control and operations were described by Reddy et al. (2001).

Additionally, chilled ethylene glycol was provided via parallel solenoid valves to the cooling system, which opened or closed based on the cooling requirement. Two electrical resistance heaters, which give off short pulses of heat to regulate the air temperature, provided the required heat. Humidity and temperature sensors (HMV 70Y, Vaisala Inc., San Jose, CA, USA) installed in the returning path of the airline ducts helped to monitor the relative humidity. Different density of shaded cloths placed around the perimeter of the plant canopy designed to

simulate canopy spectral properties was readjusted to match the canopy height daily, and this also eliminated the need for border plants. The CO_2 concentration, the air temperature inside the chamber, an irrigation system in each SPAR unit, and the continuous monitoring of plant and environmental gas exchange variables were automatically controlled and monitored every 10 seconds by a dedicated network system, also equipped to record and store data automatically. Soil moisture was monitored in all SPAR units using soil moisture probes (5TM Soil Moisture and Temperature Sensor, Decagon Devices, Inc., Pullman, WA). These probes were inserted at a depth of 15 cm from the surface of five pots in each temperature treatment and set to measure soil moisture content every 60 seconds and record it at 15-minute intervals. The CO_2 concentration inside of the chamber was measured and maintained at 420 μmol mol^{-1} daily. Irrigation was done with installed fertigation systems with full strength Hoagland plant nutrient solution. This process was carried out three times daily using an automatic drip system.

Seed materials and temperature treatments

For this study, seed material of 11 advanced carinata genotypes of three breeding types, namely inbred, double haploid, and hybrid, that is close to commercial deployment and one commercial check entry, were evaluated (Table 4.1). All seeds were sourced from Agrisoma Biosciences Inc. Canada (now Nuseed Australia). All seeds were treated with Helix Vibrance, which contains four fungicides (difenoconazole, metalaxyl-M, fludioxonil, and sedaxane), and one insecticide, (thiamethoxam), to minimize fungal infections and insects. The treated seeds were sown in 150 polyvinyl-chloride (PVC) pots (5.24-cm diameter, 30.5-cm height, and 5.5-L volume) filled with a soil medium consisting of pure fine sand. All pots contained a small hole at the bottom, filled with 500 g of gravel to facilitate natural drainage of excess water. The pots were initially sown with four seeds, and 11 days after planting were thinned, leaving only one

plant per pot. Pots were set up in a completely randomized design inside of the SPAR chambers, in 15 rows with four pots per row. Three SPAR chambers were used. Each carinata genotype replicated five times (thus, there were 60 pots per chamber) within each temperature treatment. Three day/night temperature treatments were tested in this study, 17/09ºC (low), 22/14ºC (optimum), and 29/19ºC (high), imposed at 11 days after planting (DAP) and plants were harvested at 24 days after treatment application (DAT). These treatments were selected based on a preliminary study to test temperature extremes.

Table 4.1 Type and origin of *Brassica carinata* genotypes sourced from Agrisoma Biosciences, 2019 (now Nuseed Australia).

Genotype	Type†	Justification
AX17001	I	Selection from SE16-17 AYT (Avanza family selection) Florida
AX17002	I	Selection from SE16-17 AYT (Avanza family selection) Florida
AX17004	I	High shatter tolerance family, good potential in a winter environment
AX17005	I	High shatter tolerance family, good potential in a winter environment
AX17006	I	High shatter tolerance family, good potential in a winter environment
AX17007	DH	Among the highest Sclerotinia incidence, Jay and Quincy, FL
AX17008	DH	Selection from SE16-17 PYTB Florida
AX17009	DH	Selection from SE16-17 PYTA Florida
AX17010	DH	Selection from SE16-17 PYTB Florida
AX17014	H	Top 2016-17 Quincy test hybrid Florida
AX17015	H	Promising test hybrid from 2017, frost tolerant female
Avanza 641	Check	Commercial check

†Genotypes are classified into three types (I = inbred, DH = double haploid, and H = hybrid). Seed trials (SE - Southeast, AYT - advanced yield trial, PYT - preliminary yield trial).

Measurements

Shoot growth and developmental parameters

The shoot growth and developmental components evaluated in this study included plant height (PH), the total number of leaves (LN), leaf area (LA), leaf dry weight (LDW), stem dry weight (SDW), above-ground dry weight (AGW), root dry weight (RDW), total dry weight (TDW) and root/shoot ratio (RS) for all 12 carinata genotypes evaluated in this experiment. The

PH and LN were measured and counted one day (23 DAT or 34 DAP) before harvesting, and LA was recorded on the day this study was terminated (24 DAT or 35 DAP), using an LI-3100 leaf-area meter (LI-COR, Inc., Lincoln, NE). Leaves and stems were separated and dried in a forced-air oven at 60ºC for 72 h, after which final dry biomass was recorded.

Physiological parameters

At 23 DAT, one day before final harvesting, physiological parameters, including chlorophyll (Chl), flavonoids (Flav), anthocyanin (Anth), and nitrogen balance index (NBI), were using a Dualex® Scientific Polyphenols and Chlorophyll Meter (FORCE-A, Orsay, France). Additionally, a FluorPen FP 100 (Photon Systems Instruments, Drasov, Czech Republic) was used to collect the quantum efficiency (FvFm). All measurements were collected from the second fully expanded leaf from the top of each plant at 23 DAT.

Root image acquisition and analysis

At 24 DAT, all plants were harvested by separating the stem of each plant at ground level from its root system. Roots were then removed from the pots, placed on a wire screen, and washed thoroughly to remove the soil medium, using a moderate hydro flow speed and exercising maximum caution to avoid damages to the root structures. For each plant root, the longest root length (LRL) was recorded using a meter ruler. The individually cleaned root system was scanned using a WinRHIZO optical scanner (Regent Instruments, Inc., Quebec, Canada), attached to a computer system. A similar procedure was described by Wijewardana et al. (2018) and Reddy et al. (2017). The individual cleaned roots structure was placed onto a waterproof Plexiglas tray (40-cm length × 30-cm width) filled with approximately 5 mm of water and fitted onto the scanner. The roots were submerged, and the crossings and tips were spread

using a small paintbrush to avoid overlapping. The acquired gray-scale root images were obtained through a high accuracy setting (resolution of 800 by 800 dpi) for the parameters measured by the WinRHIZO Pro Software (Regent Instruments, 2009). The software measured the following components: cumulative root length (CRL), root surface area (RSA), average root diameter (RD), lens per volume (LVP), root volume (RV), number of root tips (RT), forks (RF), and root crossings (RC).

Thermotolerance classification of genotypes based on cumulative low and high-temperature response index

The 12 genotypes were categorized into cold-tolerant and heat-tolerant groups based on a cumulative low-temperature response index (CLTRI) and cumulative high-temperature response index (CHTRI) calculated according to the methodology used by Wijewardana et al. (2015, 2018). Initially, the individual stress response index (ISRI) for low temperature (17/09ºC) was calculated as the value of a parameter (P_l) for a given genotype at the low temperature divided by the value of the same parameter at the optimum temperature (P_o; 22/14ºC; Equation 4.1). Likewise, the ISRI for high temperature (27/19ºC) was calculated for each genotype as the value of the parameter at high temperature (P_h) divided by the constant recorded for the same parameter at the optimum temperature (P_o; Equation 4.2). The CLTRI (Equation 4.3) and CHTRI (Equation 4.4) were determined for each genotype by summing all the ISRI calculated for all the shoot and growth developmental, physiological, and root parameters measured across all genotypes.

$$\text{ISRI (low)} = P_l / P_o \tag{4.1}$$

$$\text{ISRI (high)} = P_h / P_o \tag{4.2}$$

$$\text{CLTRI} = \left(\frac{PH_l}{PH_o}\right) + \left(\frac{LN_l}{LN_o}\right) + \left(\frac{LA_l}{LA_o}\right) + \left(\frac{LDW_l}{LDW_o}\right) + \left(\frac{SDW_l}{SDW_o}\right) + \left(\frac{RDW_l}{RDW_o}\right) + \left(\frac{AGW_l}{AGW_o}\right) + \left(\frac{TDW_l}{TDW_o}\right)\left(\frac{RS_l}{RS_o}\right) + \left(\frac{LRL_l}{LRL_o}\right) + \left(\frac{L_l}{L_o}\right) + \left(\frac{RSA_l}{RSA_o}\right) + \left(\frac{AD_l}{AD_o}\right) + \left(\frac{LPV_l}{LPV_o}\right) + \left(\frac{RV_l}{RV_o}\right) + \left(\frac{RT_l}{RT_o}\right) + \left(\frac{RF_l}{RF_o}\right) + \left(\frac{RV_l}{RV_o}\right) + \left(\frac{Chl_l}{Chl_o}\right) + \left(\frac{Flav_l}{Flav_o}\right) + \left(\frac{Anth_l}{Anth_o}\right) + \left(\frac{NBI_l}{NBI_o}\right) + \left(\frac{FvFm_l}{FvFm_o}\right) \tag{4.3}$$

$$\text{CHTRI} = \left(\frac{PH_h}{PH_o}\right) + \left(\frac{LN_h}{LN_o}\right) + \left(\frac{LA_h}{LA_o}\right) + \left(\frac{LDW_h}{LDW_o}\right) + \left(\frac{SDW_h}{SDW_o}\right) + \left(\frac{RDW_h}{RDW_o}\right) + \left(\frac{AGW_h}{AGW_o}\right) + \left(\frac{TDW_h}{TDW_o}\right)\left(\frac{RS_h}{RS_o}\right) + \left(\frac{LRL_h}{LRL_o}\right) + \left(\frac{L_h}{L_o}\right) + \left(\frac{RSA_h}{RSA_o}\right) + \left(\frac{AD_h}{AD_o}\right) + \left(\frac{LPV_h}{LPV_o}\right) + \left(\frac{RV_h}{RV_o}\right) + \left(\frac{RT_h}{RT_o}\right) + \left(\frac{RF_h}{RF_o}\right) + \left(\frac{RV_h}{RV_o}\right) + \left(\frac{Chl_h}{Chl_o}\right) + \left(\frac{Flav_h}{Flav_o}\right) + \left(\frac{Anth_h}{Anth_o}\right) + \left(\frac{NBI_h}{NBI_o}\right) + \left(\frac{FvFm_h}{FvFm_o}\right) \tag{4.4}$$

The genotypes were classified into four cold-tolerant groups based on CLTRI and standard deviation (SD); cold-sensitive (between minimum CLTRI and minimum CLTRI + 1 SD), moderately cold-sensitive (between minimum CLTRI + 1 SD and minimum CLTRI + 2 SD), moderately cold-tolerant (between minimum CLTRI + 2 SD and minimum CLTRI + 3 SD) and cold tolerant (> minimum CLTRI + 3 SD). Similarly, all genotypes were categorized into four heat-tolerant groups based on the CHTRI derived, and SD, which includes heat tolerant (> minimum CHTRI + 3 SD), moderately heat tolerant (between minimum CHTRI +2 SD and minimum CHTRI + 3 SD), moderately heat sensitive (between minimum CHTRI + 1 SD and minimum CHTRI + 2 SD) and heat-sensitive (between minimum CHTRI and minimum CHTRI + 1 SD).

Data analysis

Data were analyzed using the PROC GLIMMIX procedure in SAS (9.4, SAS Institute, Inc.) to determine the effect of temperature, genotype, and their interactions on the shoot, root, and physiological parameters. Responses were considered different at the $\alpha = 0.05$ level. Besides, regression analysis was used to determine the relationship between temperature response indices and among these response indices and growth parameters. Based on r^2 values, best-fit regression functions were selected. Graphical analysis was done using Sigmaplot® 13.0.

Results and Discussion

Based on our knowledge, this is the first study that provided data on the shoot and root morphological traits, along with genotype-specific cold- and heat-tolerant characteristics, to evaluate the variation across advanced carinata genotypes (Table 4.1). Information provided from this investigation on carinata response to different temperatures will be beneficial for the selection of genotypes for advanced carinata breeding programs for thermotolerance.

Shoot traits

For the shoot traits measured, the only temperature × genotype interaction that occurred was for RS ($p < 0.05$; Table 4.2). At the optimum day/night temperature, there were no differences among genotypes, but at high day/night temperature, AX17006 had the greatest and AX17009 had the least RS ratio. Also, at the low day/night temperature, AX17009 had the greatest, and AX17002 had the least RS ratio. Despite the variation from most significant to least, it was observed that the RS ratio of many of the entries was not different ($p > 0.05$) from each other (Table 4.3). It should be noted that AX17009 expressed extremes of RS ratio at the high and low-temperature treatments. The treatment × interactions are supported by findings of

Wijewardana et al. (2015) and Reddy et al. (2017), showing that cultivars of various plant species differ in their response to temperature and even vary in their sensitivity to different thermal conditions.

For all other shoot traits measured, there was a main effect of temperature ($p < 0.001$) (Table 4.2). Also, there was a main effect of genotype ($p < 0.01$) for all the other shoot parameters except RDW (Table 4.2). Two traits, PH and LN, had apparent differences among all three temperatures ranking greatest to least from the high to low temperatures regimes (Table 4.3). The mean PH at the high temperature (27/19ºC) was 12.5% taller than at the optimum temperature (22/14ºC), and the height reduction from optimum to the low temperature (17/09ºC) was substantial (67%; Table 4.3), showing the strong effect of temperature on this trait. Under the low-temperature (17/09ºC), the least LN was observed, while genotypes were grown under the optimal (22/14ºC) and high-temperature (27/19ºC) recorded higher LN. For both of these traits, AX17015 had the tallest plant, and the greatest LN and AX17004 had the shortest plants and least LN (Table 4.3). Carinata stem elongation and leaf area expansion is crucial for the light interception, which determines crop development and biomass accumulation at early season establishment. Across all the genotypes, shorter plants observed under low temperatures may be attributed to a reduction in cell division and elongation activities caused by low thermal conditions, which in turn affects cellular functions and photosynthetic processes (Miedema, 1982).

Table 4.2 Analysis of variance of three day/night temperatures (T) of low (17/09°C), optimum (22/14°C) and high (27/19°C) of 12 advanced *Brassica carinata* genotypes (G) and their interactions (T × G) for the various shoot, root, and physiological parameters measured 35 d after planting (24 d after temperature treatments imposition).

	Shoot traits								
Source	Plant height	No. of leaf	Leaf area	Leaf dry weight	Stem dry weight	Root dry weight	Above ground weight	Total dry weight	Root/ shoot ratio
T	***	***	***	***	***	***	***	***	***
G	***	**	***	***	**	NS	***	***	NS
T × G	NS	NS	NS	NS	NS	NS	NS	NS	*
	Root traits								
Source	Longest root length	Cumulative root length	Root surface area	Root diameter	Root volume	Root tips	Root forks	Root crossings	
T	*	**	*	*	*	***	***	***	
G	NS	NS	NS	*	NS	NS	NS	NS	
T × G	NS	NS	NS	NS	NS	NS	NS	NS	
	Physiological traits								
Source	Chlorophyll	Flavonoids	Anthocyanin	N balance index	Fluorescence				
T	NS	***	***	***	***				
G	**	**	*	***	*				
T × G	NS	NS	NS	NS	NS				

*Significant at the 0.05 probability level. **Significant at the 0.01 probability level. *** Significant at the 0.001 probability level. NS, nonsignificant.

Table 4.3 Least square means of shoot responses of 12 advanced *Brassica carinata* genotypes to three day/night temperatures of low (17/09°C), optimum (22/14°C), and high (27/19°C).

Traits	Temperature	Carinata genotypes												Mean
		AX17001	AX17002	AX17004	AX17005	AX17006	AX17007	AX17008	AX17009	AX17010	AX17014	AX17015	AVANZA 641	
Plant height	Low	3.0	3.0	2.8	3.2	2.8	3.0	3.6	3.2	3.0	3.4	4.6	3.2	3.2c†
	Optimum	9.2	10.4	5.6	10.4	9.0	10.0	12.8	9.2	9.4	9.0	12.6	10.4	9.8b
	High	9.2	10.8	7.4	12.6	13.6	9.6	14.0	10.0	10.2	10.6	14.6	12.0	11.2a
	Mean	7.13D	8.07CD	5.27E	8.73BC	8.47CD	7.53CD	10.13AB	7.47CD	7.53CD	7.67CD	10.60A	8.53CD	
No. of leaf	Low	3.4	2.8	3.2	3.4	3.0	3.2	3.2	3.4	3	3.6	4.0	3.2	3.3c
	Optimum	5.4	5.0	4.0	5.2	4.4	5.0	5.2	5.8	5.2	4.2	6.0	5.0	5.03b
	High	5.2	5.4	4.6	5.6	5.8	5.8	5.2	5.8	6.0	5.0	6.0	5.6	5.5a
	Mean	4.67BC	4.40CD	3.93D	4.73BC	4.40CD	4.67BC	4.53BC	5.0AB	4.73BC	4.27CD	5.33A	4.60BC	
Leaf area, cm²	Low	247.19	167.22	214.76	304.04	227.82	261.79	282.27	366.81	244.85	264.84	373.59	228.17	265.28b
	Optimum	538.3	397.44	406.38	635.61	468.02	587.96	590.2	715.55	696.64	607.77	792.4	575.02	584.2a
	High	464.69	523.37	450.63	757.33	618.51	481.07	430.51	595.84	811.05	544.69	949.56	517.26	595.3a
	Mean	416.75D	362.68D	357.26D	565.66BC	438.12CD	443.61CD	434.33CD	559.40BC	584.18AB	472.43BCD	705.18A	440.15CD	
Leaf dry weight g	Low	0.94	0.59	0.76	1.2	0.82	0.9	0.84	1.33	0.78	1.05	1.59	0.82	0.9b
	Optimum	1.82	1.21	1.35	2.44	1.41	1.78	1.69	2.35	2.18	2.21	3.09	2.09	1.9a
	High	1.52	1.67	1.43	2.71	2	1.38	1.52	1.77	2.34	1.77	3.32	1.69	1.8a
	Means	1.43CD	1.16D	1.18D	2.12AB	1.41CD	1.35CD	1.35CD	1.82BC	1.77BCD	1.68BCD	2.67A	1.553BCD	
Stem dry weight g	Low	0.31	0.22	0.26	0.42	0.24	0.28	0.38	0.4	0.25	0.3	0.53	0.28	0.3b
	Optimum	0.84	0.69	0.54	1.03	0.72	0.94	1.1	0.9	0.85	0.9	1.31	1.03	0.9a
	High	0.73	0.78	0.67	1.22	1.03	0.62	0.7	0.74	0.99	0.7	1.32	0.83	0.8a
	Mean	0.63C	0.56C	0.49C	0.89AB	0.66BC	0.61C	0.73BC	0.68AC	0.70BC	0.63BC	1.05A	0.71BC	
Root dry weight g	Low	0.1	0.06	0.11	0.15	0.09	0.1	0.11	0.22	0.09	0.15	0.18	0.09	0.12b
	Optimum	0.18	0.15	0.19	0.26	0.15	0.25	0.27	0.22	0.28	0.28	0.38	0.27	0.24a
	High	0.2	0.24	0.13	0.33	0.32	0.13	0.13	0.16	0.2	0.19	0.38	0.24	0.22a
	Means	0.16B	0.15B	0.14B	0.25AB	0.19B	0.16B	0.17B	0.20A	0.19B	0.21AB	0.31A	0.20B	
Above ground weight, g	Low	1.26	0.82	1.03	1.62	1.07	1.19	1.22	1.73	1.04	1.35	2.12	1.11	1.2b
	Optimum	2.67	1.91	1.9	3.47	2.13	2.72	2.8	3.26	3.04	3.14	4.4	3.12	2.8a
	High	2.26	2.45	2.1	3.93	3.04	2	1.85	2.51	3.33	2.53	4.65	2.52	2.7a
	Mean	2.06C	1.73C	1.68C	3.01AB	2.08C	1.97C	1.96C	2.50BC	2.47BC	2.34BC	3.72A	2.25BC	
Total dry weight g	Low	1.36	0.88	1.14	1.78	1.16	1.29	1.33	1.96	1.13	1.51	2.31	1.2	1.4b
	Optimum	2.85	2.06	2.09	3.74	2.29	2.98	3.08	3.48	3.32	3.43	4.78	3.39	3.1a
	High	2.46	2.7	2.24	4.27	3.36	2.14	1.99	2.67	3.54	2.72	5.03	2.76	2.9a
	Means	2.22C	1.88C	1.82C	3.26AB	2.27C	2.14C	2.13C	2.70BC	2.66BC	2.55BC	4.04A	2.45BC	
Root/ shoot ratio	Low	0.08BCa	0.07Ca	0.11ABa	0.09ABCa	0.09ABCa	0.08BCa	0.09ABCa	0.12Aa	0.08BCa	0.11ABa	0.09ABCa	0.08BCa	0.09
	Optimum	0.07Aa	0.07Aa	0.08Aab	0.07Aa	0.07Aa	0.09Aa	0.09Aab	0.06Ab	0.09Aa	0.07Aab	0.07Aa	0.07Aa	0.07
	High	0.08ABCa	0.08ABa	0.06BCb	0.08ABCa	0.09Aa	0.06BCa	0.06ABCb	0.05Cb	0.06BCa	0.06ABCb	0.08ABaC	0.08ABaC	0.07

†For each parameter, means followed by different lower-case letters within columns and different upper-case letters within rows are different ($p < 0.05$).

For the other shoot traits, that is, LA and weight of the various components, it was observed that responses at the low temperature were always lesser than at optimum and high-temperatures, but the response at high temperature was not different from at the optimum (Table 4.3). Previous studies noted that high temperature increases the rate of multiplication and cell expansion, hence speeding up the growth process (Wijewardana et al., 2018). Note that as with PH and LN, the genotype AX17015 always had the highest response values, and AX17004 still had the least. The other genotypes ranked similar to both of those in several instances (Table 4.3).

Biomass production

Under low temperature, less biomass production was observed for the plant components evaluated, accounting for a 52, 67, 50, and 55% reduction, respectively, for leaf, stem, root, and total biomass when compared to the optimum temperature treatment (Table 4.3). This trend is similar to findings in studies on other brassica species where a yield reduction was observed in canola grown in both winter and spring (Reddy et al., 2005). Also, Indian mustard plants exposed to high temperatures during the early season showed a decline in growth (Shamsul et al., 2009). Additionally, low temperature reduced AGW by 57% and RDW by 50% from the mean weight produced at the optimum temperature, showing that the relationship between these two traits is crucial since one seems to depend on the other. Poor root structure development during early crop establishment can limit shoot and canopy growth at later stages of growth (Gajanayake et al., 2014; Wijewardana et al., 2017).

The responses to low temperatures in our study are supported by observations of Marillia et al. (2014), indicating that although carinata is reported to be a cool-season crop, there is limited literature describing carinata growth in low temperatures. Given that this oilseed crop is

also deemed tolerant to heat and drought-like conditions (Kumar et al., 1984; Malik, 1990; Getinet et al., 1996; Schreiner et al., 2009), these characteristics may have attributed to the increased growth of some traits under higher temperature.

Additionally, Alsajri et al. (2019), in a study conducted with soybean, indicated that each developmental aspect or crop event has its specific temperature optimum, above which plant growth processes will decline. This suggests the importance of future studies of carinata genotypes at different growth stages under different temperature conditions. As observed in our research, most growth traits had significant growth and the developmental rate at the optimum temperature. Although some characteristics expressed greater tolerance to higher temperatures, they did not differ significantly at this temperature level when compared to the optimum temperature.

Table 4.4 Least square means of root trait responses of 12 advanced *Brassica carinata* genotypes to three day/night temperatures of low (17/09°C), optimum (22/14°C), and high (27/19°C).

Traits	Temperature	Carinata genotypes												Mean
		AX17001	AX17002	AX17004	AX17005	AX17006	AX17007	AX17008	AX17009	AX17010	AX17014	AX17015	AVANZA 641	
Longest root length, cm	Low	43.0	35.0	40.8	38.8	34.8	38.2	39.0	43.0	46.4	45.0	37.8	34.6	39.70b†
	Optimum	42.6	47.2	38.6	42.4	42.0	46.2	42.6	44.2	49.4	51.8	45.0	44.0	44.66a
	High	41.4	41.4	45.0	41.8	48.2	46.8	40.8	38.0	49.6	44.0	44.8	43.6	43.78ab
	Means	42.33	41.20	41.47	41.00	41.67	43.73	40.80	41.73	48.47	46.93	42.53	40.73	
Cumulative root length, cm	Low	1316.63	992.37	1584.10	1562.29	1408.87	1660.90	1579.25	3324.80	1595.72	2308.60	2423.37	1329.40	1757.20b
	Optimum	2366.02	2142.54	2740.25	1653.38	1221.51	3438.07	3052.58	2386.83	4616.81	2038.22	3146.70	2800.99	2633.70a
	High	2244.26	2024.04	1337.85	2179.86	2213.52	1453.70	1381.77	1484.57	2138.38	1555.56	2498.64	1970.19	1873.50b
	Means	1975.64	1719.65	1887.40	1798.51	1614.63	2184.22	2004.53	2398.73	2783.64	1967.46	2689.57	2033.53	
Root surface area, cm^2	Low	165.92	123.25	199.85	215.83	175.25	198.3	196.69	384.63	185.99	269.53	289.02	167.89	214.35b
	Optimum	274.7	223.21	302.69	243.45	160.45	402.76	311.92	292.38	481.37	285.71	449.63	341.64	314.16a
	High	300.04	294.12	157.76	332.44	340.23	179.3	186.19	214.75	251.6	251.40	389.35	280.52	264.812ab
	Means	246.89	213.53	220.10	263.91	225.31	260.12	231.60	297.25	306.32	268.88	376.00	263.35	
Root diameter, mm	Low	0.44	0.4	0.4	0.44	0.39	0.4	0.41	0.36	0.37	0.38	0.39	0.43	0.41ab
	Optimum	0.39	0.34	0.36	0.46	0.41	0.38	0.33	0.37	0.32	0.48	0.48	0.38	0.40b
	High	0.41	0.47	0.37	0.44	0.46	0.38	0.42	0.44	0.36	0.5	0.48	0.42	0.43a
	Means	0.4133ABC	0.4033ABCD	0.3767CD	0.4467AB	0.4200ABC	0.3867BCD	0.3867BCD	0.3900BCD	0.3500D	0.4533A	0.4500A	0.4100ABC	
Root volume, cm^3	Low	1.7	1.23	2.09	2.4	1.74	1.91	1.98	3.54	1.73	2.52	2.78	1.72	2.12b
	Optimum	2.57	1.88	2.68	2.88	1.68	3.88	2.54	2.87	4	3.79	5.63	3.41	3.12a
	High	3.26	3.44	1.48	4.23	4.22	1.77	2.02	2.56	2.36	3.38	4.9	3.33	3.09a
	Means	2.51	2.18	2.08	3.17	2.55	2.52	2.18	2.99	2.70	3.23	4.44	2.82	
Root tips, number	Low	4227.4	3139.4	3988.4	4202	4541	4054.6	3813.6	7473.2	4958.8	5407.8	5615	3514.4	4578b
	Optimum	9075	8220	10158.2	6828	6840.2	11048.4	10641.2	8160.2	12068.6	8334.2	10794	10666.4	9403a
	High	10138	9253	8599.6	10917.2	8888.4	8165	8517.4	7315.4	11396.8	6257.4	12393.8	10531.6	9364a
	Means	7913	6870	7582	7316	6757	7756	7657	7650	9475	6666	9601	8237	
Root forks, number	Low	6843.4	4618.2	7933.8	9898.6	8984.2	10307.8	9469.20	20230.2	7164.2	13278.6	15795.2	7820	10195b
	Optimum	17979.4	12643	18849.4	13725.8	8229	27853.6	21334	21043.8	33499.2	16537.8	28867	22615.6	20265a
	High	19209.4	19234.2	7583.6	20796	21141.4	10130.8	9882.2	13372.6	17724	14096.6	25280.6	19050.6	16458a
	Means	14677	12165	11456	14807	12785	16097	13562	18216	19462	14638	23314	16495	
Root crossings, number	Low	757	511.4	935.4	1008	960.2	1202.8	1099.2	2481.2	985.4	1745.8	1855.4	920.2	1205b
	Optimum	2006.8	1783	2320.8	1167.4	779.4	3347	2883.2	2245.8	4617.4	1731.6	2803.8	2314.2	2333a
	High	1648	1470.2	746.6	1691.4	1803	997.6	787.2	1138.2	1801	1141.4	2255.8	1633.2	1426b
	Means	1471	1255	1334	1289	1181	1849	1590	1955	2468	1540	2306	1623	

† For each parameter, means followed by different lower-case letters within columns and different upper-case letters within rows are different ($p < 0.05$). Lack of letters indicates no differences among means ($p > 0.05$).

Root growth and developmental parameters

The essential root growth parameters including the longest root length (LRL), cumulative root length (CRL), root surface area (RSA), average root diameter (RD), root volume (RV), root tips (RT), root forks (RF) and root crossings (RC), were measured and analyzed 24 DAT. There was the main effect of temperature ($p < 0.05$) on all the root parameters measured; however, the only genotype main effect was on RD ($p < 0.05$), and there were no temperature × genotype interactions (Table 4.2). For all parameters except RD, responses were highest at the optimum temperature (Table 4.4). At the high temperature, RD was greater than at the optimum temperature, but at the low temperature, RD was intermediate and not different from at either the optimum or high temperature. In terms of RD responses due to genotype, AX17014 and AX17015 ranked largest and AX17010 smallest, but the variation among the other genotypes was not that distinct because at least five others were not different from this greatest ranking (Table 4.4). Mean LRL at the low temperature was 11% less than at the optimum temperature, while at high-temperature LRL was intermediate and not different from either the optimum or low temperature (Table 4.4). At low and high temperatures, CRL means were not different but were less than at the optimum temperature (Table 4.4). A reduction in plant root development under low temperatures may be due to limited access to soil moisture and nutrient (Wijewardana et al., 2018). Brand et al. (2016) and Wijewardana et al. (2018) reported that suboptimal temperatures (20/12ºC and 22/14ºC) had damaging effects on root development in cotton and sweet potato cultivars, respectively.

The RSA was 32% less at the low (214.35 cm^2) than that at the optimum temperature (314.16 cm^2), but the response at the high temperature was intermediate (264.81 cm^2) and not different from either of these two (Table 4.4). Mean RV was 32% less at the low temperature

(2.12 cm^3) compared to the optimum level (3.12 cm^3), and the response at the high temperature was the same as the optimum (3.09 cm^3; Table 4.4). This response suggests a profound effect of low temperature on this root trait.

The mean number of RT was 51% less at the low temperature than at the optimum but was not different from optimum at high temperature (Table 4.4). Likewise, mean RF was 50% less when carinata was planted under low temperature as compared to the optimum temperature but was not different between optimum and high-temperature regimes (Table 4.4). Mean RC across carinata genotypes was similar at low and high temperature and was 48% less (low temperature) and 39% less (high temperature) compared to the optimum temperature (Table 4.4). Based on observations in this study, there is evidence that low temperature limited most carinata root traits development compared to optimum temperature. This concurs with reports by Kaspar and Bland (1992) and Sanders and Markhart, (2001) indicating that low thermal level can restrict lateral root development, root branching, and biomass, due to reduction in enzyme activities related to membrane lipids of plant roots and decreased transport of photosynthetic products from shoots to the root system.

Physiological parameters

Except for Chl, there was a main effect of temperature ($p < 0.01$) on the physiological traits studied (Table 4.2). Also, there was a genotype main effect on all parameters ($p < 0.05$), and there were no temperature × genotype interactions (Table 4.2). The Flav concentration was different among temperatures, greatest at the low and least at the high temperature. (Table 4.5). Among genotypes, AX17015 was ranked greatest and AX17008, but there were other genotypes that were not dissimilar from the greatest or least values (Table 4.5). Anthocyanin concentration at the low temperature was greater than at the optimal and high temperature (Table 4.5).

Genotype AX17009 ranked greatest and AX17005 at least for anthocyanin responses (Table 4.5). The NBI responses were greatest at the high and least at the low temperature (Table 4.5). Genotype AX17008 had the greatest NBI, and AX17015 had the least. At low temperature, FvFm was least, and there was no difference between the optimum and high temperatures (Table 4.5). Among genotypes, AX17015 had the greatest FvFM, but this was not different from nine of the other genotypes, and AX17006 was least.

Studies with sweet potato cultivars at three temperature levels observed similar results, where Flav was more significant at low temperatures and decreased at the high and optimal level, indicating that varying climatic factors can alter Flav production. Increases in the production of Flav in leaves makes plants more resilient to environmental stresses (Wijewardana et al., 2018).

Table 4.5 Least square means of physiological trait responses of 12 advanced *Brassica carinata* genotypes to low (17/09°C), optimum (22/14°C), and high (27/19°C) day/night temperatures.

Traits	Temperature	Carinata genotypes												Mean
		AX17001	AX17002	AX17004	AX17005	AX17006	AX17007	AX17008	AX17009	AX17010	AX17014	AX17015	Avanza 641	
Chlorophyll, µg cm cm^{-2}	Low	21.69	20.38	20.72	21.2	18.36	20.09	19.98	20.29	18.41	21.83	20.76	22.05	20.5
	Optimum	19.58	19.79	20.09	23.11	18.63	19.78	19.73	18.67	18.79	21.14	22.05	20.4	20.1
	High	20.09	20.54	20.67	23.16	18.77	16.73	20.79	19.4	21.16	23.13	22.56	19.73	20.5
	Means	20.46BCD†	20.24BCD	20.49BCD	22.50A	18.59D	18.87D	20.17BCD	19.45CD	19.45CD	22.04AB	21.79AB	20.90ABC	
Flavonoids	Low	0.89	0.98	1.11	0.87	0.9	0.8	0.74	0.9	0.83	1.08	1.02	1	0.93a
	Optimum	0.74	0.61	0.77	0.68	0.64	0.63	0.45	0.77	0.71	0.65	0.85	0.61	0.68b
	High	0.53	0.53	0.57	0.56	0.59	0.46	0.4	0.56	0.53	0.6	0.72	0.51	0.55c
	Means	0.7266ABC	0.7108BC	0.8237AB	0.7119BC	0.7156BC	0.6363CD	0.5357D	0.7493ABC	0.6969BC	0.7803AB	0.8676A	0.7104BC	
Anthocyanin	Low	0.14	0.15	0.14	0.14	0.15	0.14	0.14	0.13	0.15	0.14	0.15	0.14	0.15a
	Optimum	0.14	0.13	0.15	0.12	0.14	0.13	0.11	0.15	0.14	0.12	0.13	0.13	0.14b
	High	0.12	0.14	0.13	0.11	0.13	0.15	0.13	0.15	0.13	0.12	0.13	0.13	0.13b
	Means	0.1365ABCD	0.1447AB	0.1434AB	0.1269D	0.1446AB	0.1431AB	0.1342BCD	0.1483A	0.1434AB	0.1292CD	0.1404ABC	0.1371ABCD	
N balance index	Low	24.86	22.16	20.97	25.2	21.08	25.59	28.24	22.58	23.29	20.96	21.18	23.72	23.32c
	Optimum	27.24	32.89	27.33	34.13	28.99	31.89	43.02	26.24	26.95	32.94	27.9	34.22	31.15b
	High	38.17	39.99	37.16	41.22	32.05	36.22	53	35.23	40.24	44.5	32.37	38.5	39.06a
	Means	30.09BCD	31.68BCD	28.49BCD	33.52B	27.37D	31.23BCD	41.42A	28.02CD	30.16BCD	32.80BC	27.15D	32.15BCD	
Fluorescence	Low	0.66	0.63	0.58	0.53	0.48	0.54	0.57	0.6	0.58	0.52	0.62	0.53	0.57b
	Optimum	0.66	0.67	0.68	0.67	0.65	0.65	0.69	0.64	0.69	0.62	0.68	0.61	0.66a
	High	0.6	0.65	0.71	0.6	0.57	0.62	0.68	0.67	0.64	0.68	0.63	0.6	0.64a
	Means	0.6446A	0.6556A	0.6587A	0.6063ABC	0.5703C	0.6067ABC	0.6496A	0.6422AB	0.6438A	0.6121ABC	0.6482A	0.5851BC	

† For each parameter, means followed by different lower-case letters within columns and different upper case letters within rows are different ($p < 0.05$). Lack of letters indicate no differences among means ($p > 0.05$).

Low temperature also resulted in greater Anth than at the optimum and high temperature, but there was not a difference between high and the optimum temperature (Table 4.5). Mean NBI decreased with decreasing temperature regimes and was reduced by 25% from the optimum to the low-temperature regime. Mean FvFm was not different between high and optimum temperature treatments but decreased by 16% at the low temperature (Table 4.5). The effect of temperature on Anth, Flav, and NBI in this study indicates a robust thermal impact on the N status for carinata genotypes. Several studies have used different plant traits such as reproductive, morphological and physiological factors to quantify various stress tolerance in several crops (Reddy and Kakani, 2007; Salem et al., 2007; Singh et al., 2008; Gajanayake et al., 2011; Wijewardana et al., 2016a, 2017, 2018) and found that variability for the physiological traits existed among the different cultivars studied across all species examined. There is limited literature available, however, regarding these traits in carinata genotypes and how it affects plant adaptation to varying climatic conditions, indicating the need for future evaluation of these traits at later growth stages before concluding its overall effect on crop growth.

Thermotolerance classification of carinata genotypes based on cumulative low and high-temperature response index

Low- and high-temperature indices

The CLTRI and CHTRI were calculated to help determine the relationship between shoot, root, and physiological components for the 12 advanced carinata genotypes grown under three temperature treatments during seedling growth and development. The relationship was characterized based on the highest coefficient of determination value (r^2). A poor linear relationship ($r^2 = 0.09$) between CLTRI and CHTRI was observed (Fig. 4.1). This is an indication that both low and high-temperature tolerance mechanisms vary among the genotypes,

and when breeding or evaluating for these traits, selection must be carried out separately in accordance.

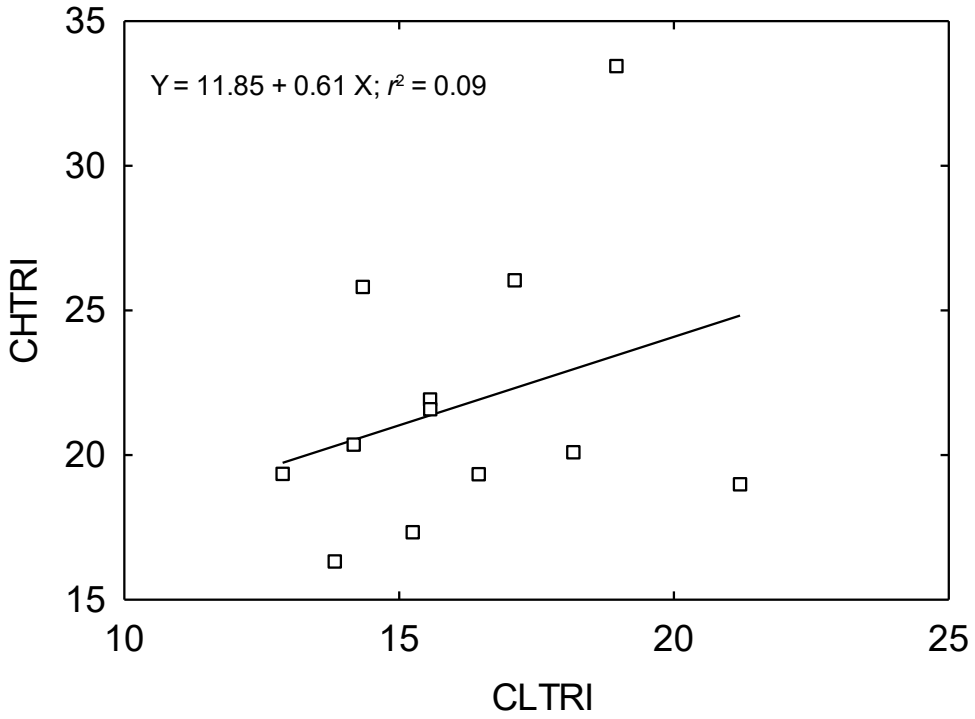

Figure 4.1 The relationship between cumulative low- and high-temperature stress response index (CLTRI; CHTRI) for 12 *Brassica carinata* genotypes.

Brassica carinata were grown under three day/night temperatures of low (17/09°C), optimum (22/14°C), and high (27/19°C) and harvested at 35 d after planting (24 d after temperature treatment imposition).

To have a greater understanding of the relationship between root, shoot, and physiological factors, the cumulative response indices were calculated for each of these components and plotted against the CLTRI (Fig 4.2) and CHTRI (Fig. 4.3), respectively. Under the low temperature (17/19ºC), a strong relationship was observed between CLTRI and shoot (r^2 = 0.42) and root (r^2 = 0.98) components, indicating the importance of these two traits when selecting carinata genotypes for cold tolerance during the early vegetative growth stage (Fig 4.2).

Physiological traits did not play a crucial role in the selection process for cold-tolerant genotypes, due to a poor relationship ($r^2 = 0.07$) observed when plotted against CLTRI (Fig 4.2).

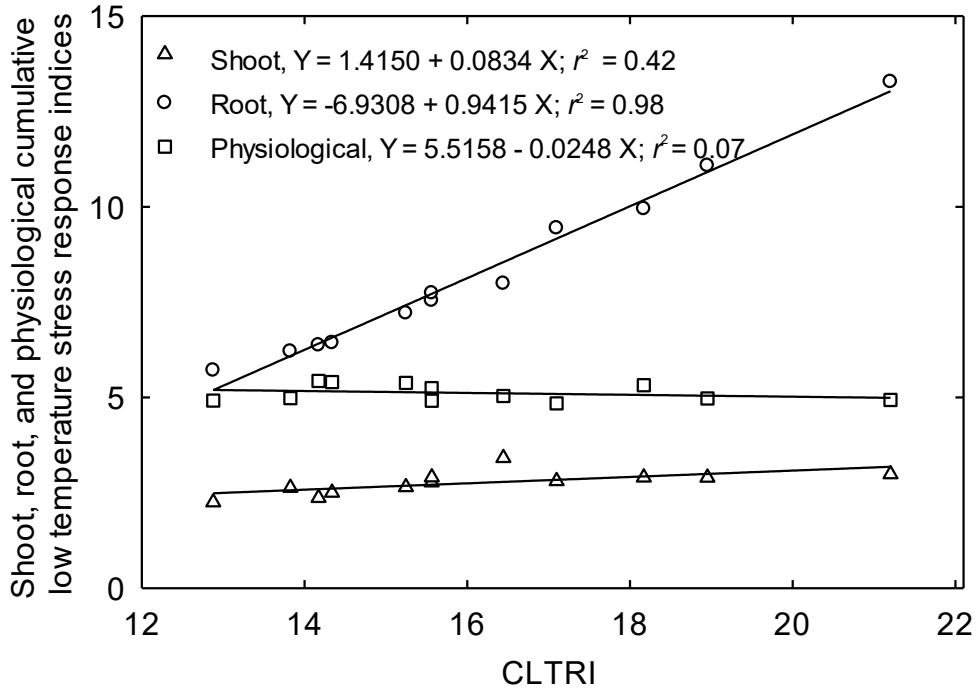

Figure 4.2 The relationship between cumulative low-temperature response index (CLTRI) and cumulative shoot, root, and physiological low response indices for 12 *Brassica carinata* genotypes.

Brassica carinata were grown under three day/night temperatures of low (17/09°C), optimum (22/14°C), and high (27/19°C) and harvested at 35 d after planting (24 d after temperature treatment imposition). Shoot, root, and physiological parameters were measured at 35 d.

A similar observation was made for genotypes grown under high temperature (27/19ºC), where a robust linear relationship was observed between CHTRI and shoot ($r^2 = 0.68$) and root ($r^2 = 0.97$) components, which also emphasized the crucial role these two traits play in the selection for heat tolerance of carinata genotypes during early growth stage (Fig. 4.3). Also, a

poor relationship was observed between CHTRI and the physiological traits (r^2 = 0.10; Figure 4.3), indicated that this trait might be the least desirable trait for the selection of heat-tolerant carinata genotypes. The data gathered from this evaluation will be beneficial for future screening of carinata for cold and heat-tolerance since it gives a more unambiguous indication of which traits are most relevant and should be considered when selecting for tolerance level.

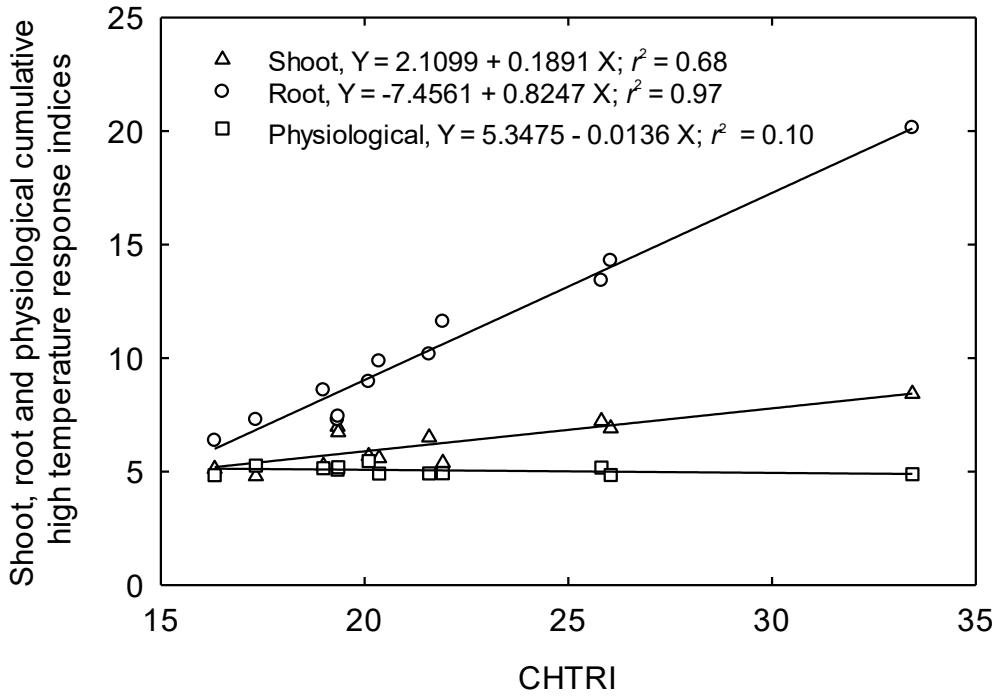

Figure 4.3 The relationship between cumulative high-temperature response index (CHTRI) and cumulative shoot, root, and physiological high response indices for 12 *Brassica carinata* genotypes.

Brassica carinata were grown under three day/night temperatures of low (17/09°C), optimum (22/14°C), and high (27/19°C) and harvested at 35 d after planting (24 d after temperature treatment imposition). Shoot, root, and physiological parameters were measured at 35 d.

Thermotolerance classification of carinata genotypes

The CLTRI and CHTRI values determined for all the shoot, root, and physiological parameters measured for carinata genotypes at the early growth stage and their standard deviations were applied for the classification of the 12 advanced carinata genotypes into four heat- (Table 4.6) and cold-tolerant groups (Table 4.7). Across the genotypes studied, seven (58%) were categorized as heat-sensitive, two (17%) as moderately heat-sensitive, two (17%) as moderately heat-tolerant, and one (8%) as heat-tolerant (Table 4.6). The CHTRI scores varied between 16.32 and 35.18 among the genotypes. The genotype AX17006 was identified as the most heat tolerant, while the heat-sensitive group consisted of AX17007, AX17008, AX17009, AX17004, AX17010, AX17014, and AVANZA 641 (Table 4.6).

Table 4.6 Classification of 12 *Brassica carinata* genotypes into heat-tolerant groups using cumulative high-temperature response indices (CHTRI; with individual CHTRI scores in parenthesis).

Heat-sensitive CHTRI (16.32 - 21.03)	Moderately heat-sensitive CHTRI (21.04 - 25.75)	Moderately heat-tolerant CHTRI (25.76 - 30.47)	Heat-tolerant CHTRI (30.48 - 35.18)
AX17007 (16.32)	AX17015 (21.58)	AX17002 (25.81)	AX17006 (33.45)
AX17008 (17.33)	AX17001 (21.92)	AX17005 (26.04)	
AX17009 (18.99)			
AX17004 (19.34			
AX17010 (19.35)			
AX17014 (20.10)			
AVANZA 641 (20.36)			
7	2	2	1
58%	17%	17%	8%

The carinata genotypes were classified into four cold-tolerant groups also. Of the 12 genotypes evaluated, one (8%) was identified as cold-tolerant, two (17%) as moderately cold-tolerant, four (33%) as moderately cold-sensitive, and five (42%) as cold-sensitive (Fig 4.7). The CLTRI scores ranged from 12.88 to 22.47, with genotype AX17010 being the most cold-

sensitive and AX17009 the most cold-tolerant genotype. Among the 12 genotypes, the heat-tolerant genotype, AX17006, performed 78, 54, and 29% better than the heat susceptible, moderately heat-susceptible, and moderately heat tolerant-groups, respectively. Similarly, the most cold-tolerant genotype, AX17009, exhibited an average of 50, 31, and 14% more cold-tolerance traits than the cold-sensitive, moderately cold-sensitive, and moderately cold-tolerant groups, respectively.

Table 4.7 Classification of 12 *Brassica carinata* genotypes into cold-tolerant groups using cumulative low-temperature response indices (CLTRI; with individual CLTRI scores in parenthesis).

Cold-sensitive CLTRI (12.88 - 15.28)	Moderately cold-sensitive CLTRI (15.29 - 17.67)	Moderately cold-tolerant CLTRI (17.68 - 20.07)	Cold-tolerant CLTRI (20.08 - 22.47)
AX17010 (12.88)	AX17001 (15.56)	AX17014 (18.17)	AX17009 (21.20)
AX17007 (13.83)	AX17015 (15.56)	AX17006 (18.95)	
AVANZA 641 (14.17)	AX17004 (16.44)		
AX17002 (14.34)	AX17005 (17.10)		
AX17008 (15.24)			
5	4	2	1
42%	33%	17%	8%

Since there is a deficiency in information regarding carinata heat- and cold-tolerance characteristics, this study aids in a better understanding of how these genotypes react to different temperature treatments at the early growth stage. This indicates that responses of the traits evaluated may vary among genotypes across varying temperature conditions. Hence, studying the shoot, root, and physiological traits and combining these for tolerance classification is essential to better understanding the genetic variation that exists among these genotypes. Furthermore, given that this study was conducted under enclosed sunlit environmental conditions that mimic open field settings, these results obtained could be transferred to natural open field conditions, as was suggested in a similar study with cotton (Reddy et al., 1997). Those authors

validated predictions of a cotton simulation model, and data gathered matched the actual results obtained under open field conditions.

Table 4.8 Means of shoot, root, and physiological traits responses of four *Brassica carinata* breed types to temperature.

Traits	Carinata breed types			
	Hybrid	Inbred	Double haploid	Check
Plant height, cm	9.13A[†]	7.53B	8.17AB	8.53AB
No. of leaf	4.80	4.43	4.73	4.60
Leaf area, cm^2	588.81A	428.09C	505.38AB	440.16BC
Leaf dry weight g	2.17A	1.46B	1.54B	1.53B
Stem dry weight g	0.86A	0.65B	0.68B	0.72AB
Root dry weight g	0.26	0.18	0.18	0.20
Above ground weight, g	3.03A	2.11B	2.23B	2.25B
Total dry weight g	3.29A	2.29B	2.41B	2.45B
Root/ shoot ratio	0.085	0.082	0.081	0.081
Longest root length, cm	44.73	41.53	43.68	40.73
Cumulative root length, cm	2328.52	1799.17	2342.79	2033.53
Root surface area, cm^2	322.44	233.95	273.83	263.36
Root diameter, mm	0.46A	0.42B	0.38B	0.42AB
Root volume, cm^3	3.84A	2.50B	2.60B	2.83AB
Root tips, number	8133.70	7267.75	8134.43	8237.47
Root forks, number	18976.00	13178.00	16834.00	16495.00
Root crossings, number	1922.30	1305.91	1965.50	1622.53
Chlorophyll, µg cm cm^{-2}	21.92A	20.46B	19.49B	20.91AB
Flavonoids	0.82A	0.74A	0.65B	0.71AB
Anthocyanin	0.13	0.14	0.14	0.14
N balance index	29.98	30.23	32.71	32.15
Fluorescence	0.63	0.63	0.64	0.59

[†]For each parameter, means followed by different upper-case letters within rows are different ($p < 0.05$). Lack of letters indicate that there were no differences ($p > 0.05$).

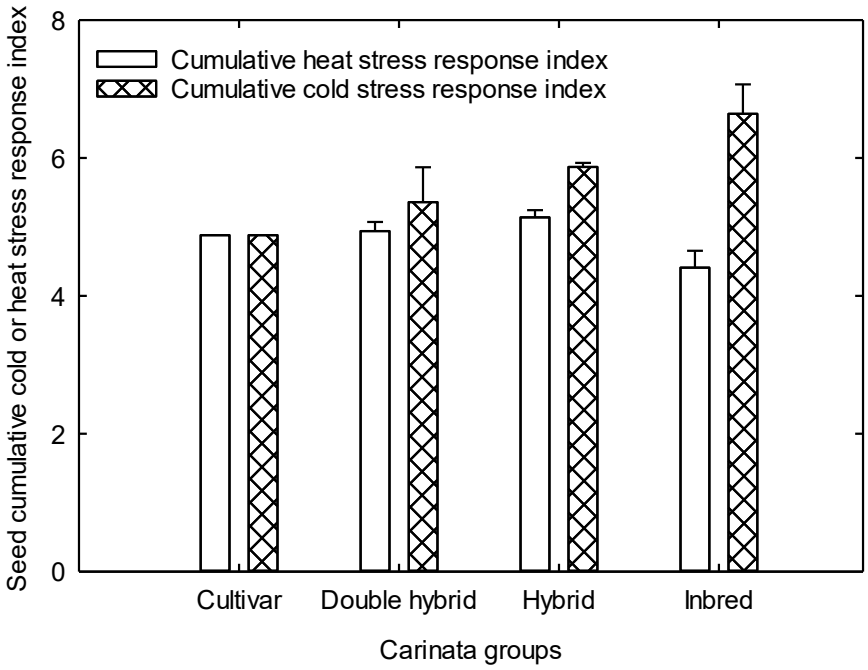

Figure 4.4 Cumulative cold or heat stress response indices of seed vigor responses of *Brassica carinata* breed types

Bars represent four *Brassica carinata* breed types; group mean cold or heat cumulative stress response indices and their standard errors. Plain bars represent cumulative heat temperature stress response indices (CHTRI), and crossed bars represent cumulative cold temperature stress response indices (CLTRI) for the four breed types germinated under eight temperatures; 8.2, 12.73, 15.65, 19.93, 23.8,29.28, 34.22, and 36.96ºC.

When genotypes were grouped according to breed types, hybrids generally had better responses to temperature than the inbred lines, and double haploids and the check responses were intermediate in early season growth response traits measured (Table 4.8). Furthermore, the variability observed for the heat and cold response indices at both seed germination (Chapter 3), and early-growth stages indicated that screening for heat or cold tolerance at the early growth stage is superior compare to the germination stage (Figures 4.4 and 4.5). The inbred group exhibited better cold tolerance relative to the other groups, and even greater tolerance compared to the commercially grown cultivar, AVANZA 641 (Figure 4.4.) at the germination stage. At the

early growth stage, the hybrid group showed greater cold tolerance compared to the other groups, while the inbred group shows greater heat tolerance (Figure 4.5). The double hybrid group expressed greater heat and cold tolerance during early growth, while the commercial cultivar thermotolerance was the same at both growth stages.

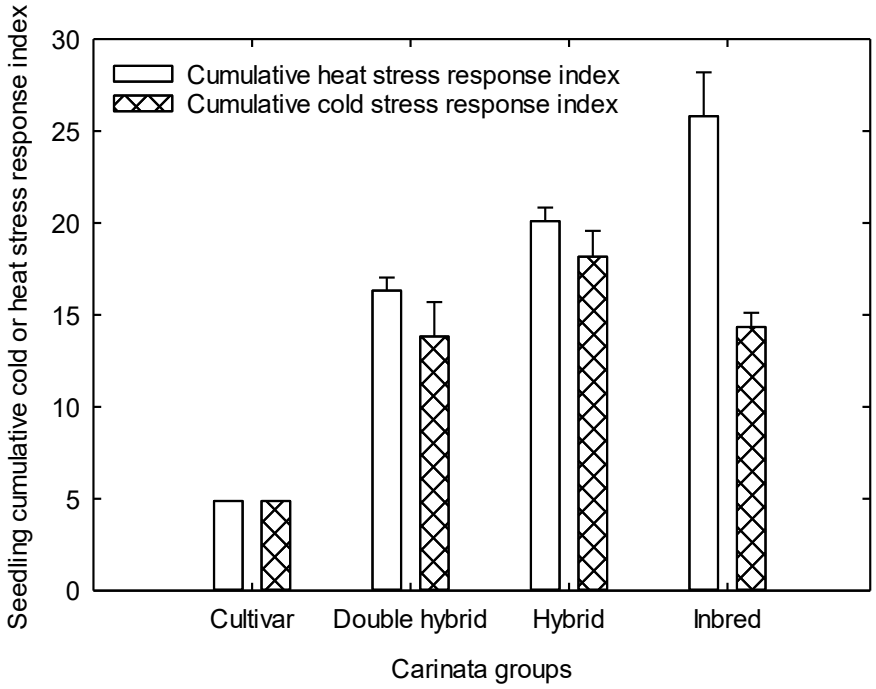

Figure 4.5 Cumulative cold or heat stress response indices of seedling vigor responses of four *Brassica carinata* breed types.

Bars represent four *Brassica carinata* breed types; group mean cold or heat cumulative stress response indices and their standard errors. Plain bars represent cumulative heat temperature stress response indices (CHTRI), and crossed bars represent cumulative cold temperature stress response indices (CLTRI) for the four breed types grown under three day/night temperatures; low (17/09°C), optimum (22/14°C), and high (27/19°C) and harvested at 35 d after planting (24 d after temperature treatment imposition).

Summary and Conclusions

The 12 advanced carinata genotypes evaluated had substantial variability for the shoot, root, and physiological traits measured from plants grown under three different temperature treatments. The low temperature affected various shoot traits, causing a 67, 34, 55, and 55% reduction on plant height, total leaf number, leaf area, and total biomass, respectively. Suboptimal temperature also impacted root traits such as the most extended root length, cumulative root length, root surface area, root volume, root tips, root forks, root crossings, as well as physiological trait NBI. Also, carinata genotypes grown under high temperatures recorded greater values for plant height, leaf area, root diameter, and NBI than the mean value observed under optimal and low temperatures for the same traits. A poor relationship between CLTRI and CHTRI highlighted the variation in response mechanisms at low and high-temperature among genotypes, and therefore separate selection is necessary for developing cold and heat tolerant carinata genotypes. When the genotypes were grouped according to breed type, the hybrids generally were superior compared to the inbred lines, and the double haploids and the commercial check were broadly similar and intermediate between hybrids and inbred lines in response to temperature during early season growth. Also, the inbred group had greater cold tolerance at the germination stage, while the hybrid group was more cold-tolerant at the early growth stage. Further research is required to assess how carinata genotypes vary in their response to low and high-temperature at later growth stages, and open field conditions. The heat and cold tolerant genotypes identified in this study would be beneficial for plant breeders in the development of genotypes adaptable to different climatic zones.

CHAPTER V

GENERAL SUMMARY AND CONCLUSIONS

To successfully cultivate carinata [*Brassica carinata* (A. Braun)] in Mississippi during the winter period requires information that aids in the correct selection of genotypes, cultivation area, planting window, and crop management. Hence, understanding how carinata genotypes respond to different temperature conditions during the seed germination and early-season growth stages will be beneficial for breeding programs and developing a model for field applications. Two studies were conducted to quantify temperature effects on seed germination and early-season growth and development of 12 carinata genotypes. In the first study, *in vitro* seed germination assays and regression models were used determined seed germination response functions, MSG, SGR, and to estimate genotype-specific cardinal temperatures and TAR. Then, seed germination traits were used to develop genotype-specific thermotolerance classification based on seed germination traits. In the second study, early-season vigor responses of 12 carinata genotypes were evaluated at three different temperatures, low, optimum, and high-temperature conditions. Then, low- and high-temperature response indices were calculated to differentiate the 12 carinata genotypes into different thermo-tolerance groups.

The quadratic model functions best described the MSG, time to 50% MSG, and SGR in all carinata genotypes to temperature. The cardinal temperatures (T_{min}, T_{opt}, and T_{max}) for each genotype and their TAR (T_{max}-T_{min}) varied among carinata genotypes. The individual and cumulative temperature response indices (ITRI; CTRI), the sum of all individual trait responses, varied among the 12 carinata genotypes for low and high temperatures. A weak relationship

between cumulative low (CLTRI) and high (CHTRI) temperature response indices indicated that cold- and heat-tolerance responses among these genotypes were divergent. Therefore, carinata genotypes were classified into four cold- and heat-tolerant groups based on seed germination traits. The genotypes AX17002 and AX17004 were the least and most heat tolerant, respectively. In addition, AX17004, was the most cold-tolerant, while AVANZA 641, AX17010, AX17008, and AX17007 were the most cold-sensitive among 12 genotypes tested in this study. The relationship between the two growth stages at low and high temperatures showed that double haploid and hybrid carinata breeding groups had a stable thermotolerance response at both stages. In contrast, the inbred group had a wider cluster at both stages in response to a minimum and maximum temperatures.

In the second study, shoot, root, and physiological traits were measured for 12 carinata genotypes grown under low (17/09°C), optimum (22/14°C), and high (27/19°C) day/night temperature conditions in the sunlit, but environment-controlled plant growth chambers. All plants were harvested 35 d after seeding (24 days after temperature treatment imposition). There was substantial variability for the shoot, root, and physiological traits measured among the 12 carinata genotypes. The low-temperature treatment decreased plant height (67%) and reduced the total number of the leaves (34%), leaf area (55%), and total biomass (55%) compared to genotypes grown at optimum temperature conditions. The low temperature also reduced the growth of various root parameters, including the longest root length, cumulative root length, root surface area, root volume, number of root tips, number of root forks, and number of root crossings, as well as nitrogen balance index, a physiological trait. On the other hand, genotypes grown at the high-temperature treatment showed greater plant height, leaf area, root diameter, and nitrogen balance index than genotypes at the optimum temperature.

Based on early-season growth responses to low and high temperatures for each genotype, CLTRI and CHTRI were used to classify genotypes into four cold- and heat-tolerant groups. A strong relationship between CLTRI and CHTRI with a shoot and root traits indicates the importance of these traits for stress tolerance in breeding and selection. Weak correlation between seed-based CLTRI and CHTRI as well as seedling-based CLTRI and CHTRI indicated that stress tolerance at seed and seedling stages operate differently, and efforts to breed new genotypes should be stage-specific. According to CHTRI calculations, the most heat tolerant genotype, AX17006, showed 105% more heat-tolerance than the least heat-tolerant genotype, AX17007. Besides, AX17006 performed 78, 54, and 29% better than the heat susceptible, moderately heat-susceptible, and moderately heat tolerant-groups, respectively.

Similarly, the most cold-tolerant genotype, AX17009, exhibited 50, 31, and 14% more cold-tolerance than the cold-sensitive, moderately cold-sensitive, and moderately cold-tolerant groups, respectively. When the genotypes were grouped according to breed type, the hybrids generally were superior compared to the inbred lines, and the double haploids and the commercial check were generally similar and intermediate between hybrids and inbred lines in response to temperature during early season growth. Also, the inbred group had greater cold tolerance at the germination stage, while the hybrid group was more cold-tolerant at the early growth stage.

Classifying carinata genotypes using both seed germination traits and early season growth and developmental parameters would help in understanding responses to temperature stress at these stages. The cardinal temperatures determined will be beneficial for carinata crop model development and application in field production systems. The identified stress-tolerant genotypes will help in the selection of genotypes best suited for different production regions

based on existing temperature conditions. Furthermore, the heat and cold tolerant genotypes identified in this study would be beneficial for plant breeders in the development of genotypes adaptable to specific environments and production regions.

REFERENCES

Aburas, H., and A. Demirbas. 2015. Evaluation of beech for production of biochar, bio-oil, and gaseous materials. Process Saf. Environ. 94: 29-36.

Acharya, S.N., J. Dueck, and R.K. Downey. 1983. Selection and heritability studies on canola/rapeseed for low temperature germination. Can. J. Plant Sci. 3: 377-384.

Adak, T., N.V.K. Chakravarty, and R. Saxena. 2009. Growth and yield prediction in mustard using InfoCrop simulation model. J. Agrometeorol. 11: 156-161.

Aggarwal, P.K., and N. Kalra. 1994. Analyzing the limitations set by climatic factors, genotype, and water and nitrogen availability on productivity of wheat. II. Climatically potential yields and optimal management strategies. Field Crops Res. 38: 93-103.

Aggarwal, P.K., N. Kalra, A.K. Singh, and S.K. Singh. 1994. Analyzing the limitations set by climatic factors, genotype, water and nitrogen availability on productivity of wheat-I. The model documentation, parameterization and validation. Field Crops Res. 38: 73-91.

Agriculture and Agri-Food Canada. 2015. Carinata: an oilseed ready for lift-off. Available from http://www.agr.gc.ca/eng/news/scientific-achievements-in-agriculture/bioproducts/carinata-an-oilseed-ready-for-lift-off/?id=1389626028671 (Accessed July 18, 2017.)

Agrisoma Biosciences Inc. 2017. Carinata management handbook, southeastern US 2017-18. Agrisoma Biosciences Inc. USA, Tifton, GA.

Alagumalai, A. 2014. Bio-diesel-A global scenario. Renew. Sustain. Energy Rev. 29: 517-527.

Al-Ahmadi, M.J., and M. Kafi. 2007. Cardinal temperatures for germination of *Kochia scoparia* (L). J. Arid Environ. 68(2): 308-314.

Alberti, P.K. 2017. Development of best management practices for production of Ethiopian mustard (*Brassica carinata*) in South Dakota. MS thesis, South Dakota State Univ., Brookings.

Alemayehu, N., and H. Becker. 2002. Genotypic diversity and patterns of variation in a germplasm material of Ethiopian mustard (*Brassica carinata* A. Braun). Genet. Resour. Crop Evol. 49: 573-582.

Aloni, B., M. Peet, M. Pharr, and L. Karni. 2001. The effect of high temperature and high atmospheric CO_2 on carbohydrate changes in bell pepper (*Capsicum annuum*) pollen in relation to its germination. Physiol. Plant. 112: 505-512.

Alsajri, F., B. Singh, C. Wijewardana, J. Irby, W. Gao, and K.R. Reddy. 2019. Evaluating soybean cultivars for low- and high-temperature tolerance during the seedling growth stage. Agronomy. 9: 13.

Alsajri, F., C. Wijewardana, L. Krutz, J. Irby, B. Golden, and K.R. Reddy. 2019. Quantifying and validating soybean seed emergence model as a function of temperature. Am. J. Plant Sci. 10(01): 111-124.

Altun, S., and F. Yasar. 2013. Biodiesel production from leather industry wastes as an alternative feedstock and its use in diesel engines. Energ. Explor. Exploit. 31: 759-770.

Alvarado, V., and K.J. Braford. 2002. A hydrothermal time model explains the cardinal temperatures for seed germination. Plant Cell Environ. 25: 1061-1069.

Anapalli, S.S., L. Ma, D.C. Nielsen, M.F. Vigil, and L.R. Ahuja. 2005. Simulating planting date effects on corn production using RZWQM and CERES-maize models. Agron. J. 97: 58-71.

Andreucci, M.P., D.J. Moot, A.D. Black, and R. Sedcole. 2016. A comparison of cardinal temperatures estimated by linear and nonlinear models for germination and bulb growth of forage brassicas. Eur. J. Agron. 81: 52-63.

Angadi, S.V., H.W. Cutforth, B.G. McConkey, M.H. Entz, S.A. Brandt, and K.M. Volkmar. 2000. Response of three *Brassica* species to high temperature stress during reproductive growth. Can. J. Plant Sci. 80: 693-701.

Appels, L., J. Lauwers, J. Degrève, L. Helsen, B. Lievens, K. Willems, et al. 2011. Anaerobic digestion in global bio-energy production: Potential and research challenges. Renew. Sustain. Energy. Rev. 15: 4295-4301.

Asamenew, G., H. Beyene, W. Negatu, and G. Alele. 1993. A survey of farming systems of the vertisol areas of the Ethiopian highlands in Tekalign, M. et al., eds. Improved management of vertisols for sustainable crop-livestock production in the Ethiopian highlands: Synthesis report 1986-92. Technical Committee of the joint Vertisol Poject, Addis Ababa, Ethiopia.

Association of Official Seed Analysts. 1983. Seed Vigor Testing Handbook. AOSA, Ithaca, NY, USA. (Contribution to the Handbook on Seed Testing, 32).

Atabani, A.E., A.S. Silitonga, H.C., Ong, T.M.I. Mahlia, H.H. Masjuki, I.A. Badruddin, et al. 2013. Non-edible vegetable oils: A critical evaluation of oil extraction, fatty acid compositions, biodiesel production, characteristics, engine performance and emissions production. Renew. Sust. Energ. Rev. 18(C): 211-245.

Baalbaki, R., S. Elias, J. Marcos-Filho, M.B. McDonald. 2009. Seed vigor testing handbook, AOSA, Ithaca, NY, USA.

Balat, M., and H. Balat. 2009. Recent trends in global production and utilization of bio-ethanol fuel. Appl. Energy. 86: 2273-2282.

Ban, Y., N.A. Khan, and P. Yu. 2017. Nutritional and metabolic characteristics of *Brassica carinata* co-products from biofuel processing in dairy cows. J. Agri. Food Chem. 65: 5994-6001.

Barthet, V.J. 2008. (n-7) and (n-9) cis-monosaturated fatty acid contents of 12 *Brassica* species. Phytochemistry. 69(2): 411-417.

Basili, M., and M.A. Rossi. 2018. *Brassica carinata*-derived biodiesel production: Economics, sustainability and policies. The Italian case. J. Clean. Prod. 191: 40-47.

Baskin, C.C., and J.M. Baskin. 2001. Seeds: Ecology, biogeography, and evolution of dormancy and germination. Academic Press, San Diego, California, pp. 666.

Bassel, G.W. 2016. To grow or not to grow. Trends in Plant Sci. 21: 498-505.

Basu, P.S., and J.S. Minhas. 1991. Heat tolerance and assimilate transport in different potato genotypes. J. Exp. Bot. 42(7): 861-866.

Bewley, J.D. 1997. Seed germination and dormancy. Plant Cell 9: 1055-1066.

Bhuiya, M.M.K, M.G. Rasul, M.M.K. Khan, N. Ashwath, and A.K. Azad. 2016. Prospects of 2nd generation biodiesel as a sustainable fuel-Part: 1 Selection of feedstocks, oil extraction techniques and conversion technologies. Renew. Sust. Energ. Rev. 55: 1109-1128.

Biello, D. 2008. Wild green yonder: Flying the environmentally friendly skies on alternative fuels. Sci. Am. https://www.scientificamerican.com/article/flying-environmentally-friendlyskies-on-alternative-fuels/ (Accessed 12 May 2018).

Bierhuizen, J.F., and W.A. Wagenvoort. 1974. Some aspects of seed germination in vegetables. 1. The determination and application of heat sums and minimum temperature for germination. Sci. Hortic. 2: 213-219.

Bona, S., G. Mosca, and T. Vamerali. 1999. Oil crops for biodiesel production in Italy. Renew. Energy 16: 1053-1056.

Bonner, F.T., and T.R. Dell. 1976. The Weibull function: a new method of comparing seed vigor. J. Seed Technol. 1: 96-103.

Bouaid, A., M. Martinez, and J. Aracil. 2009. Production of biodiesel from bioethanol and *Brassica carinata* oil: Oxidation stability study. Bioresour. Technol. 100: 2234-2239.

Boyer, J.S.1982. Plant productivity and the environment. Science 218: 443-448.

Bradford, K.J. 1990. A water relation analysis of seed germination rates. Plant Physiol. 94: 840-849.

Bradford, K.J. 1995. Water relations in seed germination. In: Kigel, J., G. Galili, eds. Seed development and germination. New York: Marcel Dekker Inc. 351-96.

Bradford, K.J., A.M. Tarquis, and J.M. Duran. 1993. A population- based threshold model describing the relationship between germination rates and seed deterioration. J. Exp. Bot. 44*:* 1225-1234.

Bradford, K.J., and O.A. Somasco. 1994. Water relations of lettuce seed thermoinhibition. I. Priming and endosperm effects on base water potential. Seed Sci. Res. 4**:** 1-10.

Brand, D., C. Wijewardana, W. Gao, and K.R. Reddy. 2016. Interactive effects of carbon dioxide, low temperature, and ultraviolet-B radiation on cotton seedling root and shoot morphology and growth. Front. Earth Sci. 10: 607-620.

Brown, R.F., and D.G. Mayer. 1988a. Representing cumulative germination. 1. A critical analysis of single-value germination indices. Ann. Bot. 61: 117-125.

Brown, R.F., and D.G. Mayer. 1988b. Representing cumulative germination. 2. The use of the Weibull function and other empirically derived curves. Ann. Bot. (London) 61: 127-138.

Brown, R.F.1987. Germination of *Aristida armata* under constant and alternating temperatures and its analysis with the cumulative Weibull distribution as a model. Aust. J. Bot. 35: 581-591.]

Butnar, I., J. Rodrigo, C. Gasol, and F. Castells. 2010. Life-cycle assessment of electricity from biomass: Case studies of two biocrops in Spain. Biomass Bioenergy. 34: 1780-1788.

Cabin, R.J., A.S. Evans, and R.J. Mitchell. 1997. Genetic effects of germination timing and environment: An experimental investigation. Evolution 51: 1427-1434.

Cahoon, E.B., J.M. Shockey, C.R. Dietrich, S.K. Gidda, R.T. Mullen, and J.M. Dyer. 2007. Engineering oilseeds for sustainable production of industrial and nutritional feedstocks: Solving bottlenecks in fatty acid flux. Curr. Opin. Plant Biol. 10 : 236-244.

Canvin, D.T. 1965. The effect of temperature on the oil content fatty acid composition of the oils from several oilseed crops. Can. J. Bot. 43: 63-69.

Cardone, M., M. Mazzoncini, S. Menini, V. Rocco, A. Senatore, M. Seggiani, et al. 2003. *Brassica carinata* as an alternative oil crop for the production of biodiesel in Italy: Agronomic evaluation, fuel production by transesterification and characterization. Biom. Bioenerg. 25: 623-636.

Carlsson, A. 2009. Plant oils as feedstock alternatives to petroleum-a short survey of potential oil crop platforms. Biochimie 91: 665-670.

Carneiro, J.W.P. 1994. Determinacao do numero de sementes para avaliar o desempenho germinativo de sementes de capim braquiaria (*Brachiaria brizantha* cv. Marandu). Rev. Bras. Sementes 16: 156-158.

Cherubini, F., S. Bargigli, and S. Ulgiati. 2009. Life cycle assessment (LCA) of waste management strategies: Landfilling, sorting plant and incineration. Energy 34: 2116-23.

Chhetri, A.B., M.S. Tango, and S.M. Budge. 2008. Non-edible plant oils as new sources for biodiesel production. Int. J. Mol. Sci. 9: 169-180.

Chiotti, Q., and T. Johnston. 1995. Extending the boundaries of climate change research: A discussion on agriculture. J. Rural Stud. 11: 335-350.

Choudhary, B., P. Joshi, and S. Ramarao. 2000. Interspecific hybridization between *Brassica carinata* and *Brassica rapa*. Plant Breed. 119: 417-420.

Christensen, J.V., A.M.F. Hennig , J.S. McKenzie, W.G. Legge, R.M. DePauw, B. Siemens, et al. 1985. Effect of seeding date, nitrogen and phosphate fertilizer on growth, yield and quality of rapeseed in northwest Alberta. Can. J. Plant Sci. 65: 275-284.

Chu, P.L., C. Vanerghem, H.L. MacLean, and B.A. Saville. 2017. Financial analysis and risk assessment of hydro processed renewable jet fuel production from camelina, carinata and used cooking oil. Appl. Energy 198: 401-409.

Cosentino, S.L. 1996. Crop physiology of sweet sorghum (*Sorghum bicolor* (L.) Moench). Proc. 1st Eur. Sem. Sorghum for Energy and Indus. 1-3 April, Toulouse, France. p. 30-41.

Covell, S., R. Ellis, E. Roberts, and R. Summerfield, R.1986. The influence of temperature on seed germination rate in grain legumes. I. A comparison of chickpea, lentil, soybean and cowpea at constant temperatures. J. Exp. Bot. 37: 705-715.

Dawadi, D., R Seepaul, S George, J Groot and D Wright. 2019. Drought tolerance classification of common oilseed species using seed germination assay. J. Oilseed Brassica 10: 97-105.

de Koff, J., P. Illukpitiya, S. Chawla, and R. Link. 2017. Industrial oilseed crops for Tennessee. Tennessee State Univ. Ext. ANR-B15.

De La Soujeole, A.A., and F.R. Miller. 1984. Cold tolerance of sorghum during early developmental stages. Proc. 39th Ann. Corn and Sorghum Res. Conf. 44: 18-32. Chicago, IL. 5-6 Dec. 1984. Am. Seed Trade Assoc., Washington, DC.

Drenth, A.C., D.B. Olsen, P.E. Cabot, and J.J. Johnson. 2014. Compression ignition engine performance and emission evaluation of industrial oilseed biofuel feedstocks across three fuel pathways. Fuel 136: 143-155.

Du Ploy, J., and R. Nel. 2012. A study of a feebate policy aimed at vehicle manufacturers to reduce CO_2 emissions. Int. Bus. and Econ. Res. 11: 1029-1040.

Duca, D., G. Toscano, G. Riva, C. Mengarelli, G. Rossini, A. Pizzi, et al. 2015. Quality of residues of the biodiesel chain in the energy field. Ind. Crops Prod. 75: 91-97.

Dürr, C., J.B. Dickie, X.Y. Yang, and H.W. Pritchard. 2015. Ranges of critical temperature and water potential values for the germination of species worldwide: contribution to a seed trait database. Agric. For. Meteorol. 200: 222-232.

EIA. 2017a. As U. S. airlines carry more passengers, jet fuel use remains well below its previous peak. U. S. Energy Information Administration. https: //www.eia.gov/todayinenergy/detail.php?id=31512 (Accessed 12 Oct. 2018).

El-Kassaby, Y., I. Moss, and D. Kolotelo. (2008). Seed germination: Mathematical representation and parameters extraction. Forest Sci. 54: 220-227.

Ellis R.H, G. Simon, and S. Covell. 1987. The influence of temperature on seed germination rate in grain legumes. III. A comparison of five faba bean genotypes at constant temperatures using a new screening method. J. Exp. Bot. 38: 1033-43.

Ellis, R.H., and E.H. Roberts. 1981. The quantification of aging and survival in orthodox seeds. Seed Sci. Technol. 9(2): 373-409.

Ellis, R.H., S. Covell, E.H. Roberts and R.J. Summerfield, 1986. The influence of temperature on seed germination rate in grain legumes. II. Interspecific variation in chickpea (*Cicer arietinum* L.) at temperature. J. Exp. Bot. 37: 1503-1515.

Emerson, B.N., and H.C. Minor. 1979. Response of soybean to high temperature during germination. Crop Sci. 19: 553-556.

Energy Information Administration. 2017b. Ethanol-Energy explained, your guide to understanding energy. https: //www.eia.gov/energyexplained/biofuels/ethanol.php (Accessed 18 July 2018).

Energy Information Administration. 2017c. Monthly energy review: Table 10.4 Biodiesel and other renewable fuels overview. EIA-0035, DOE, Washington, D.C.

Enjalbert, J., S. Zheng, J.J. Johnson, J.L. Mullen, P.F. Byrne, and J. K. MacKay. 2013. Brassicaceae germplasm diversity for agronomic and seed quality traits under drought stress. Ind. Crop. Prod. 47: 176-185.

Entz, M.H., and D.B. Fowler. 1991. Agronomic performance of winter versus spring wheat. Agron. J. 83: 527-532.

Environmental Protection Agency. 2011. Notice of decision granting partial waiver. Federal Register, Volume 76 Issue17 (Wednesday January 26 2011). https: //www.govinfo.gov/content/pkg/FR-2011-01-26/html/2011-1646.htm (Accessed 18 Nov. 2018).

Environmental Protection Agency. 2015. Inventory of U.S. greenhouse gas emissions and sinks. https: //www.epa.gov/ghgemissions/inventory-us-greenhouse-gas-emissions-and-sinks (Accessed 26 May 2018).

Escobar, J.C., E.S. Lora, O.J. Venturini, E.E. Yáñez, E.F. Castillo, and O. Almazan. 2009. Biofuels: Environment, technology and food security. Renew. Sustain. Energy Rev.13: 1275-1287.

FAO. 2008. The state of food and agriculture. http: //www.fao.org/3/a-i0100e.pdf (Accessed 18 July 2018).

Finch-Savage, W.E., and G. Leubner-Metzger. 2006. Seed dormancy and the control of germination. New Phytol. 171: 501-523.

Foley, M.E., and S.A. Fennimore. 1998. Genetic basis for seed dormancy. Seed Sci. Res. 8: 173-179.

Fracheboud, Y., P. Haldimann. J. Leipner, and P. Stamp. 1999. Chlorophyll fluorescence as a selection tool for cold tolerance of photosynthesis in maize (*Zea mays* L.). J. Exp. Bot. 50: 10.

Fulbright, T.E. 1988. Effects of temperature, water potential, and sodium chloride on indiangrass germination. J. Range. Manage. 41: 207-210.

Gajanayake, B., B.W. Trader, K. Reddy, and R. Harkess. 2011. Thermotolerance classification of ornamental pepper cultivars using seed germination traits. Seed Tech. 33: 134-142.

Gajanayake, B., K.R. Reddy, M.W. Shankle, R.A. Arancibia, and A.O. Villordon. 2014. Quantifying storage root initiation, growth, and developmental responses of sweetpotato to early season temperature. Agron. J. 106: 1795-1804.

Gan, Y.T., C.A. Campbell, H.H. Janzen, R. Lemke, L.P. Liu, P. Basnyat, et al. 2009. Root mass for oilseed and pulse crops: Growth and distribution in the soil profile. Can. J. Plant Sci. 89: 883-893.

Gao, J.P., D.Y. Chao, and H.X. Lin. 2007. Understanding abiotic stress tolerance mechanisms: Recent studies on stress response in rice. J. Integr. Plant Biol. 49: 742-750.

Garcia-Huidobro, J., J.L. Monteith, and G.R. Squire. 1982a. Time, temperature and germination of pearl millet (*Pennisetum typhoides*, S & H). J. Exp. Bot. 33: 288-296.

Garcia-Huidobro, J., J.L. Monteith, and G.R. Squire. 1982b. Time, temperature and germination of pearl millet (*Pennisetum typhoides* S and H.): I. Constant temperature. J. Exp. Bot. 33: 288-296.

Gasol, C., D. Gabarrell, A. Xavier, R. Assumpció, C. Miquel, C. Juan, et al. 2009. LCA of poplar bioenergy system compared with *Brassica carinata* energy crop and natural gas in regional scenario. Biomass Bioenerg. 33 : 119-129.

Gasol, C.M., X. Gabarrell, A. Anton, M. Rigola, J. Carrasco, P. Ciria, et al. 2007. Life cycle assessment of a *Brassica carinata* bioenergy cropping system in southern Europe. Biomass Energy. 31: 543-555.

Gesch, R.W., T.A. Isbell, E.A. Oblath, B.L. Allen, D.W. Archer, J. Brown, et al. 2015. Comparison of several *Brassica* species in the north-central U. S. for potential jet fuel feedstock. Ind. Crops Prod. 75: 2-7.

Getinet, A., G. Rakow, and R.K. Downey. 1996. Agronomic performance and seed quality of Ethiopian mustard in Saskatchewan. Can. J. Plant Sci. 76: 378-392.

Ghaderi, F.A., A. Soltani, and H. Sadeghipour, 2008. Cardinal temperature of germination in medicinal pumpkin, borago and black cumin. Asian J. Plant Sci. 7: 574-578.

Ghosh, R.K., and B.N. Chatterjee.1988. Effect of dates of sowing on oil content and fatty acid profiles of Indian mustard. Indian J. Oil Seed Res. 5: 144-149.

Goldemberg, J., S. Coelho, and P. Guardabassi. 2008. The sustainability of ethanol production from sugarcane. Energy Policy 36: 2086-2097.

Gómez-Campo, C., and S. Prakash. 1999. Origin and domestication. In: C. Gómez-Campo, editor, Developments in plant genetics and breeding. Elsevier, Amsterdam. p. 33-58.

González-Garcia, S., J. Bacenetti, R. Murphy, and M. Fiala. 2012. Present and future environmental impact of poplar cultivation in Po valley (Italy) under different crop management systems. J. Clean. Prod. 26: 56-66.

Goodchild, N.A., and M.G. Walker. 1971. A method of measuring seed germination in physiological studies. Ann. Bot. 35: 615-621.

Gugel, R.K., G. Séguin-Swartz, and A. Petrie. 1990. Pathogenicity of three isolates of *Leptosphaeria maculans* on *Brassica* species and other crucifers. Can. J. Plant Pathol. 12: 75-82.

Gutterman, Y. 2000. Genotypic and phenotypic germination survival strategies of ecotypes and annual plant species in the Negev Desert of Israel. In: Black, M., Bradford, K.J., Vázquez-Ramos, J., (Eds.), Seed Biology: Advances and applications: proceedings of the sixth International Workshop on Seeds, Mérida, México, 1999. CABI Pub., Wallingford, Oxon., UK, New York, pp. 389.

Hacisalihoglu, G. 2008. Responses of three switchgrass (*Panicum virgatum* L.) cultivars to seed priming and differential aging conditions, Acta Agric. Scand., Section B-Soil and Plant Science, 58: 280-284.

Hall, A.E. 1992: Breeding for heat-tolerance. Plant Breed. Rev. 10: 129-168.

Hampton, J.G. 2000. Producing quality seed: The problem of seed vigour. Spec. Publ. Soc. N. Z. 12: 53-61.

Hampton, J.G., B. Boelt, M.P. Rolston, and T.G. Chastain. 2013. Effects of elevated CO2 and temperature on seed quality. J. Agr. Sci. 151: 154-162.

Hanson, J.D., and H.A. Johnson. 2005. Germination of switchgrass under different temperature and pH regimes. Seed Technol. J. 27: 203-210.

Hardegree, S.P. 2006. Predicting germination response to temperature. I. Cardinal-temperature models and subpopulation-specific regression. Ann. Bot. 97(6): 1115-1125.

Hardegree, S.P., T.A. Jones, F.B. Pierson, P.E. Clark, and G.N. Flerchinger. 2008. Dynamic variability in thermal-germination response of squirreltail (*Elymus elymoides* and *Elymus multisetus*). Environ. Exp. Bot. 62: 120-128.

Hegarty, T.W. 1973. Temperature sensitivity of germination in carrots: its frequency of occurrence and response to seed advancement. J. Hortic. Sci. 48: 43-50.

Hellstrom, A., and C. Kjellstrom. 1989. Plant stand geometry. In: Research on Rapeseed and Mustard. Proc. Indo-Swedish Sym., September 3-6, 1989.

Ho, D.P., H.H. Ngo, and W.Guo. 2014. A mini review on renewable sources for biofuel. Bioresour. Technol. 169: 742-749.

Hotchkiss, J.R., P.Revilla, and W.F Tracy. 1997. Cold tolerance among open- pollinated sweet corn cultivars. HortScience 32: 719-723.

Hou, F.F., and F.S. Thseng. 1992. Studies on the screening technique for pregermination flooding tolerance in soybean. Jpn. J. Crop Sci. 61: 447-453.

Hsu, F.H., C.J. Nelson, and A.G. Matches. 1985. Temperature effects on germination of perennial warm-season forage grasses. Crop Sci. 25: 215-220.

Hsu, F.H., C.J. Nelson, and W.S. Chow. 1984. A mathematical model to utilize the logistic function in germination and seedling growth. J. Exp. Bot. 35: 1629-1640.

Hund, A., Y. Fracheboud, A. Soldati, and P. Stamp. 2008. Cold tolerance of maize seedlings as determined by root morphology and photosynthetic traits. Eur. J. Agron. 28: 178-185.

Iannucci, A., N. Di Fonzo, and P. Martiniello. 2000. Temperature requirements for seed germination in four annual clovers grown under two irrigation treatments. Seed Sci. Technol. 28: 59-66.

Igartua, E., M.P. Gracia, and J.M. Lasa. 1995. Field responses of grain sorghum to a salinity gradient. Field Crops Res. 42: 15-25.

Impallomeni, G., A. Ballistreri., G.M. Carnemolla, S. Guglielmino, M. Nicolo, and M. G. Cambria. 2010. Synthesis and characterization of poly(3-hydroxyalkanoates) from *Brassica carinata* oil with high content of erucic acid and from very long chain fatty acids. Int. J. Biol. Macromol. 48 : 137-45.

ISTA (International Seed Testing Association). 1985. International rules for seed testing. Seed Sci. Technol. 13: 307-513.

Jagadish, S.V.K., E.M. Septiningsih, A. Kohli, M.J. Thomson , C. Ye, E. Redona, et al., 2012. Genetic advances in adapting rice to a rapidly changing climate. J. Agron. Crop Sci. 198: 360-373.

Janssen, J.G.M. 1973. A method of recording germination curves. Ann. Bot. 37: 705-708.

JinLing, M.1997. Pollen selection for cold resistance at flowering time in *Brassica napus*. Cruciferae Newslett. 19: 85-86.

Jones, J.W., G. Hoogenboom, C.H. Porter, K.J. Boote, W.D. Batchelor, L.A. Hunt, et al. 2003. The DSSAT cropping system model. Eur. J. Agron. 18(3-4): 235-265.

Jordan, G.L., and M.R. Haferkamp. 1989. Temperature responses and calculated heat units for germination of several range grasses and shrubs. J. Range Manage. 42: 41-45.

Jordan, W.R. 1983. Whole plant responses to water deficits-An overview. In: Taylor, H.M., W.R. Jordan and T.R. Sinclair (Eds.) Limitation to Efficient Water Use in Crop Production. Madison, Wisconsin, USA: A.S. A. 289-317.

Kakani, V.G., K.R. Reddy, S. Koti, T.P. Wallace, P.V. Prasad, V.R. Reddy, et al. 2005. Differences in *in vitro* pollen germination and pollen tube growth of cotton cultivars in response to high temperature. Ann. Bot. 96(1): 59-67.

Kakani, V.G., P.V.V. Prasad, P.Q. Craufurd, and T.R. Wheeler. 2002. Response of *in vitro* pollen germination and pollen tube growth of groundnut (*Arachis hypogaea* L.) genotypes to temperature. Plant Cell Environ. 25: 1651-1661.

Kaspar, T.E., and W.L. Bland. 1992. Soil temperature and root growth. Soil Sci. 154: 290-299.

Kebreab, E., and A.J. Murdoch. 2000. The effect of water stress on the temperature range for germination of *Orobanches aegyptiaca* seeds. Seed Sci. Res. 10: 127-133.

Khan, M.A., D.C. Gemenet, and A. Villordon. 2016. Root system architecture and abiotic stress tolerance: current knowledge in root and tuber crops. Front. Plant Sci. 7: 1584. https://doi.org/10.3389/fpls.2016.01584 (Accessed 8 Oct. 2019).

King, J.J., and M.P. Bridgen. 1990. Environmental and genotypic regulation of *Alstroemeria* seed germination. HortScience 25: 1607-1609.

Kiniry, J.R., K.A. Cassida, M.A. Hussey, J.P. Muir, W.R. Ocumpaugh, J.C. Read, et al. 2005. Switchgrass simulation by the ALMANAC model at diverse sites in the southern US. Biomass Bioenerg. 29: 419-425.

Knoll, K., B. West, W. Clark, R. Graves, J. Orban, S. Przesmitzki, et al. 2009. Thesis: Effects of intermediate ethanol blends on legacy vehicles and small non-road engines. In: 2009 Sci.Tech. Connect. Rep. no. TP-540-43543. NREL, Golden, CO. p. 3-3.

Koti, S., K.R. Reddy, V.G. Kakani, D. Zhao, and V.R. Reddy. 2004. Soybean (*Glycine max*) pollen germination characteristics, flower and pollen morphology in response to enhanced Ultraviolet-B radiation. Ann. Bot. 94(6): 855-864.

Kotowski, F. 1926. Temperature relations to germination of vegetable seed. Proc. Am. Soc. for Hort. Sci. 23: 176-184.

Kumar, A., P. Singh, D.P. Singh, H. Singh, and H.C. Sharma. 1984. Differences in osmoregulation in *Brassica* species. Ann. Bot. 54: 537-541.

Larkindale, J., J.D. Hall, M.R. Knight, and E Vierling. 2005. Heat stress phenotypes of Arabidopsis mutants implicate multiple signaling pathways in the acquisition of thermotolerance. Plant Physiol. 138: 882-897.

Li, P.H., D.W. Davis, and Z.Y. Shen. 1991. High-temperature -acclimation potential of the common bean: Can it be used as a selection criterion for improving crop performance in high-temperature environments. Field Crops Res. 27: 241-256.

Lichtenthaler, H.K., G. Langsdorf, S. Lenk, and C. Buschamann. 2005. Chlorophyll fluorescence imaging of photosynthetic activity with the flesh lamp fluorescence imaging system. Photosynthetica 43: 355-369.Liu, H.L., J.Y. Yang, C.F. Drury, W.D. Reynolds, C.S. Tan, Y.L. Bai, et al. 2011. Using the DSSAT-CERES-Maize model to simulate crop yield and nitrogen cycling in fields under long-term continuous maize production. Nutr. Cycl. Agroecosys. 89: 313-328.

Lobell, D.B., and G.P. Asner. 2003. Climate and management contribution to recent trends in US agricultural yields. Science 299: 1032.

Luo, T., M. Xian, M.N. Khan, L. Hu and Z. Xu, 2018. Estimation of base temperature for germination of rapeseed (*Brassica napus*) using different models. Int. J. Agric. Biol. 20: 524-530.

Lynch, J., and J.J. VanBeem. 1993. Growth and architecture of seedling roots of common bean genotypes. Crop Sci. 33: 1253-1257.

Ma, Z., C.W. Wood, and D.I Bransby. 2001. Impact of row spacing, nitrogen rate, and time on carbon partitioning of switchgrass. Biomass Bioenerg. 20: 413-419.

Madakadze, I. B. Prithiviraj, K.A. Stewart, .PR. Peterson, B.E. Coulman, and D.L. Smith. 2001. Variation in base temperatures for germination in warm season grasses. Seed Sci. Technol. 29: 31-38.

Magarey, R.D., D.M. Borchert, and J.W. Schlegel. 2008. Global plant hardiness zones for phytosanitary risk analysis. Sci. Agric. 65: 54-59.

Malik, R.S. 1990. Prospects for *Brassica Carinata* as an Oilseed crop in India. Exp. Agric. 26: 125-129.

Marillia, E., T. Francis, K.C. Falk, M. Smith, and D.C. Taylor. 2014. Palliser´s promise: *Brassica carinata*, an emerging western Canadian crop for delivery of new bioindustrial oil feedstocks. Biocatal. Agric. Biotechnol. 3: 56-74.

Marois, J., D. Males, D. Wright, R. Bennett, and M. Lindenbaum. 2015. *Brassica carinata* as a winter cover crop in the southestern United States. In: 14th Int. Rapeseed Congr., Saskatoon, Canada p. 492.

Matile, P.1975. The lytic compartment of plant cells, cell biology monographs, Vol. 1. Springer, New York, NY.

McNair, J.N., A. Sunkara, and D. Frobish. 2012. How to analyse seed germination data using statistical time-to-event analysis: Non- parametric and semi-parametric methods. Seed Sci. Res. 22: 77-95.

Meehl, G.A., T.F. Stocker, W.D. Collins, P. Friedlingstein, A.T. Gaye., J.M. Gregory, et al. 2007. Global climate projections. In: S. Solomon, et al., editors, Climate Change 2007: The physical science basis. contribution of working Group I to the fourth assessment report of the intergovernmental panel on climate change [Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Meehl, T.G.A., and C. Tebaldi. 2004. More intense, more frequent, and longer lasting heat waves in the 21st century. Science 305: 994-997.

Mendham, N.J., and P.A. Salisbury. 1995. Physiology: Crop development, growth and yield. In: D. Kimber and D.I. McGregor, editors, *Brassica* oilseeds: Production and utilization. CAB International, Slough, UK. p. 11-64.

Meng, T.C., S. Somani, and P. Dhar. 2004. Modeling and simulation of biological systems with stochasticity. Silico Biol. 4(3): 293-309.

Meyer, S.E., and R.L. Pendleton. 2000. Genetic regulation of seed dormancy in *Purshia tridentata* (*Rosaceae*). Ann. Bot. 85: 521-529.

Miedema, P., 1982. The effect of low temperature on *Zea mays*. Advn. Agron. 35: 93-128.

Milbau, A., B.J. Graae, A. Shevtsova, and I Nijs. 2009. Effects of a warmer climate on seed germination in the subarctic. Ann. Bot. 104: 287-296.

Mittler, R. 2006. Abiotic stress, the field environment and stress combination. Trends Plant Sci. 11: 15-19.

Mnzava, N.A., and R.R, Schippers. 2007. *Brassica carinata* A. Braun. In: H.A.M. van der Vossen and G.S. Mkamilo, eds, Plant resources of tropical Africa 14: Vegetable oils. PROTA Foundation, Wageningen, Netherlands, Backhuys Publishers, Leiden, Netherlands. p. 42-46.

Mohammad, S., A. Haghnazari, F. Shekari, and A. Faramarzi. 2008. The study of seed yield and seed yield components of lentil (*Lens culinaris* Medik) under normal and drought conditions. Pak. J. Bio. Sci. 11: 758-762.

Monti, A., G. Bezzi, and G. Venturi. 2009. Internal conductance under different light conditions along the plant profile of Ethiopian mustard (*Brassica carinata* A. Brown.). J. Exp. Bot. 60: 2341-2350.

Mulvaney, M.J., R.G. Leon, R. Seepaul, D.L. Wright, and T.L. Hoffman. 2019. *Brassica carinata* seeding rate and row spacing effects on morphology, yield, and oil. Agron. J. 111: 528-535.

Murillo-Amador, B., E. Troyo-Dieguez, H.G. Jones, F. Ayala-Chairez, C. Tinoco-Ojanguren, and A. Lopez-Cortes. 2000. Screening and classification of cowpea genotypes for salt tolerance during germination. Phyton. 67: 71-84.

Mwale, S.S., S.N. Azam-Ali, J.A. Clark, R.G. Bradley and M.R. Chatha, 1994.Effect of temperature on the germination of sunflower (*Helianthus annus* L.). Seed Sci. Technol. 22: 565-571.

Nabloussi, A., A. Marquez-Lema, J.M. Fernandez-Martinez, and L. Velasco. 2008. Novel seed oil types of Ethiopian mustard with high levels of polyunsaturated fatty acids. Ind. Crop Prod. 27: 359-363.

National Alliance for Advanced Biofuels and Bio-products. 2014. Synopsis. https: //energy.gov/sites/prod/files/2014/07/f18/naabb_synopsis_report_0.pdf (Accessed 18 Oct. 2018).

Nelson, G.C., H. Valin, R.D. Sands, P. Havlík, H. Ahammad, D. Deryng, et al. 2014. Climate change effects on agriculture: economic responses to biophysical shocks. Proc. Natl. Acad. Sci. USA. 111: 3274-3279.

Newson, W.R, R. Kuktaite, M.S. Hedenqvist, M. Gällstedt, and E. Johansson 2013. Oilseed meal based plastics from plasticized, hot pressed *Crambe abyssinica* and *Brassica carinata* residuals. J. Am. Oil Chem. Soc. 90: 1229-1237.

Ozturk, H. 2014. Energy analysis for biodiesel production from rapeseed oil. Energ. Explor. Exploit. 32: 1005-1032.

Patane, C., and S. Tringali. 2011. Hydrotime analysis of Ethiopian mustard seed germination under different temperatures. J. Agron. Crop Sci. 197: 94-102.

Pathak, H., and R. Wassman. 2009. Quantitative evaluation of climatic variability and risks for wheat yield in India. Clim. Change 93: 157-175.

Paulsen, G.M. 1994. High temperature responses of crop plants. In: Boote, K.J., J.M. Bennett, T.R. Sinclair and G.M. Paulsen (eds). Physiology and determination of crop yield. ASA, Madison, pp 365-389.

Peet, M.M., S. Sato, and R.G. Gardner. 1998. Comparing heat stress effects on male-fertile and male-sterile tomatoes. Plant Cell Environ. 21: 225-231.

Perlack, R., L. Wright, A.F. Turhollow, R.L. Graham, B. Stokes, and D.C. Erbach. 2005. Biomass as feedstock for a bioenergy and bioproducts industry: The technical feasibility of a billion-ton annual supply. Oak Ridge National Laboratory, Oak Ridge, TN. p. 1-78.

Petkova, V., I. Denev, D. Cholakov, and I. Porjazov. 2007. Field screening for heat tolerant common bean cultivars (*Phaseolus vulgaris* L.) by measuring chlorophyll fluorescence induction parameters. Sci. Hortic. 111: 101-106.

Platenkamp, G.A.J., and R.G. Shaw. 1993. Environmental and genetic maternal effects on seed characters in *Nemophila menziesii*. Evolution 47: 540-555.

Porch, T.G. 2006. Application of stress indices for heat tolerance screening of common bean. J. Agron. Crop Sci. 192(5): 390-394.

Porter, J.R., 2005. Rising temperatures are likely to reduce crop yields. Nature 436: 174.

Potaczek, H., and E. Kozik. 2000. Low temperature seed germination of several tomato genotypes. Acta. Physiol. Plant. 22: 293-294.

Prakash, S., and K. Hinata. 1980. Taxonomy, cytogenetics and origin of crop Brassicas, a review. Opera. Bot. 55: 1-57.

Prakash, S., and V. Chopra. 1988. Introgression of resistance to shattering in *Brassica napus* from *Brassica juncea* through non-homologous recombination. Plant Breed. 101: 167-168.

Prasch, C.M., and U. Sonnewald. 2015. Signaling events in plants: Stress factors in combination change the picture. Environ. Exp. Bot. 114: 4-14.

Qin, S.J., Y.Z. Sun, and X.C. Meng, 2010. Production and analysis of biodiesel from non-edible seed oil of *Pistacia Chinensis*. Energ. Explor. Exploit. 28: 37-46.

Rakow, G. 2004. Species origin and economic importance of *Brassica*. In: G. Rakow, E.C. Pua, and C.J. Douglas, eds, biotechnology in agriculture and forestry, *Brassica*. Springer-Verlag, Berlin Heidelberg. 54: 3-12.

Rakow, G., and A. Getinet. 1998. *Brassica carinata* an oilseed crop for Canada. Acta Hortic. 459: 419-428.

Ramin, A.A. 1997. The influence of temperature on germination Taree irani (*Allium ampeloprasum* L.). Seed Sci. Thecnol. 25: 419-426.

Rao, T.R. 1992. Growth and development of mustard and pearlmillet in relation to light interception and photosynthesis and modeling for *Brassica* species. Ph.D. thesis, IARI, New Delhi.

Rasmussen, S., P. Barah, M.C. Suarez-Rodriguez, S. Bressendorff, P. Friis, P. Costantino, A.M. Bones, et al. 2013. Transcriptome responses to combinations of stresses in *Arabidopsis*. Plant Physiol. 161: 1783-1794.

Reddy, K.R., and H.F. Hodges. 2000. Climate change and global crop productivity. CAB International, Oxon.

Reddy, K.R., and V.G. Kakani. 2007. Screening *Capsicum* species of different origins for high temperature tolerance by in vitro pollen germination and pollen tube length. Sci. Hortic. 112: 130-135.

Reddy, K.R., D. Brand, C. Wijewardana, and W. Gao, 2017. Temperature effects on cotton seedling emergence, growth, and development. Agron. J. 109: 1379-1387.

Reddy, K.R., H.F. Hodges, and J.M. McKinion, 1997: A comparison of scenarios for the effect of global climate change on cotton growth and yield. Aust. J. Plant Physiol. 24: 707-713.

Reddy, K.R., H.F. Hodges, and J.M. McKinion. 1995. Carbon dioxide and temperature effects on Pima cotton development. Agron. J. 87: 820-826.

Reddy, K.R., H.F. Hodges, and V.R. Reddy, 1992: Temperature effects on cotton fruit retention. Agron. J. 84: 26-30.

Reddy, K.R., H.F. Hodges, J.J. Read, J.M. McKinion, J.T. Baker, L. Tarpley, et al. 2001. Soil-Plant-Atmosphere-Research (SPAR) facility: A tool for plant research and modeling. Biotronics 30: 27-50.

Reddy, K.R., V.G. Kakani, and H.F. Hodges 2008. Exploring the use of the environmental productivity index concept for crop production and modeling. In: Ahuja, L.R., V.R. Reddy, S.A. Saseendran, Q. Yu, editors, Response of crops to limited water: Understanding and modeling water stress effects on plant growth processes, Adv. Agric. Syst. Model. 1. ASA, CSSA, SSSA, Madison, WI. p. 387-410.

Resonance Carinata. 2015. Production Manual: A guide to best management practices. 2015. Agrisoma Biosciences Inc., Saskatoon, SK, CA.

Revilla, P., J.R. Hotchkiss, and W.F. Tracy. 2003. Cold tolerance evaluation in a diallel among open-pollinated sweet corn cultivars. HortScience 38: 88-91.

Ripley, V.L, K.C. Falk, and V. Roslinsky. 2006. Genetic diversity of *Brassica carinata* populations selected for improved seed quality. Acta Hort. 706: 135-140.

Rivero, R.M., T.C. Mestre, R. Mittler, F. Rubio, F. Garcia-Sanchez, and V. Martinez. 2014. The combined effect of salinity and heat reveals a specific physiological, biochemical and molecular response in tomato plants. Plant Cell Environ. 37: 1059-1073.

Roche, C., B. Shafii, D.C. Thill, and W.J. Price. 1996. Estimation of cardinal temperatures in germination data analysis. Appl. Stat. Agric. G.A. Milliken (Ed.). Kansas State University, Manhattan, Kansas, pp. 33-46.

Rosenthal, E.J., J.A. Clapper, G.A. Perry, and D.W. Brake. 2017. Effects of solvent or mechanically extracted *Brassica carinata* meal on performance of cows. J. Anim. Sci. 95: 311.

Sage, R.F, and T.D. Sharkey. 1987. The effect of temperature on the occurrence of O_2 and CO_2 insensitive photosynthesis in field-grown plants. Plant Physiol. 84(3): 658-664.

Saha, G., and S.A. Khan. 2008. Predicting yield and yield attributes of yellow sarson with agrometeorological parameters. J. Agromet., (Special issue-Part 1): 115-119.

Salem, M.A., V.G. Kakani, S. Koti, and K.R. Reddy. 2007. Pollen based screening of soybean genotypes for high temperatures. Crop Sci. 47: 219-231.

Salmerón, M., E.E. Gbur, F.M. Bourland, L. Earnest, B.R. Golden, and L.C. Purcell. 2015. Soybean maturity group choices for maximizing radiation interception across planting dates in the Midsouth United States. Agron. J. 107: 2132-2142.

Salmerón, M.E., E.E. Gbur, F.M. Bourland, N.W. Buehring, L. Earnest, F.B. Fritschi, et al. 2014. Soybean maturity group choices for early and late plantings in the US Midsouth. Agron. J. 106: 1893-1901.

Sanders, P.L., and A.H. Markhart. 2001. Root system functions during chilling temperatures: injury and acclimation. In: Basra, A.S. (Ed). Crop responses and adaptation to temperature stress. The Haworth Press, Inc. Binghampton, New York, USA pp 77-90.

Schimpf, D.J., S.D. Flint, and I.G. Palmblad. 1977. Representation of germination curves with the logistic function. Ann. Bot. 41: 1357-1360.

Schneider, M.P. 2006. Plant-oil-based lubricants and hydraulic fluids. J. Sci. Food Agric. 86: 1769-1780.

Schnepf, R., and B. Yacobucci. 2010. Selected issues related to an expansion of the renewable fuel standard (RFS) 141-164.

Schnepf, R., and B.D. Yacobucci. 2012. Renewable Fuel Standard (RFS): Overview and issues.27. Washington, DC: Congressional Res. Ser.Rep.

Schnepf, R., and B.D. Yacobucci. 2013. Renewable Fuel Standard (RFS): Overview and Issues. Washington, DC: Congressional Res. Ser.Rep. R40155.

Schreiner, M., B. Beyene, A. Rumbein, and H. Stutzel. 2009. Ontogenetic changes of 2-propenyl and 3-indolylmethyl glucosinolates in *Brassica carinata* leaves as affected by water supply. J. Agric. Food Chem. 57: 7259-7263.

Seepaul, R., B. Macoon, and K.R. Reddy. 2012. Ecotypic differences in switchgrass seed germination responses to *in vitro* osmotic stress. Seed Technol. 34: 173-182.

Seepaul, R., B. Macoon, K.R. Reddy, and B. Baldwin. 2011. Switchgrass (*Panicum virgatum* L.) intraspecific variation and thermotolerance classification using in vitro seed germination assay. Am. J. Plant Sci. 2: 134-147.

Seepaul, R., C.M. Bliss, D.L. Wright, J.J. Marois, R. Leon, N. Dufault, et al. 2015. Carinata, the jet fuel cover crop: 2015 production manual for the southeastern United States. The University of Florida/ Institute of Food and Agricultural Sciences (UF/IFAS) and North Florida Research and Education Center (NFREC), Quincy. SS-AGR-384.

Seepaul, R., I.M. Small, M.J. Mulvaney, S. George, R.G. Leon, S.V. Paula-Moraes, et al. 2019. Carinata, the sustainable crop for a bio-based economy: 2018-2019 Production Recommendations for the Southeastern United States. University of Florida/ IFAS Extension, Agronomy Department. SS-AGR-384. http: //edis.ifas.ufl.edu/pdffiles/AG/AG38900.pdf (Accessed 30 April 2019).

Seepaul, R., S. George, and D.L. Wright. 2016. Comparative response of *Brassica carinata* and *B. napus* vegetative growth, development and photosynthesis to nitrogen nutrition. Ind. Crops Prod. 94: 872-883.

Selvakumar, M.J., and S.J. Alexis. 2016. Renewable fuel production technologies. MEJSR 24: 2502-2509.

Setimela, P.S., D.J. Andrews, J. Partridge, and K.M. Eskridge. 2005. Screening sorghum seedlings for heat tolerance using a laboratory method. Eur. J. Agron. 23: 103-107.

Shafii, B., and W.J. Price. 2001. Estimation of cardinal temperatures in germination data analysis. J. Agric. Bio. Environ. Statis. 6(3): 356-366.

Shaftii, B., W.I. Price, I.B. Swensen, and G.A. Murray. 1991. Nonlinear estimation of growth curve models for germination data analysis. Proc. 1991 Kansas State Uni. Con. on Appl. Stat. Agric. pp 19-36.

Shamsul H., M. Asim, Y. Mohammad, F. Quazi, and A. Aqil. 2009. Growth of Indian mustard (*Brassica juncea* L.) in response to salicyclic acid under high-temperature stress. Brazilian J. Plant Physiol. 21(3): 187-195.

Shelef, O., T. Gendle, Y. Gutterman, and S. Rachmilevitch. 2016. Low water availability and salinity effects on seedling viability of *Brassica indica* compared to *B. iranica* and *B. prostrata* (Amaranthaceae). Seed Sci. Res. 26: 77-83.

Shinohara T., J.G. Hampton, and M.J. Hill. 2006. Location of deterioration within garden pea (*Pisum sativum*) cotyledons is associated with the timing of exposure to high temperature. N. Z. J. Crop Hort. 34: 299-309.

Shivpuri, A., H.P. Chhipa, R.B.L. Gupta, and K.N. Sharma. 1997. Field evaluation of mustard genotypes against white rust, powdery mildew and stem rot. Ann. Arid Zone. 36: 387-389.

Singh, A., S. Gangopadhyay, P. Nanda, S. Bhattacharya, C. Sharma, and C. Bhan. 2008. Trends of greenhouse gas emissions from the road transport sector in India. Sci. Total Environ. 390: 124-31.

Singh, B., K.R. Reddy, E.D. Redoña, and T. Walker. 2017a. Developing a screening tool for osmotic stress tolerance classification of rice cultivars based on *in vitro* seed germination. Crop Sci. 57: 387-394.

Singh, B., K.R. Reddy, E.D. Redoña, and T. Walker. 2017b. Screening of rice cultivars for morpho-physiological responses to early-season soil moisture stress. Rice Sci.. 24: 322-335.

Singh, B., S.K. Singh, S.K. Matcha, V.G. Kakani, C. Wijewardana, D. Chastain, et al. 2019. Parental environmental effects on seed quality and germination response to temperature of *Andropogon gerardii*. Agronomy. 9*:* 304.

Singh, C., P. Singh, and R. Singh. 2007. Modern techniques of raising field crops. 2nd ed. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, India.

Singh, D. 2003. Genetic improvement in Ethiopian mustard (*Brassica carinata* A. Brun) vis a vis Indian mustard (*Brassica juncea* L. Czern and Coss.) p 513. In: Proc. 11th Int. Rapeseed Conf. 4-7 July 2003, Copenhagen, Denmark.

Singh, D., V. Rao, S. Singh, and R. Singh. 2002. Testing the performance and application of BRASSICA model.

Singh, K., C. Wijewardana, B. Gajanayake, S. Lokhande, T. Wallace, D. Jones, et al. 2018. Genotypic variability among cotton cultivars for heat and drought tolerance using reproductive and physiological traits Euphytica. 214: 57.

Singh, R., P.V.V. Prasad, K. Sunita, S.N. Giri, and K. Reddy. 2007. Influence of high temperature and breeding for heat tolerance in cotton: A Review. Adv. Agron. 93: 313-385.

Singh, R., V. Rao, and D. Singh. 2001. Validation of BRASSICA model for Brassica juncea at Hisar. Indian J. Agr. Sci. 71: 94-107.

Singh, S.K., V.G. Kakani, D. Brand, B. Baldwin, and K.R. Reddy. 2008. Assessment of cold and heat tolerance of winter-grown canola (*Brassica napus* L.) cultivars by pollen-based parameters. J. Agron. Crop Sci. 194: 225-236.

Snapp, S., R. Koide, and J. Lynch. 1995. Exploitation of localized phosphorus patches by common bean roots. *Plant and Soil* 177: 211-218.

Soltani, A., M.J. Robertson, and B. Torabi. 2006. Modeling seedling emergence in chickpea as influenced by temperature and sowing depth. Agric. For. Meteorol. 138(1-4): 156-167.

Spears, J.F., D.M. TeKrony, and D.B. Egli. 1997. Temperature during seed filling and soybean seed germination and vigour. Seed Sci. Technol. 25: 233-244.

Srinivasan, A., H. Takeda, and T. Senboku. 1996. Heat tolerance in food legumes as evaluated by cell membrane thermostability and chlorophyll fluorescence techniques. Euphytica 88(1)35-45.

Tacarindua, C.R.P., T. Shiraiwa, K. Homma, E. Kumagai, and R. Sameshima. 2013. The effects of increased temperature on crop growth and yield of soybean grown in a temperature gradient chamber. Field Crops Res. 154: 74-81.

Tan, K.T., K.T. Lee, and A.R. Mohamed. 2008. Role of energy policy in renewable energy accomplishment: The case of second-generation bioethanol. Energy Pol. 36: 3360-5.

Timsina, J., and E. Humphreys. 2006. Application of CERES Rice and CERES-Wheat in research, policy and climate change studies in Asia: A review. Int. J. Agri. Res. 1: 202-225.

Tipton, J.L. 1984. Evauation of three growth curve models for germination data analysis. J. Am. Soc. Hortic. Sci. 109: 451-454.

Tiryaki, I., and D.J. Andrews. 2001. Germination and seedling cold tolerance in sorghum: 1. Evaluation of rapid screening methods. Agron. J. 93: 1386-1391.

Torabi, B., E. Soltani, S.V. Archontoulis, and A. Rabii. 2016. Temperature and water potential effects on *Carthamus tinctorius* L. seed germination: measurements and modeling using hydrothermal and multiplicative approaches. Br. J. Bot. 39: 427-436.

Torssell, B.W.R., and A. Kornher. 1983. Validation of a yield prediction model for temporary grasslands. Swedish J. Agric. Res. 13: 125-135.

USDA. 2013. Agriculture, Navy secretaries promote U.S. military energy independence with farm-to-fleet. Agriculture, Navy Secretaries Promote U.S. Military Energy Independence with Farm-to-Fleet. USDA. (Accessed 20 March 2019).

Vandendriessche, H.J., and M.K. van Ittersum. 1995. Crop models and decision support systems for yield forecasting and management of the sugar beet crop. Eur. J. Agron. 4: 269-279.

Wang, C., H. Xiao, L. Zhao, J. Liu, L. Wang, F. Zhang, et al., 2016. The allelopathic effects of invasive plant *Solidago canadensis* on seed germination and growth of *Lactuca sativa* enhanced by different types of acid deposition. Ecotoxicology. 25(3): 555-62.

Warwick, S.I. 2011. Brassicaceae in agriculture. In: I. Bancroft and R. Schmidt, eds, Genetics and genomics of the *Brassicaceae*. Springer, New York. p. 33-67.

Warwick, S.I., R.K. Gugel, T. McDonald, and K.C. Falk. 2006. Genetic variation and agronomic potential of Ethiopian mustard (*Brassica carinata* A. Braun) in western Canada. Genet. Res. Crop Evol. 53: 297-312.

Whisler, F.D., B. Acock, D.N. Baker, R.E. Fye, H.F. Hodges, J.R. Lambert, et al. .1986. Crop simulation models in agronomic system. Advances Agron. 40: 141-208.

Wijewardana, C., K.R. Reddy, M.W. Shankle, S. Meyers, and W. Gao. 2018. Low and high temperature effects on sweetpotato storage root initiation and early transplant establishment. Sci. Hort. 240: 38-48.

Wijewardana, C., M. Hock, B. Henry, and K. Reddy. 2015. Screening corn hybrids for cold tolerance using morphological traits for early-season seeding. Crop Sci. 55: 851-867.

Wijewardana, C., M. Hock, W.B. Henry, and K.R. Reddy. 2015. Screening corn hybrids for cold tolerance using morphological traits for early season seeding. Crop Sci. 55: 851-867.

Wijewardana, C., W.B. Henry, and K.R. Reddy. 2017. Evaluation of drought tolerant maize germplasm to induced drought stress. J. Miss. Acad. Sci. 62: 316-329.

Wijewardana, C., W.B. Henry, M. Hock, and K.R. Reddy. 2016a. Growth and physiological trait variation among corn (*Zea mays* L.) hybrids for cold tolerance. Can. J. Plant Sci. 96: 639-656.

Wijewardana, C., W.B. Henry, W. Gao, and K.R. Reddy. 2016b. Interactive effects on CO_2, drought, and ultraviolet-B radiation on maize growth and development. J. Photochem. Photobiol. 160: 198-209.

Wilkes, M.A., I. Takei, R.A. Caldwell, and R.M. Trethowan. 2013. The effect of genotype and environment on biodiesel quality prepared from Indian mustard (*Brassica juncea*) grown in Australia. Ind. Crops Prod. 48: 124-132.

Witcombe, J.R., and W.J. Whittington. 1971. A study of the genotype by environment interaction shown by germinating seeds of *B. napus*. Heredity 26: 397-411.

Yu, J., M.R. Tuinstra, M.M. Claassen, W.B. Gordon, and M.D. Witt. 2004. Analysis of cold tolerance in sorghum under controlled environment conditions. Field Crops Res. 85: 21-30.

Zanetti, F., A. Monti, and M.T. Berti. 2013. Challenges and opportunities for new industrial oilseed crops in EU-27: A review. Ind. Crops Prod. 50: 580-595.

Zhao, F.Y., W. Liu, and S.Y. Zhang. 2009. Different responses of plant growth and antioxidant system to the combination of cadmium and heat stress in transgenic and non-transgenic rice. J. of Integr. Plant Biol. 51: 942-950.

Zhao, X., L. Wei, S. Cheng, Y. Cao, J. Julson, and Z. Gu. 2015. Catalytic cracking of carinata oil for hydrocarbon biofuel over fresh and regenerated Zn/Na-ZSM-5. Appl. Catal. A: Gen. 507: 44-55.

Zhu, L., F. Krens, M.A. Smith, X. Li, W. Qi, E.N.van Loo, et al. 2016. Dedicated industrial oilseed crops as metabolic engineering platforms to sustainable industrial feedstock production. Sci. Rep. 6: 22181.