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Identifying Drought Stress Tolerance Characteristics in Brassica carinata Lines at Germination

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By

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ABSTRACT

Efforts to reduce greenhouse gas emissions from petroleum-based fuels has led to an increasing interest in the development and use of biofuels. The Departments of Defense, Energy, and Agriculture, as well as commercial airlines, are all in support of developing bio-jet fuels from nonfood source oilseed crops. *Brassica carinata* is a non-edible oilseed species grown as a replacement fuel for airliners that has the potential to be a successful winter crop in the southeastern United States. Because *B. carinata* has not been widely commercially grown, suitable varieties must be identified for southeastern production as a winter crop. Drought tolerance is an essential trait for suitable varieties to have, both because the crop would be planted at the driest time of the season and because the southeast has experienced increasingly unpredictable precipitation patterns that have contributed to increased drought. A series of germination tests were performed on twelve varieties of *B. carinata* to determine their drought tolerance. Polyethene Glycol was used to decrease osmotic potential and simulate drought. There were five total tests: control and four tests with osmotic potential decreasing by -0.1 each time. In each test, there were four replications of 100 seeds per variety that were incubated at 25 °C. After a 24-hour incubation period, seeds were monitored every two hours for 48 hours to track germination. Analysis of the results showed significant variance in the drought tolerance of the twelve tested varieties. Varieties AX17004 and AX17015 were found to perform best across all tested parameters, and the commercial variety, Avanza, was found to be one of the least tolerant to osmotic stress among varieties tested.

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INTRODUCTION

The Brassicaceae family (syn. Cruciferae) is highly diverse, containing many important agricultural species ranging from food vegetables (mustards, broccoli, and cabbage) to oilseed crops (rapeseed (canola)). One of the prominent oilseed species, *Brassica carinata* is grown predominantly as a biofuel crop – more specifically, as a replacement fuel for jet airliners. Commonly referred to as 'carinata', this species also has the potential to be a successful winter cover crop in the southeastern United States, both because it is naturally well-suited to the local winter climate and because it establishes rapidly and provides ground cover when other beneficial plant species are lacking.

Climate has always heavily influenced agricultural systems, but increasingly unpredictable or unusual weather patterns are taking their toll on farmers all over the world. Water-related stressors, particularly drought, are already a problem in many areas and promise to increasingly cause trouble. Drought stress not only poses a significant potential for crop damage during the growing season, but it can also impact germinating crop seeds, and prevent plants from developing robust root systems. In Mississippi, carinata is usually planted mid to late October, which is one of the driest parts of the year. If planting is delayed until rainfall increases, there is a risk of frost damage to young, tender plants. Because of this, tolerance to drought is an essential characteristic for varieties of carinata grown in Mississippi and other similar regions of the southeast.

Current research is being conducted by a multi-state research initiative into the viability of carinata as potential cash and cover crops in the southeastern United States. Germplasm resources for all research currently being conducted in the United States are derived from experimental lines owned by (Gatineau, Quebec, CA). These experimental lines represent the majority of all useable germplasm in carinata research. Determining the drought tolerance of these cultivars and other experimental varieties is an important step towards identifying which lines would be ideal for producers to utilize in the southeast. Physiological response to drought stress can be evaluated at multiple growth stages, but the seed germination stage is the most applicable for potential producers in the southeastern United States.

LITERATURE REVIEW

Brassica carinata

Origin

Commonly called Ethiopian mustard, Ethiopian rape, Abyssinian mustard, or – more succinctly – 'carinata', *Brassica carinata* (A.Braun) is native to the highland plateaus of Ethiopia and East Africa (CFIA, 2017). Carinata is believed to be a cross between *B. nigra* and *B. oleracea*, because both of those species grew in the same regions of Africa at the time carinata is thought to have originated (CFIA, 2017). *B. nigra* is maternal - and more closely related - parent (Stewart, 2002). Carinata has been cultivated since as early as 4000 B.C. (Mulvaney et al., 2019, CFIA, 2017). In addition to its native region, carinata has been grown in other parts of Africa, Southern Europe, India, and Australia (CFIA, 2017). Beginning in the 1980s, interest in growing carinata in other parts of Europe and in North America has continued to increase (CFIA, 2017).

Botanical Description

Carinata is an erect, herbaceous annual that grows 30 – 200 cm tall (Gasol et al., 2007, APHIS, 2014). It grows well in cool, moist areas like its native climate, the Ethiopian highland plateaus, but also thrives in semi-arid climates because of its natural resistance to abiotic stressors (CFIA, 2017, Agrisoma, 2017). Carinata has an upright, highly branched morphology, with leaves arranged alternately around the stem (APHIS, 2014). It has a determinate growth habit, which is a desirable agronomic trait, generally leading to uniformly tall plants, thus increasing harvestability (Zanettia et al., 2013). Carinata reproduces sexually and, similar to other *Brassica* species, has not shown potential for vegetative reproduction. Carinata is considered an 'often-crossed' species, meaning it can cross-pollinate as well as tolerate self-pollination. This is a common evolutionary characteristic in other members of the Brassicaceae family, due to the production of heavy, sticky pollen that is not well dispersed by wind (CFIA, 2017).

The Brassica genus has three identified genomes [A, B, and C (Figure 1)]. Carinata exists as an allotetraploid - with a BBCC genome (2n=4x=34) – having inherited the B genome from *B. nigra* and the C genome from *B. oleracea.* (Navabi et al., 2010; Stewart, 2002). In nature, however, it behaves as a diploid (APHIS, 2014). The B genome contains many agronomically desirable traits, such as heat, drought, and salinity tolerance (Navabi et al., 2010). Interspecific crosses between carinata and its parent species indicate that the B genome chromosomes may not form homeologous pairs with chromosomes from the A and C genomes, and observation showed that A-C pairings were more common than either A-B or B-C pairings (Navabi et al., 2010).

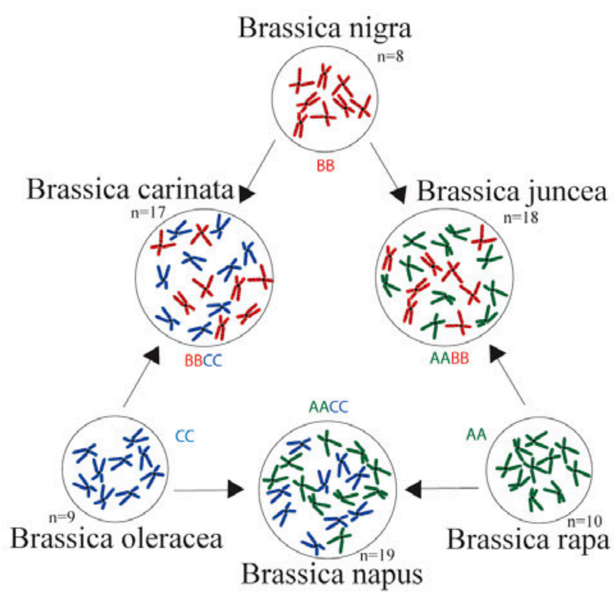

Figure 1. The "triangle of U" diagram. Genetic relationships among six species of the Brassica genus are represented with arrows illustrating cross-fertile pairings and their offspring. Chromosomes from the A (green), B (red) and C (blue) genomes are represented. Adapted from National Institute of Agricultural Botany (www.niab.com)

Growth and Development

The complete lifecycle for carinata is generally reported as 200 days, depending mainly on latitude, but also cultivar, row spacing, and environmental conditions (Seepaul et al., 2019b). Following germination, cotyledons emerge within 7-14 days after planting (DAP) depending on planting depth and environmental conditions. In the seedling stage, which generally lasts 14-21 days, plants develop 3-5 true leaves. This vegetative stage slowly develops into the rosette stage, lasting another 60-80 days, where plants produce several true leaves but do not generally reach heights over 35 cm. Approximately 90 DAP, bolting begins when plants quickly develop an elongated meristem with buds produced at the terminal. At 100 DAP, flowers appear and continue to appear as the meristem grows. Throughout the flowering stage, as flowers are pollinated and senesce, pods immediately begin to form. Pods are elongated, generally less than 5cm in length, with 10-16 seed in each pod (Agrisoma, 2017). Carinata produces small, orthodox seed - considered significant when compared to other Brassica species - with a 1,000 seed weight of approximately 4.2 g (Seepaul et al., 2019b). At maturity, the seed is fully formed and begins to turn from green to yellow. Pods turn yellow, then brown or purple, and become dry. The seed is ready to be harvested when it has under 10% moisture. The plant will dry out from the top down, but stems may still be green at harvest (Agrisoma, 2017).

Current uses

In Europe, *Carinata* is being grown in regions with a Mediterranean climate, such as Spain, Italy, and France (Gasol et al., 2007). In North America, it is being developed as a winter crop in the southeastern region.

Cover Crop

As a cover crop, Carinata provides benefits such as deeper recycling of soil nutrients, which improves soil quality and reduces nutrient leaching (Agrisoma, 2017; Seepaul et al., 2019b). Its extensive rooting system can also increase soil organic matter and improve soil structure (Seepaul et al., 2019b). Carinata can help reduce erosion, and, after harvest, the remnant biomass provides ground cover and ads to soil organic matter and carbon sequestering. It also ads diversity into the field, helping control weeds and diseases (Agrisoma, 2017). Carinata may also have some capacity to reduce nematode and fungal pathogens in soil (Seepaul et al., 2019b).

Biofuel

There has been a growing interest in the last decade to developing biofuels from non-food source oilseed crops (Gibbons, 2020). The Departments of Defense, Energy, and Agriculture have all provided funding and support to such initiatives. Commercial airlines are also interested in the "Farm to Fly" initiative (Gibbons, 2020).

Carinata seeds generally have an oil content of at least 40%, which is relatively higher than other Brassica oilseed species (Mulvaney et al., 2019; Seepaul et al., 2019b). About half of the oil is comprised of long-chain fatty acids with between 14 and 18 carbon backbones (C14-C18) in length. The other half is very-long-chain fatty acids that have 19 or more carbons (Mulvaney et al., 2019). About 36% of the total oil content is erucic acid, a C22:1 monounsaturated fatty acid that can be more easily converted to C10-C11 hydrocarbon chains than shorter fatty acids can be (Mulvaney et al., 2019; Zanettia et al., 2013; Gibbons, 2020).

The concentration of very-long-chain fatty acids allows carinata oil to be refined into high-energy biofuels using less energy than needed to improve similar *Brassica* species

(Mulvaney et al., 2019). Since vegetable oil has a high viscosity that cannot be used directly in engines, its viscosity is lowered through transesterification (Kasim et al., 2017). Transesterification exchanges the heavier glycerol components of the triglyceride molecules that make up vegetable oil for lighter components from alcohol-like ethanol or methanol (Cardone et al., 2003). This produces fatty acid ethyl or methyl esters made of saturated and unsaturated hydrocarbon chains (Cardone et al., 2003). This process requires a catalyst, which is usually alkaline (Kasim et al., 2017). *Carinata* oil can be converted to biodiesel using potassium hydroxide (Vicente et al., 2005). These carinata oil-derived products have similar chemical and physical properties and performance to petroleum-based products (Seepaul et al., 2019a). Fuel derived from carinata has many desirable traits for biofuel, including low-temperature tolerance, density, and viscosity (Gibbons, 2020).

Biofuels burn more cleanly than fossil fuels, resulting in lower emission of greenhouse gasses as well as decreased emission of harmful compounds like sulfur, a major pollutant. However, biodiesel only produces about 90% of the energy of petroleum diesel, and its chemical structure makes it less suitable for use in cold temperatures. Additionally, because each vegetable oil has a different structure, biofuels can have variances in physical qualities (Kasim et al., 2017).

Cultural Practices in Carinata Production

Because of its recent introduction as a potential cash/cover crop in the United States, research into carinata production standards has been relegated mostly to the southeastern United States (Figure 2) (Mulvaney et al., 2019). Like other Brassica species, carinata is a small-seeded crop, so it is crucial that seedbed conditions, planting date, seeding rate, seeding depth, and fertility are optimal for stand success. (Mulvaney et al., 2019).

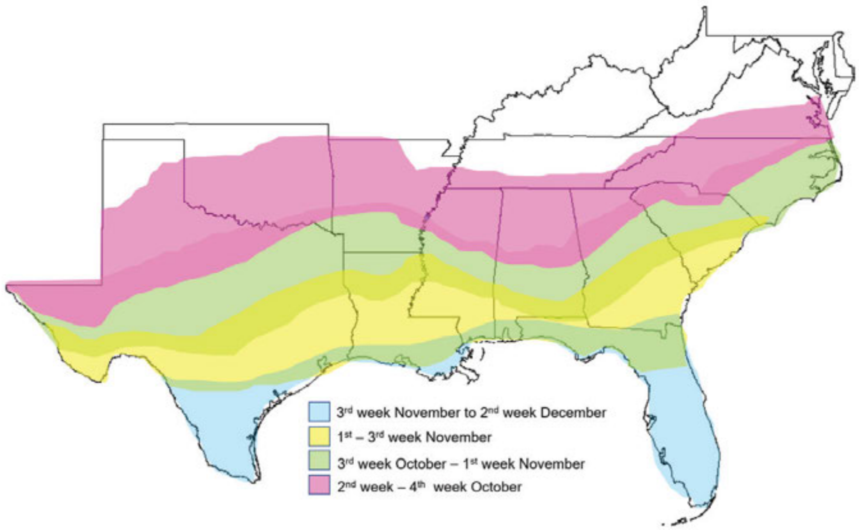

Figure 2. Recommended planting windows for Brassica carinata across the southeastern United States. Adapted from Agrisoma Production Manual, 2019. (www.agrisoma.com)

Seeding

In the southeastern United States, the optimal range for seeding is late September to mid-November, or about three to four weeks before the first frost, depending on location (Agrisoma, 2017; Seepaul et al., 2019b). Seeding depth should be about 1.3 cm, as deeper planting may delay emergence and result in a reduced stand. Depending on the cultivar, recommended seeding rates often range from 4.5 to 6.7 kg/ha (Agrisoma, 2017). Row spacing of 18 to 36 cm has been shown to maximize seed yield, with 36 cm rows being optimal (Seepaul et al., 2019b). Row spacing greater than 36 cm can decrease both seed and biomass yield as a result of low plant density and may also increase weed pressure due to lack of canopy closure (Seepaul et al., 2019b). Canopy closure and rapid, early growth are important for suppressing weeds in carinata because of limited herbicide options for the crop (Mulvaney et al., 2019).

Fertility

Fertility requirements for seed production vary based on field conditions, but typically carinata will remove 67-135 kg nitrogen, 56 kg phosphorus, and 90 kg potassium, and 1 kg boron per hectare per season. Carinata also uses a significant amount of sulfur, removing 17-34 kg per hectare per season. For most soil types, fertilizer is most efficiently utilized by plants when splitting into two applications. A 30:70 split is standard in carinata, with the first application occurring at planting or within a week of emergence, and supplying 30% of the total needed N and S, along with the full amount of P, K, and B. The remaining 70% of N and S should be applied at bolting. Boron can be used as a granular at planting or by a foliar application at bolting. Fertilizer management in coarser-textured (sand) soils may utilize a three-way (20:40:40) split application, applying 20% of N, S, and K, and the needed amount of P and B at planting. This is followed by 40% of N, S, and remaining K at bolting or about 30 days after the initial application. Then 40% N and S at flowering (Agrisoma, 2017).

Seed Germination

Current Standards

Germination is the process in which orthodox seeds are hydrated, begin respiration, and begin elongation of roots and embryonic axis (Bewley and Black, 1994). For optimum germination, seeds require adequate moisture, oxygen, and a favorable temperature (Ferguson et al., 1991). The optimum levels of those three things vary among species, and determining optimum conditions for germination of target species is critical before beginning agronomic production. Germination tests are standard in determining seed lot quality and seed viability in agronomic crops. Still, they are also often used in newly adopted crops to develop best management practices (BMPs) for growing plants *in situ* (Davies et al., 2015).

The most common form of germination test is the warm germination test, as described in the *Rules for Testing Seeds* handbook, published by the Association of Official Seed Certifying Agencies' (AOSCA, 2018) in which replicated sets of 100 seeds are placed on a moist piece of germination paper and placed in a controlled environment chamber. For a standard germination trial, the temperature is set at 25 °C and humidity levels are kept high, typically greater than 90%, to maintain the moist conditions needed for seed germination (Paulsen, 2008). When testing newly adopted crops, multiple combinations of temperature, moisture, and light levels are used to determine the optimum level of each variable necessary to maximize germination. During a germination test, seeds are periodically checked for germination, which is defined by the emergence of an embryonic axis – usually the root radicle – from the seed coat (Davies et al., 2015). Standard metrics used to determine BMPs for seed germination in a specific crop include maximum germination percentage (G_{max}) and time to 50% germination (G_{50}).

Polyethylene Glycol (PEG)

Polyethylene glycol (PEG) is a flexible, water-soluble, polyether compound used for a variety of industrial, scientific, and medical purposes. It is hydrophilic, highly biocompatible, and does not harm live cells, which makes it an ideal osmoticum for biological experimentation (Manoukian et al., 2019). It can be constituted at different molecular weights (polyethylene oxide, polyoxyethylene), which gives it a wide range of uses. At higher molecular weights, it cannot pass through cell walls, and so it is used to regulate water intake and simulate drought in germination tests (Hellala et al., 2018). Although there is no mention of the approved use of PEG in the Association of Official Seed Certifying Agencies (AOSCA) seed testing guidelines, multiple experiments using PEG to simulate drought stress during seed germination have been recognized in the literature. The successful use of PEG in simulating drought stress has been documented in grasses (Emmerich and Hardegree, 1990), lentil (Muscolo et al., 2014), sunflower (Luan et al., 2014), *B. juncea* (Toosi et al., 2014), and several oilseed species, including soybean (Dawadi et al., 2019), among others.

Drought

Drought in the Southeastern United States

The Southeast is characterized by relatively consistent year-round rainfall; however, there are frequent periods of drought as well. Mississippi has experienced some level of drought for over half of the years since monitoring by the United States Drought Map began in 2000. Over 50% of the state experienced drought for approximately 12% of the monitored time period (Runkle et al., 2017). As a result of climate change and warming trends, changes in rainfall patterns are also projected across the United States. Droughts may also become more intense and prolonged, leading to substantial impacts on agricultural production in the southeast (Runkle et al., 2017).

Historically, September and October have been the driest months for southeastern states (NOAA, 2020). In September 2019, the southeast United States experienced a flash drought, which is a short period characterized by high surface temperature and low soil moisture (Di Liberto, 2019; Kingtse and Lettenmaier, 2015). Drought can occur as a result of either prolonged high temperature, or acute precipitation deficits. The latter is more common in southern states (Kingtse and Lettenmaier, 2015). The 2019 flash drought followed a period of exceptionally high rainfall and high temperatures (Di Liberto, 2019). These kinds of droughts impact agriculture because the combination of high temperatures and low rainfall rapidly depletes field-level groundwater supplies. For fall-planted crops, this can be extremely problematic, as immature plants generally have not developed root systems adequate enough to access moisture deeper in the soil profile.

Physiological Effects of Drought in Brassica

The uptake of water is an essential step in seed germination. While drought can severely impact plant growth and development, it is one of the most detrimental abiotic factors impairing germination (Li et al., 2013; Hatzig et al., 2018). To ensure stand survival and productivity, the germination and early seedling stages are potentially the most critical times for producers to limit drought stress (Li et al., 2013).

B. napus, the second most important oilseed crop in the world following soybean, was shown to have a decrease in seed yield and quality as a result of drought (Hatzig et al., 2018). *B. juncea*, another important oilseed crop, has also showed decreased germination percentage, dry matter yield, and shoot and root length of seedlings as a result of drought stress. Additionally, seed collected from maternal plants that had been exposed to stress was found to have decreased quality compared to non-stressed controls; however, it was also found to have increased vigor. These qualities were not affected by the performance of the maternal plant (Hatzig et al., 2018). Another study looking at the effects of drought on five varieties of *B. napus* showed a decrease in germination percentage, germination rate, shoot length, and root length due to drought stress across varieties (Channaoui et al., 2017).

The effects of drought stress may be determined more by when in the growth cycle, the stress occurs than the intensity of the stress (Zirgoli and Kahrizi, 2015). Drought stress at the early stages of germination was found to decrease the ability to imbibe water and eventually led to metabolic changes that reduced the ability of the seed to access its energy reserve (Toosi et al., 2014). During the stem elongation and flowering stages are when the reproductive period is most likely to be affected by drought stress, ultimately leading to yield losses (Zirgoli and Kahrizi, 2015). At flowering, drought stress can lead to a decline in floral production and, eventually, decreased seed yield (Saeed et al., 2016). The oil content of *Brassica* species can be drastically affected by drought stress, with oil levels generally decreasing and the lipid content changing. In *B. napus*, drought stress decreased the linolenic acid content and increased glucosinolates. *B. juncea* and *B. rapa* also showed a decrease in productivity and oil content as a result of drought stress. Drought stress can also result in decreased seed pod production, reduced number of seed per pod, and decreased seed weight (Saeed et al., 2016).

Because of the potential for drought cycles in the fall throughout the southeastern United States, it is imperative that research is conducted into the effects of drought stress on the germination of *Brassica carinata*. Identifying drought-tolerant varieties is essential to establishing carinata as a successful winter crop in the southeast.

OBJECTIVES

Before *Brassica carinata* can be adopted as a potential cash crop in the southeastern United States, basic morphological responses to extreme environmental conditions must be investigated. Those investigations began by endeavoring to meet the following objective: quantify the effects of drought stress on *B. carinata* during the seed germination stage.

METHODS AND MATERIALS

Germination was studied in twelve varieties of *B. carinata* (Table 1). The twelve varieties were sourced from Agrisoma Biosciences, and all seed resources came from the parent company with a surface treatment of fungicide and rodenticide. Distilled water was used for the control, and solutions of distilled water and PEG 8000 with osmotic potentials of -0.1, -0.2, -0.3, and -0.4 MPa were used for the four trials. In each trial, four replications of 100 seeds of each variety were tested.

Entry	Genotype ID	Type	Description
1	**AX17001**	Inbred	Selection from SE16-17 AYT (Avanza family selection)
2	**AX17002**	Inbred	Selection from SE16-17 AYT (Avanza family selection)
4	**AX17004**	Inbred	High shatter tolerance family, good potential in the winter environment
5	**AX17005**	Inbred	High shatter tolerance family, good potential in a winter environment
6	**AX17006**	Inbred	High shatter tolerance family, good potential in a winter environment
7	**AX17007**	DH	Among the highest sclerotinia incidence, Jay and Quincy, FL 16-17
8	**AX17008**	DH	Selection from SE16-17 PYTB
9	**AX17009**	DH	Selection from SE16-17 PYTA
10	**AX17010**	DH	Selection from SE16-17 PYTB
14	**AX17014**	Hybrid	Top 2016-17 Quincy, FL test hybrid
15	**AX17015**	Hybrid	Promising test hybrid from 2017, frost tolerant female
16	**Avanza 641**	Released cultivar	Commercial cultivar

Table 1. Description of 12 Brassica carinata *genotypes in this study with a genotype identification number and production type. (All description information courtesy of Agrisoma Biosciences, Inc., Gatineau, Quebec, CA)*

The seeds were placed on paper toweling, which was placed on a tray that had been disinfected using a solution of sodium hypochlorite (bleach) and water. The paper toweling was then moistened with distilled water or the appropriate PEG solution. The trays were covered and placed in an incubator set at 25 °C. They were incubated for 24 hours. Beginning at the 24th hour, seeds were monitored for germination every two hours for the following 48-hour period. Seeds were considered germinated when the radicle was at least the length of the seed coat. At each two-hour interval, the number of germinated seeds was recorded.

Data were analyzed using Statistical Analysis Software (SAS Institute, Cary, NC) at $\alpha = 0.05$ level of significance. Dependent variables were maximum seed germination, time to 50 percent germination (T_{50}), and rate of germination. Main effects included cultivar (n=12), treatment (n=5) and replication (n=4).

RESULTS

Influence of Cultivar on Germination Parameters

Before the evaluation of PEG treatment effects, it was necessary to assess cultivar effects on the three dependent variables of interest: maximum percent germination (MPG), time to 50% germination (T_{50}), and rate of germination ($1/T_{50}$). There was a significant effect on MPG, T_{50}, and $1/ T_{50}$, due to cultivar ($P < 0.0001$). Replication had no marked impact on MPG ($P = 0.0753$), T_{50} ($P = 0.3480$), or $1/T_{50}$ ($P = 0.9073$).

Maximum Percent Germination (MPG)

Before exposure to drought stress, cultivar AX17009 had the highest MPG with 99.38%. Cultivars AX17004 and AX17015 had similarly high performances, which were not statistically significantly different than AX17009. Cultivars AX17002 and AX17006, as well as the commercially available cultivar Avanza, had the lowest MPG before treatment (Figure 3).

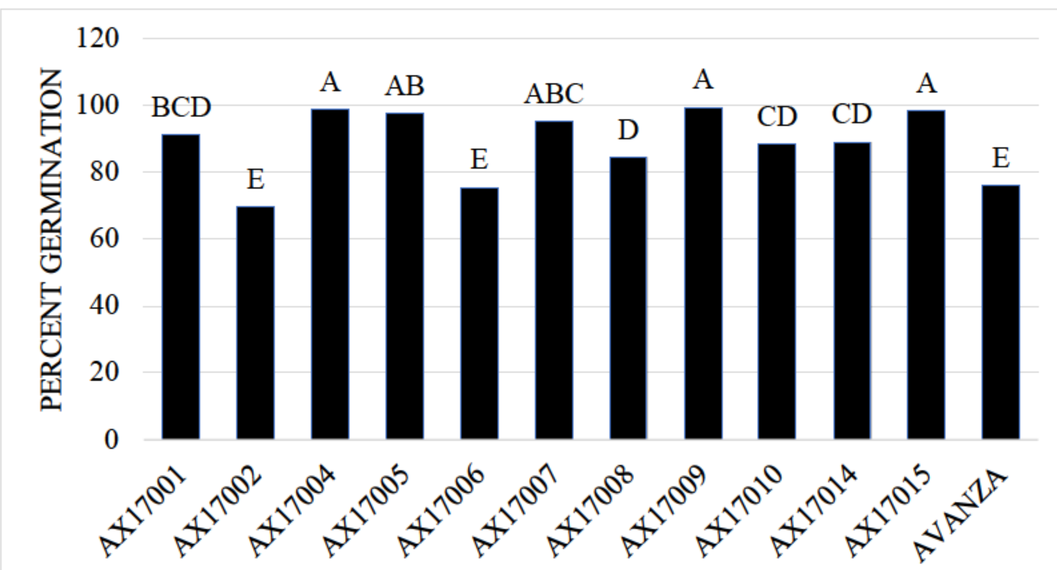

Figure 3. Influence of cultivar (genotype) on maximum seed germination of 12 Brassica carinata entries. Data are means of four replications.

Time to 50% Germination (T50)

Cultivar AX17009 had the shortest T_{50}a cross all cultivars pre-treatment. Cultivars AX17015, AX17005, AX17004, and AX17014 were not significantly different than AX17009. Cultivar AX17002 had the highest T_{50}, and Avanza had the second highest (Figure 4).

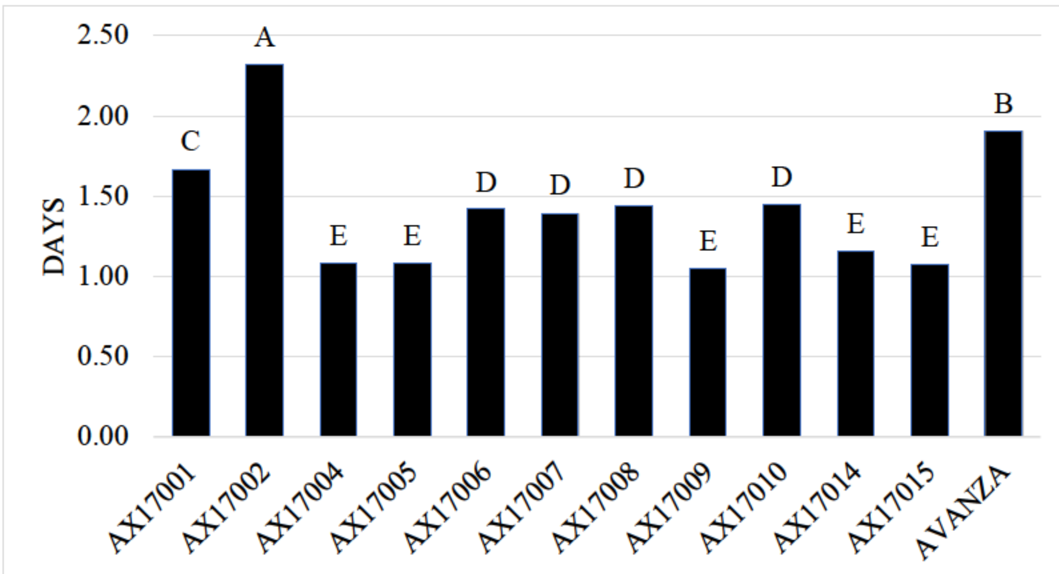

Figure 4. Influence of genotype on time to 50% seed germination of 12 Brassica carinata genotypes. Data are means of four replications.

Rate of Germination (1/ T_{50})

Cultivar AX17009 had the highest rate of germination before exposure to drought stress. Cultivars AX17004, AX17005, AX17014, and AX17015 had similarly high rates of germination that did not significantly differ from AX17009 or each other. Cultivar AX17002 had the lowest rate of germination, followed by cultivars AX17001 and Avanza (Figure 5).

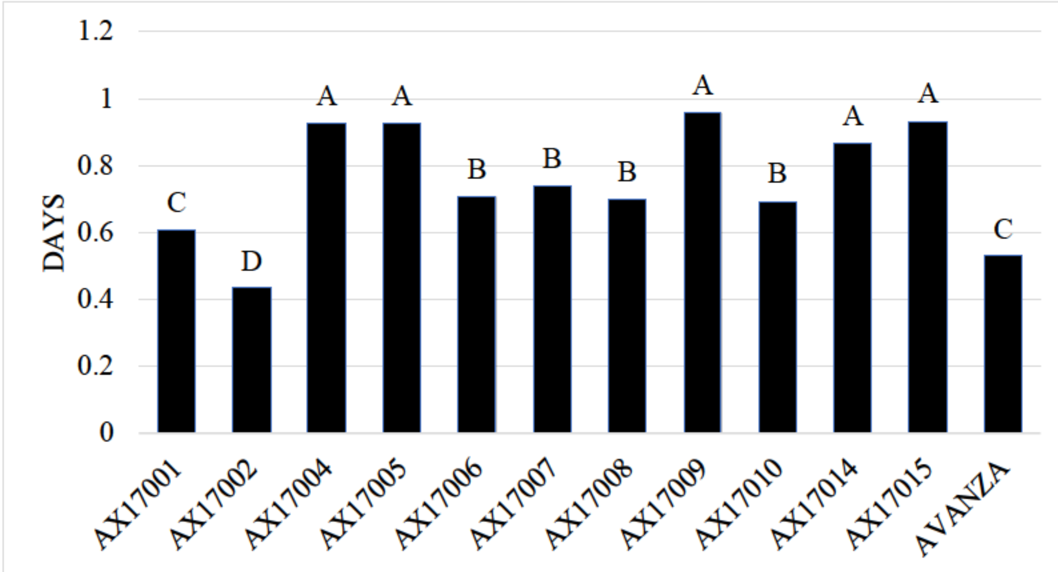

Figure 5. Influence of genotype on the rate of germination of 12 Brassica carinata genotypes. Data are means of four replications.

Statistical Analysis

Analysis of variance showed significant effects of cultivar on maximum seed germination, T_{50}, and rate of germination. There was also a genotype x treatment interaction for all three dependent variables (Table 2).

Source	Parameters		
	MSG (%)	T_{50} (d)	Germ Rate (d^{-1})
PEG Treatment (D)	***	***	***
Genotype (G)	***	***	***
D × G	***	***	***

*Table 2. Analysis of variance of 12 Brassica carinata genotypes, osmotic potential treatments, and their interaction for germination parameters: maximum seed germination (MSG), time to fifty-percent germination (T_{50}), germination rate (1/t50) estimated from the three-parameter sigmoid functions fitted to cumulative seed germination and incubation time, and seed weight (mg). *** Denotes significance level < 0.01*

Influence of Osmotic Potential on Germination Parameters

There was a significant effect on the maximum percent germination due to cultivar ($P < 0.0001$) and PEG treatment level ($P < 0.0001$)

Maximum Percent Germination (MPG)

The cultivar AX17004 exhibited the greatest MPG with 93.15% when compared to all other entries across all four trials. Cultivars AX17009 and AX17015 had similar performances, with MPG of 90.49% and 87.96%, respectively, which was not significantly different. Cultivars AX17002 and AX17005 had the lowest MPG. The commercially available cultivar Avanza also exhibited low maximum germination (Figure 6).

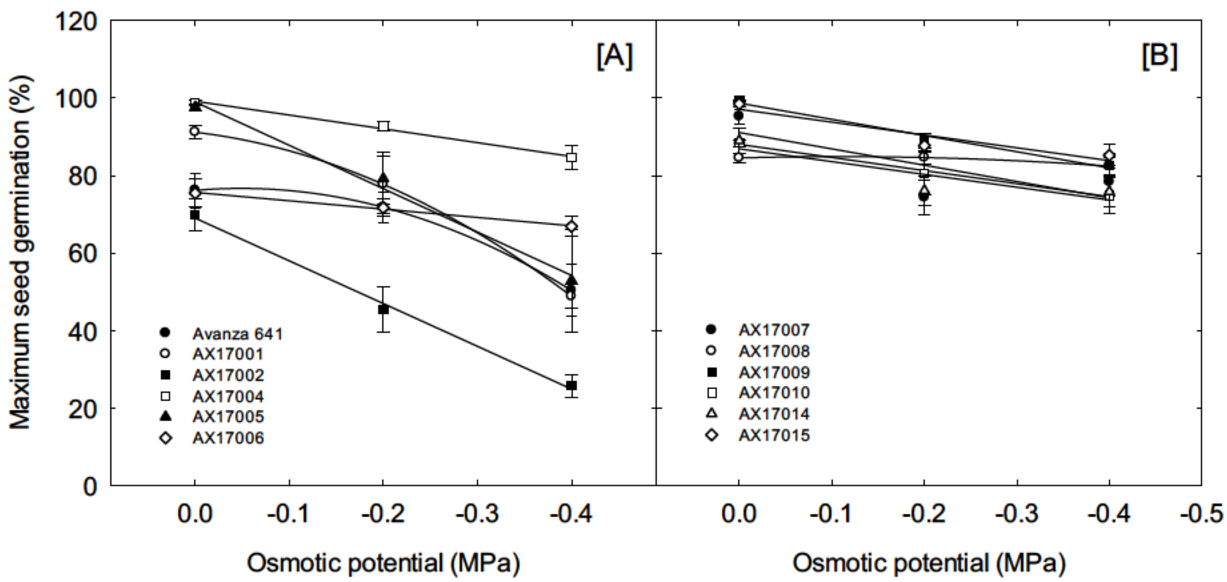

Figure 6. Influence of osmotic potential on maximum seed germination of 12 Brassica carinata genotypes. Data are means and ± SE of four replications.

Time to 50% Germination (T_{50})

Cultivar AX17004 had the shortest mean T_{50} across the four trials. Cultivar AX17015 had a slightly higher T_{50} but was not statistically different. Cultivar AX17002 had the highest mean T_{50}, followed by cultivars AX17001 and Avanza (Figure 7).

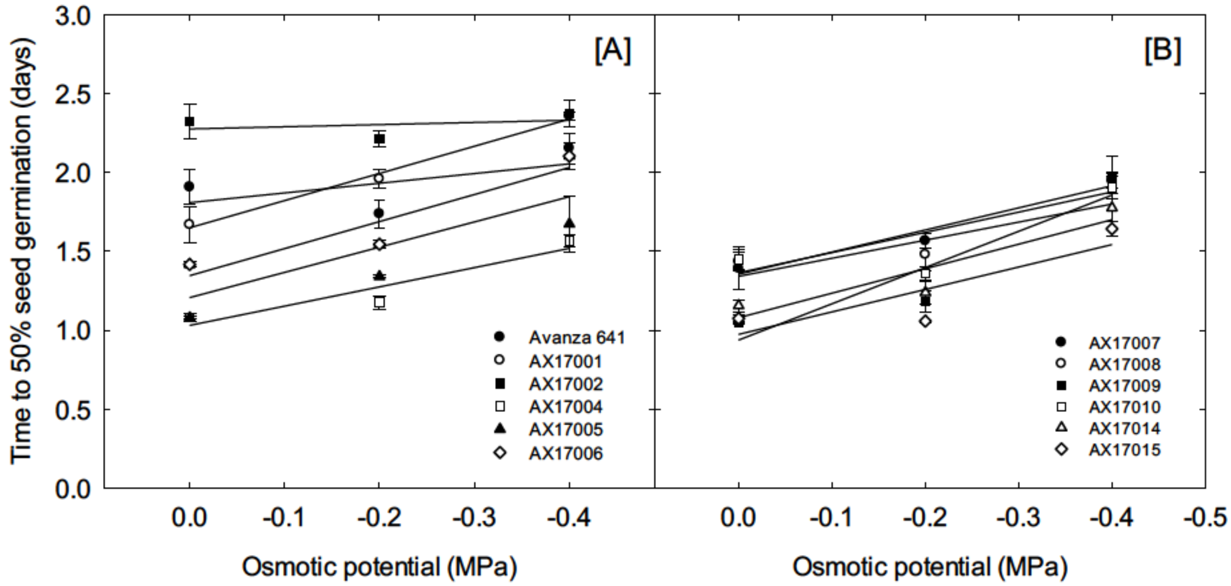

Figure 7. Influence of osmotic potential on time to 50% seed germination rate of 12 Brassica carinata genotypes. Data are means and ± SE of four replications.

Rate of Germination ($1/ T_{50}$)

Cultivar AX17004 had one of the greatest rates of germination. AVANZA had one of the lowest rates of germination, which was relatively unaffected by decreasing osmotic potential. AX17002 was also relatively unaffected by changes in osmotic potential but suffered from a slow rate of germination across all four trials. AX17009 seemed to have a severe response to decreasing osmotic potential resulting in a much steeper slope (Figure 8).

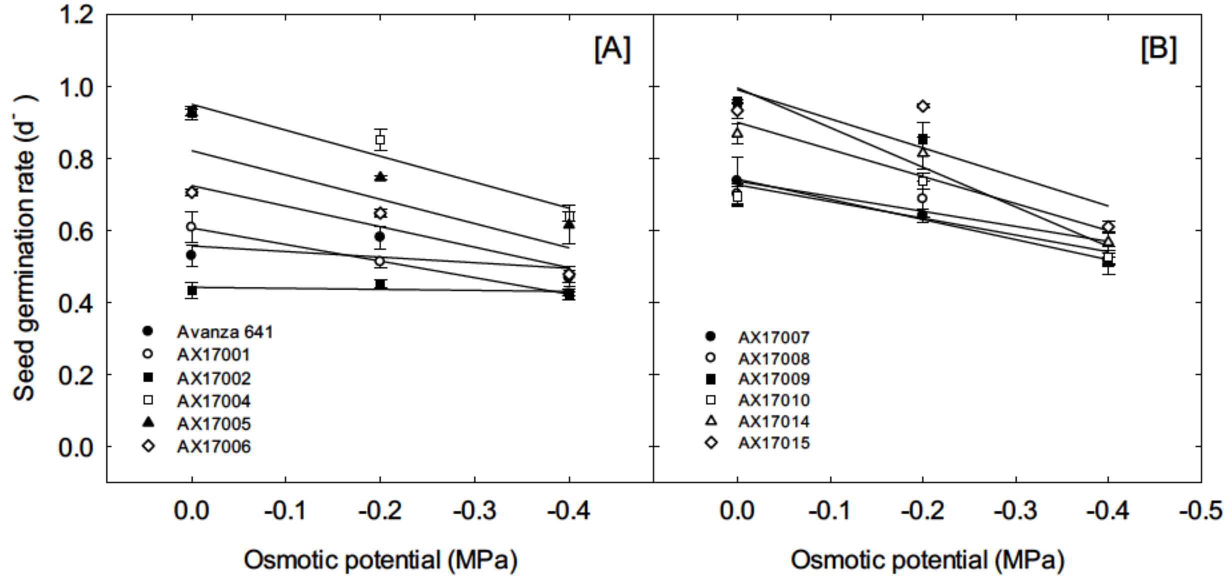

Figure 8. Influence of osmotic potential on the seed germination rate of 12 Brassica carinata genotypes along with fitted quadratic equations. Data are means and ± SE of four replications.

Summary

Cultivars AX17004 and AX17015 showed the statistically greatest rates of germination, the statistically shortest T_{50} values, and were among the highest-ranked cultivars in maximum germination. These two cultivars were consistently in the highest-ranking groups and should be considered for breeding germplasm in the development of drought-tolerant cultivars of carinata.

DISCUSSION AND CONCLUSION

This research aimed to identify varieties of *B. carinata* that germinate well under drought stress to develop varieties suitable for use as a winter crop in the southeast. Based on the analysis of germination under decreasing osmotic potential, there were two cultivars that consistently showed the most promising performance: AX17005 AND AX17015. It was expected for this study were that MPG and 1/ T_{50} would generally decrease and T_{50}would generally increase for all varieties as osmotic potential decreased, and that performance would vary among varieties. MPG, T_{50}, and 1/ T_{50} did all trend in the expected directions across the four trials, and performance did vary by variety to a statistically significant extent. An unexpected finding was the poor performance of the commercial variety, Avanza. That it was consistently one of the lowest-performing varieties for all three parameters means that the commercially available variety is falling far below this crop's potential for drought tolerance.

All three of the seed germination parameters measured in this study were affected by drought stress. The findings of previous studies of drought tolerance in Brassica species are in agreement with this result. A study performed a study on *B. napus* using PEG to simulate drought found that drought stress significantly affected germination percentage, germination rate index, and mean germination time (Channaoui et al., 2017). In a similar study, it was found that *B. juncea* var. Ensabi showed a decrease in percent, rate, and final germination as osmotic potential decreased (Toosi et al., 2014). One of the strengths of these studies, as well as other similar ones, is that they test germination at more levels of osmotic potential as well as lower levels. This gives more complete data on the behavior of the varieties being studied and allows for greater distinction between varieties if multiple varieties are being tested. Having only four trials and only testing to - 0.4 MPa was a limiting factor of this study. To further distinguish which varieties germinated best under drought stress, they ought to be tested at even lower levels of osmotic potential.

Germination trials are a first-step procedure in selecting and developing good agronomic varieties. By performing germination trials on the twelve tested varieties, strong performers in the area of drought stress tolerance could be identified for further research. Further research could either test further decreasing osmotic potential at the germination stage or could test growth under drought stress. Additionally, these varieties can be used in breeding commercial lines with all the necessary agronomic traits required for a successful southeastern winter crop. The best performing varieties from the trial, since they are better than the commercial variety, hold promise for use in developing new commercial varieties. As biofuel use continues to increase, so will the demand for biofuel crops. The sooner new and better performing commercial varieties of carinata can be developed, the sooner farmers in the southeast can start supplying that crop to the biofuel market.

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