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Article type : Research Review

Brassica carinata: Biology and Agronomy as a Biofuel Crop

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/GCBB.12804

Abstract

The environmental consequences of using non-renewable fossil fuels have motivated a global quest for sustainable alternatives from renewable sources. *Brassica carinata* A. Braun, has been developed as a low carbon intensity, non-food oilseed biomolecular platform to produce advanced drop-in renewable fuels, meal, and co-products. The crop is widely adaptable to grow in the humid subtropical and humid continental climatic regions of Asia, Africa, North America, South America, Europe, and Australia as a spring or winter crop. Carinata is heat tolerant, resistant to diseases and seed shattering with lower water-use requirements than other oilseed brassicas. Adopting carinata in double-cropping systems would require continuing research to integrate crop biology with agronomy, to understand growth and development and its interaction with agricultural inputs and management. Site-specific best management agronomic practices and crop improvement research to develop frost tolerant, early maturing, nutrient use efficient, and high yielding varieties with desirable oil content and fatty acid profile will enhance the crop's adaptability and economic viability. The exploitation of intra- and inter-specific and intra- and inter-generic diversity will further enhance carinata productivity and resistance to biotic and abiotic stresses. This review attempts to present a comprehensive description of carinata's biology, beginning with its origin and current state of distribution, availability of genetic and genomic resources, and a discussion of its morphology, phenology, and reproduction. A detailed analysis of the agronomy of the crop, including planting and germination and management practices, is presented in the context of crop growth and development. This will facilitate global adoption, sustainable production, and commercialization of carinata as a dedicated biofuel oilseed crop in diverse cropping systems and growing regions of the world, including the Southeast United States.

Keywords: biofuels, carinata, oilseed, renewable, germplasm resources, vegetative traits, photosynthesis, biomass

1. Introduction

Brassica carinata A. Braun, commonly referred to as "Ethiopian mustard," "Ethiopian rape," "Abyssinian mustard" or "carinata," is being developed as a low carbon intensity, non-food oilseed

feedstock to produce advanced drop-in renewable fuels, protein-rich meal, and bio-products. It exhibits a desirable oil profile, wide adaptability, and productivity under suboptimal conditions (Cardone et al., 2002; Blackshaw et al., 2011; Gesch et al., 2015). Production of carinata as a winter crop presents a unique opportunity for growers in the Southeast US to produce a significant amount of biofuel feedstock to contribute to domestic energy needs. Carinata fits into existing cropping systems as a winter crop, providing opportunities to farm over 1.4 million hectares of winter fallow land that could translate to over 1224 million liters of jet fuel, displacing 1.4 to 2.33% of petroleum-based jet fuel in the USA (Alam and Dwivedi, 2019).

Carinata seed has 18.7–28.3% protein and 42–52% oil content with a well-distributed fatty acid profile. Erucic acid (41–.43%) forms the primary fatty acid component, followed by linoleic, linolenic, and oleic acids (Kumar et al., 2020). After hexane extraction, the seed meal has 43.6% crude protein, 23.6% neutral detergent fiber, 13.2% acid detergent fiber, and 2.5% crude fats making carinata meal a high-value protein feed (Schulmeister et al., 2019). The species possesses agronomic traits allowing it to be grown either as a winter crop in the humid subtropical regions or as a spring-planted crop in humid continental climates. Carinata is heat tolerant, resistant to diseases and seed shattering, and has lower water-use requirements than other oilseed brassicas (Kumar et al., 1984; Malik, 1990; Shivpuri et al., 1997; Raman et al., 2017). Varieties (Mulvaney et al., 2018; Kumar et al., 2020) that are frost tolerant, early maturing, nutrient use efficient, high yielding with desirable oil content and fatty acid production are needed to integrate carinata into prevalent crop rotation system (Seepaul et al., 2018; Mulvaney et al., 2019; Kumar et al., 2020). Connecting biology with agronomy is critical for the sustainable cultivation of carinata in different production regions of the world.

2. Origin and Distribution

Carinata is a member of the family Brassicaceae (formerly known as Cruciferae); order Capparales; tribe Brassiceae; genus *Brassica*; and species *carinata* (Edwards *et al*., 2000). Although the name carinata was first given by A. Braun in 1841, it is known to have various scientific synonyms like *Brassica intergrifolia* var. Carinata (West) Rupr (1860), *Melanosinapis abyssinica* Hort. ex Regel, and *Sinapis abyssinica* A. Braun (1856) (Edwards *et al*., 2000). Carinata, an allotetraploid (BBCC-genome, 2n = 4x = 34, genome size ~ 1300 Mb) originated through spontaneous interspecific hybridization between wild *B. nigra* (BB-genome, 2n = 2x = 16, genome size ~ 630 Mb) and

cultivated *B. oleracea* (CC-genome, 2n = 2x = 18, genome size ~ 700 Mb) in Northeastern Africa, probably in the Ethiopian plateau and the Mediterranean coast (Hemingway, 1995; Gomez-Campo, 1999; Warwick et al., 2006). The presence of these progenitor species in the region during the emergence and domestication of carinata supports this hypothesis (Alemayehu ad Becker 2002). The origin of carinata and its relationship with diploids *B. rapa* (AA-genome, 2n = 2x = 20, genome size ~ 550 Mb), *B. nigra*, *B. oleracea* and allotetraploids *B. juncea* (AABB-genome, 2n = 4x = 36, genome size ~ 1100 Mb) and *B. napus* (AACC-genome, 2n = 4x = 38, genome size ~ 1130 Mb) species has been explained in Triangle of U (Morinaga, 1934; Nagaharu, 1935; Figure 1). Restricted Fragment Length Polymorphism (RFLP) analysis of chloroplast DNA (Palmer et al., 1983) revealed that carinata has the cytoplasm of *B. nigra*.

Cultivation of carinata is believed to have started in the 4th to 5th millennia BC in Northeastern Africa (Ethiopia, Sudan, and Eritrea) and surrounding areas like East Tropical Africa (Kenya, Tanzania, and Uganda), Westcentral Tropical Africa (Cameroon and the Democratic Republic of Congo), West Tropical Africa (Cote D'Ivoire), South Tropical Africa (Mozambique, Malawi, Zambia, and Zimbabwe), Southern Africa (Botswana), Western Indian Ocean (Madagascar) and Southwest Asia (Saudi Arabia and Yemen) where it was grown for production of leafy vegetable, fodder, and oilseed (Simmonds, 1979; Warwick et., al 2009; Delesa, 2011). Carinata was introduced to North America from Ethiopia in 1957 to be used as a source of leafy vegetables (Stephens, 2009). Due to its use as an alternative to napus and as an alternative energy crop with low to no indirect land-use changes, an increasing trend of cultivation of carinata is seen in different parts of the world, including Europe (Spain, Italy, Greece, and U.K.), Australia, New Zealand, South America (Chile and Uruguay;) and South Asia (India and Pakistan) (Malik, 1990; Velasco et al., 2003; Bozzini et al., 2007; Prakash et al., 2012; Zada et al., 2013b; Seepaul et al., 2016; Figure 2).

3. Genetic and Genomic Resources

3.1 Germplasm resources

Plant germplasm resources contain the genetic information of a plant's hereditary makeup depicting its origin and evolution. This information can identify genetically diverse parental lines for breeding and other crop improvement programs. Specifically, crop improvement leading to increased

productivity and/or resistance to biotic and abiotic stressors can be facilitated by intra- and inter-specific and intra- and inter-generic diversity. Eight gene banks across the world have an extensive repertoire of carinata. These gene banks have 1707 accessions collected from 17 countries across the world (Table 1).

3.2 Genetic and genomic advancements

The genetic diversity of the crop is influenced by natural and artificial selection (Wang et al., 2016), which helps to unravel the crop's evolutionary history. Carinata shows low genetic diversity due to a stronger genetic bottleneck during domestication (Khedikar, et al., 2020). A recent comparative analysis of different genetic and genomic resources like nucleotide sequences, protein sequences, genes, and research articles published on carinata and other common Brassicaceae members (napus, *B. juncea* and *B. rapa*) is provided in Table 2.

Phenotypic analysis of 11 carinata lines for their agronomic performance and seed quality as a new potential oilseed crop in Canada did not show wide variability (Getinet et al., 1996). In contrast, Alemayehu and Becker (2002) assessed 36 accessions of carinata for 13 morphological and seed-related traits and found a wide range of genetic variability for yield-related traits. They also reported moderate variability in oil quantity and quality (glucosinolate levels) and protein content. The use of morphological traits and biochemical markers, which are highly influenced by environmental factors, may have resulted in the detection of a wide variability among the accessions. Recently, 99 accessions of carinata were assessed for eight morphological traits and sinigrin content showed a wide variation among the traits (Teklehaymanot et al., 2019). It was found that sinigrin content, a predominant glucosinolate (GSL) in the leaves, was negatively correlated with leaf area, leaf width, primary branches, and plant height.

Genetic analysis of 39 carinata accessions using six amplified fragment length polymorphism (AFLP) primer combinations resulted in 189 polymorphic markers (Genet et al., 2005). This study segregated the accessions into 7 clusters showing the presence of substantial genetic diversity in carinata. A collection of 43 accessions from 5 different countries was genotyped using 50 random amplified polymorphic DNA (RAPD) markers and showed high genetic diversity but no apparent geographical clustering (Teklewold and Becker, 2006). In another study, a total of 296 AFLP markers produced

using four primer combinations were used to assess 66 carinata accessions showed a low level of genetic diversity in carinata in comparison to *B. nigra* and *B. juncea* (Warwick et al., 2006). In contrast, Jiang et al., (2007) assessed 110 accessions of carinata using 233 AFLP markers and showed high genetic diversity. Efforts are being made to develop genetic maps and to identify quantitative trait loci (QTL) for the crop. Priyamedha et al. (2012) constructed the first skeleton linkage map in carinata by using an F2 population of 150 individuals developed by crossing a resynthesized parental line Ar29 with natural cultivar PC5. They used 69 loci (23 RAPD, 20 ISSR, and 17SSR) spanning 2166 cM on all 17 linkage groups. Guo et al., (2012) constructed the first genetic linkage map of carinata using 212 loci (151 SSR, 44 AFLP, 12 SRAP, and 5 IBP markers) on a doubled haploid population of 183 lines covering 1703 cM assigned to the eight linkage groups of B-genome and nine linkage groups of C-genome. They were able to identify loci governing two Mendelian-inherited traits (petal and anther tip color) and one quantitative trait (seed coat color). Zou et al., (2014) constructed a high-density genetic linkage map using 4031 DArTseq loci covering 2048.4 cM on a doubled haploid population of 185 individual lines leading to the identification of QTLs governing budding and flowering time. Genes conferring black rot resistance were identified and mapped by Sharma et al., (2016) using 160 ILP and 204 SSR markers on F2 population of 212 genotypes. Zhang et al (2017) genotyped a panel of 81 accessions of carinata to generate 54510 DArTseq polymorphic markers. These markers were used for genome-wide association analysis of the panel, and seven markers were significantly associated with five seed yield and quality traits (flowering time, oleic acid, linolenic acid, pod number, and seed weight). A diversity panel of 83 carinata accessions procured from the Australian Grains Genebank was assessed for pod shatter resistance (Raman et al., 2017) and led to the identification of parental lines to develop an F2 population of 300 individuals. This population was assessed using 6464 DArTseq markers to develop a genetic linkage map and for QTL identification (5 QTLs distributed on chromosome B1, B3, B8, and C5) related to pod shattering resistance (Raman et al., 2017). Recently, Khedikar et al., (2020) assessed a worldwide panel of 620 accessions to study genetic diversity, linkage disequilibrium, and haplotype patterns using 10000 SNPs. This analysis helped in the identification of genomic regions showing evidence of selection pressure. Carinata showed lower nucleotide diversity levels than napus suggesting the development of a genetic bottleneck during domestication.

4. Morphology, Phenology, and Reproduction

Carinata is an erect, annual grown as an oilseed or as a leafy vegetable. The seedling emerges epigeally, with heart-shaped cotyledons (2–3 cm) that are photosynthetically active to offset insufficient food reserves (Seegeler, 1983; Mnzava and Schippers, 2007). Carinata shows determinate growth with height averaging 1.4 m, high branching, and elongated taproots reaching up to 1 m (Barro and Martin, 1999, Zanetti et al., 2013). Stems are glabrous, waxy, reaching up to 2 cm in diameter (Seegeler, 1983) with leaves having short petiole, simple trichomes, alternate, glabrous to slightly hairy, and waxy phenotype (Mnzava and Schippers, 2007; Al-Shehbaz, 2012). Lower leaf-blades are ovate to oblong with 1-3 deep lobes up to 20 cm long and 10 cm wide. The lower leaves' abaxial surface is green, while the adaxial surface is paler or grayish with purple or light-green veins. In comparison, upper leaves are lighter-colored and have fewer lobes, smaller in size, narrower, and less waxy (Seegeler, 1983).

The inflorescence is a loose, highly branched, and elongated compound raceme, with actinomorphous and perfect flowers borne terminally on the main stem and branches (Seegeler, 1983; Mnzava and Schippers, 2007). Pedicels are cylindrically shaped and 5–6 mm long. Flowers bear four green and oblong sepals (4–7 mm long) alternating with four yellow and obovate petals (6–10 mm long). Flowers also have six stamens and four nectaries. Most brassica species reproduce sexually through cross-pollination, contributing to the great diversity within species. However, carinata sets seed efficiently through both self and cross-pollination. Carinata was reported to self-pollinate 46%–88% of the time (Labana *et al*., 1987) due to self-compatibility, and cross-pollinated 30% of the time due to its flower structure and delayed anthesis (Velasco and Fernandez-Martinez, 2009; Cheung *et al*., 2015). Pollens are heavy, sticky, and are difficult to transfer from plant to plant by wind (Adeniji and Aloyce, 2012). Fruits are linear siliques up to 5 cm long, with 2–7 mm straight or curved conical beak (Seegelar, 1983). Siliques are green and photosynthetically active when immature and turn light brown at maturity. They contain up to 20 seeds and are non-dehiscent due to their thick and highly lignified valve margins (Barro and Martin, 1999; Banga *et al*., 2011). Seeds are globose, finely reticulated, yellow to brown with a diameter ranging between 1–1.5 mm (Getinet, 1986; Setia and Richa, 1989; Mnzava and Schippers, 2004; Rahman and Tahir, 2010).

Carinata has been described as a long-day plant (Zanetti et al., 2013). However, there is a possible interactive effect between temperature and photoperiod for flowering initiation (Friend, 1968) as high temperature has been associated with accelerated phenological development under long photoperiod (Nanda et al., 1996). Flower initiation in carinata varied from 77 to 126 days across multiple locations and years in Florida depending upon genotypic, edaphic, and climatic factors (Kumar et al., 2020). Carinata lifecycle ranged from 3949–4288 growing degree days (GDD; 4.4°C base temperature) with an average lifecycle of 154 days for an early-maturing genotype and 165 days for a late-maturing genotype when grown as a winter crop in Florida (Kumar et al., 2020) while it reached physiological maturity at 2000–2200 GDD (4°C) in Europe (Zanetti et al., 2013). Carinata variety 110994EM bolted, flowered, developed pods, and reached seed maturity around 535, 1084, 1547, and 2404 GDD, respectively (Seepaul et al., 2019b).

5. Agronomy of Carinata

Carinata is tolerant to a wide range of climatic conditions and can be fall-planted in the humid subtropical regions with mild winters and even rainfall throughout the year or spring-planted in the humid continental climate with hot and humid summers. Carinata can be fall-planted (October to November) in the Southeast US, generally 3 to 4 weeks before the first frost event (Seepaul et al., 2019c) or spring-planted (mid-March to mid-May) in Midwest and Western states in the USA (Gesch et al., 2019). Timely fall planting facilitates asynchrony between carinata phenology and incidence of biotic and abiotic stresses. For example, timely planting allows the crop to reach the rosette stage at the time of highest frost probability (Mulvaney et al., 2018) or avoidance of pest incidence and severity during periods of greatest herbivory. There is substantial variation in flowering time within the species (Rakow and Getinet, 1998). Fall-planted carinata in Florida took 102 days to flower and 161 days to mature after planting (Kumar et al., 2020), while spring-planted carinata in Saskatchewan, Canada, took 55 days to flower and 110 days after planting to mature (Getinet et al., 1996). Spring-planted carinata was the latest maturing species (111-113 days after planting) among common oilseed crops evaluated in Minnesota (Gesch et al., 2015). The relatively long growing cycle of carinata limits its commercial production in the Prairie Provinces of western Canada, requiring the development of early maturing varieties (Getinet et al., 1996). Scaling up production in the Southeast US also requires early maturing varieties to fit carinata in the double-cropped peanut-cotton rotations prevalent in the

region. Whether planted in the spring or fall, carinata's lifecycle should fit the diverse rotations with minimal delay in planting the subsequent crop within the growing regions where it is double-cropped (Christ et al., 2020).

5.1 Planting and germination

Carinata is a very shallow planted crop (Seepaul et al., 2019c) and germinates in the top portion of the soil that usually experiences moisture deficit (Patane and Tringali, 2011). Carinata should be planted not more 1.3 cm deep because of its small seed size, however deeper depths may be considered when planting in sandy soils (Seepaul et al, 2019c). Early season moisture availability in the 0.64 - 1.3 cm inches topsoil is critical for uniform and vigorous seed germination (Patane and Tringali, 2011). Carinata is generally seeded at 6.1 kg ha^{-1} (129 pure live seeds m^{-2}) (Kumar et al., 2020) but undergoes self-thinning resulting from interplant competition. Due to the high degree of compensatory ability, maximum seed yield can be achieved over a wide range of plant densities from 34 to 117 plants m^{-2} (Pan et al., 2012; Gesch et al., 2019). Relatively higher yields were obtained from plant populations ranging from 22-37 m^{-2} (Punia et al., 2001). Seed yield was responsive to seeding rate as high as 300 pure live seeds per m^2 in 3 of the 9 site-years in North Dakota (Hossain et al., 2018). Maximum seed yields (1140-1492 kg ha^{-1}) also occurred at a relatively high seeding rate (9-13.5 kg ha^{-1}) in South Dakota (Alberti, 2017). Similarly, carinata planted at 10 kg ha^{-1} produced 2890 kg seed ha^{-1} (Bozzini et al., 2007) in Italy, while 8 kg ha^{-1} produced 1592 kg ha^{-1} in Ethiopia (Tadesse et al., 2012). In Florida, maximum seed yield (2761 kg ha^{-1}) was produced at a 3 kg ha^{-1} seeding rate (Mulvaney et al., 2019). Higher seeding rates may reduce stem diameter and increase lodging potential but may be necessary for no-till systems to compensate for increased seedling mortality (Alberti, 2017). Changing the plant geometry through seeding rate and row spacing alters the leaf arrangement and canopy architecture, which controls light interception and photosynthetic productivity (Sarlikioti et al., 2011). Carinata has phenotypic plasticity to modulate plant architecture to optimize light interception (Mulvaney et al., 2019), especially at low plant populations, by producing more branches, racemes, and pods. This phenotypic plasticity is modulated by environmental conditions and resource availability (Hossain et al., 2018). Carinata growth and yield are influenced more by row spacing than seeding rate (Mulvaney et al., 2019) in Florida. Single rows spaced 36 cm apart maximized carinata yield (2761 kg ha^{-1}) in Florida (Mulvaney et al., 2019), while

a 30 cm row spacing produced 9-11% greater yield than a 60 cm row spacing carinata in India (Kaur, 2002). Wider row spacings favor branching and increase the number of pods per plant (Mulvaney et al., 2019).

5.2 Growth and Development

Carinata development is divided into vegetative (seedling, rosette), transition (bolting), and reproductive growth stages (flowering, pod development, and seed ripening). At physiological maturity, carinata typically has 30 mainstem nodes, 53% producing primary branches with 25 secondary branches bearing 280 pods per plant when fertilized with 90 kg N ha^{-1} (Seepaul et al., 2020). Leaves contributed 52% of the total dry matter (DM) during the vegetative stage and decline sharply to 0% at maturity while the stem fraction increased from 48% at the vegetative stage to a maximum of 73% at flowering and decline to 37% at maturity (Seepaul et al., 2019a). Seeds contributed 25% of the DM production (Seepaul et al., 2019a). Carinata self-defoliates with the onset of reproductive development as photo-assimilates and nutrients are translocated from leaves to the developing seeds (Seepaul et al., 2018) resulting in decreased leaf area with plant maturity (Seepaul et al., 2019b). The reduction of photosynthetic leaf area through artificial defoliation reduces seed yield by 21 and 11 kg ha^{-1} for every percent defoliation at the vegetative or reproductive stage, respectively (Silvana V. P-M, personal communication). Complete defoliation through excision at the post-flowering stage leads to decreased seed numbers per pod and 1000 seed weight by 32% and 25%, respectively in carinata (Ramana and Ghildiyal, 1997). Carinata is a high biomass producer accumulating 14224 kg ha^{-1} in spring-planting in Minnesota, USA (Gesch et al., 2015), and 7017 kg ha^{-1} as a fall-planted crop in Florida, USA (Seepaul et al., 2019a). Harvest index (HI) ranged from 0.28–0.37 in Minnesota and 0.30–0.34 in Florida (Gesch et al., 2015; Seepaul et al., 2019a).

Green pods contain chloroplasts in the outer pod wall layer and are responsible for 70-100% of assimilation of photosynthates in seeds in later stages of plant development (Andrews and Svec, 1975; Major and Charnetski, 1976; Sheoran and Randhir, 1991; Singal et al., 1995; Bennett et al., 2011; Raven and Griffiths, 2015).

Yield is dependent on the number of branches, number of pods per plant, number of seeds per pod, and 1000-seed weight (Ozturk, 2010; Setia et al., 1995). Seed size and yield are positively correlated with aboveground DM yield, suggesting that high DM accumulation at all growth stages throughout the crop growth cycle in stress-free conditions is key to optimizing yield components and yield (Enjalbert et al., 2013; Seepaul et al., 2019a).

5.3 Management practices

Nitrogen (N) availability alters the early season and post-bolting physiology, morphology, and biomass distribution patterns in carinata. Nitrogen accounts for the largest energy input and production costs in oilseed production (Gan et al., 2007); therefore, understanding the biomass accumulation and allocation, nutrient concentration, and uptake can help synchronize in-field N application with crop growth for optimum uptake and utilization. Carinata is highly responsive to N application (Alberti et al., 2019; Pan et al., 2011; Seepaul et al., 2019a) and requires adequate N fertilization for optimum seed yields (Johnson et al., 2013; Montemurro et al., 2016; Prakash et al., 1999). (Seepaul et al., 2016; Seepaul et al., 2019a; Seepaul et al., 2020; Seepaul et al., 2019b). Height, node numbers, primary branches, secondary branches, and pod numbers, and seeds per pod increased by 38.3, 6.7, 64.5, 146.1, and 128.2, % from 0 to 135 kg N ha^{-1}, respectively (Seepaul et al., 2020). Carinata grown with limited N (0 mg N l^{-1}) had 47% lower photosynthesis (21.2 µmol $m^{-2}s^{-1}$) than plants grown with optimal N (16 mg N l^{-1}) (31.0 µmol $m^{-2}s^{-1}$) (Seepaul et al., 2016). Suboptimal N availability modified carinata canopy architecture by reducing leaf size, early abscission and senescence, and vertical distribution of leaves on the main stem (Seepaul et al., 2016). Modification in canopy architecture in response to N deficiency adversely affected canopy photosynthesis and the production of flowers (Seepaul et al., 2016). Bolting is a period of rapid stem elongation in carinata and is a critical period for N fertilization. The limitation of N at the onset of bolting induces morphological changes such as reducing leaf area, light interception and canopy photosynthetic activity (Seepaul et al., 2016). Limiting N during carinata reproductive development resulted in a 62% yield penalty indicating that carinata is sensitive to N limitation (Seepaul et al., 2019). Under non-limiting N conditions (16.1 mg N l^{-1}), carinata produced 164% greater seed yield when compared to limited N (0 mg N l^{-1}) (Seepaul et al., 2019).

Seed yield response to N application rate over five years at Florida was quadratic and ranged from 1245 kg ha^{-1} with 0 kg N ha^{-1} to 2444 kg ha^{-1} with 117 kg N ha^{-1}. The economic optimum N rate occurred at 103 kg N ha^{-1}, which produced 2427 kg seed ha^{-1} (Seepaul et al., 2020). The application of 90 kg N ha^{-1} increased seed and oil yield ha^{-1} by 36% and 29%, respectively, over a non-treated control (Seepaul et al., 2020). In North Dakota, the application of 117–297 kg N ha^{-1} produced 2315 kg seed ha^{-1} (high input system), which was 14% greater than a low input system (2035 kg seed ha^{-1} at 32–97 kg N ha^{-1}) (Hossain et al., 2018). Maximum seed yield (2204 kg ha^{-1}) of spring-planted

carinata was produced at 150 kg N ha^{-1} in the Canadian Prairies (Pan et al., 2012). In Italy, the application of 100 kg N ha^{-1} produced 1770 kg seed ha^{-1}, 29% greater than the 0 N control. In India, fall-planted carinata dry matter accumulation increased linearly with N rate as high as 150 kg N ha^{-1}, but there was no benefit to seed yield above 100 kg N ha^{-1} (Kaur and Sidhu, 2004). Straw yield also showed a positive response to N application, reaching a peak at 200 kg N ha^{-1} in Canada (Johnson et al., 2013) as opposed to 100 kg N ha^{-1} (Gan et al., 2007) in various other brassica species in the Northern Great Plains. Nitrogen concentration and uptake in brassica seeds and straw increased with increasing N availability (Prakash et al., 2000; Johnson et al., 2013; Seepaul et al., 2019a). Increased nutrient uptake is related to greater biomass accumulation due to enhanced growth and photosynthetic capacity under non-limiting N conditions (Seepaul et al, 2016b). Nitrogen amount in carinata seeds (90.3 kg N ha^{-1}) is 115% greater than straw (42.0 kg N ha^{-1}) (Prakash et al., 2000). The relative comparison of different nutrient uptake in the seed and straw of carinata is presented in Table 3.

A two-way split of N increased secondary branches by 9% relative to a single or three-way split of 90 kg N ha^{-1} (Seepaul et al., 2020). Applying N at planting and bolting maximized primary and secondary branches in both the two- and three- way split of 90 kg N ha^{-1}. The application of either ammonium nitrate or Environmentally Smart Nitrogen (ESN) at bolting resulted in maximum secondary branches and pod numbers but did not affect seed yield (Seepaul et al., 2020). Nutrient uptake is closely related to biomass production. Maximum N uptake (73 kg N ha^{-1}) occurred between 50% bolting and 50% flowering while maximum P, K, Ca, Mg, S, B, Mn, Fe, Zn, and Cu uptake occurred between 50% flowering and pod formation (Seepaul et al., 2019a). Total nutrient uptake (seed + straw) in descending order is N>K>Ca>S>P>Mg>Fe>Zn>Mn>B>Cu. At 90 N kg ha^{-1}, carinata extracted 57 kg N ha^{-1} or 63% greater than the N application rate (Seepaul et al., 2019a).

Carinata growth and seed yield are also responsive to S application up to 45 kg S ha^{-1}; however, the economically optimal S rate was 36 kg S ha^{-1} (Bhattarai, 2019; Verma et al., 2018). The application of 40 kg S ha^{-1} increased seed yield by 33-34 % over a 0 S control by increasing the number of primary branches, the number of pods per plant, and seeds per pod (Bhattarai, 2019; Verma et al., 2018). There is a dearth of information on phosphorous and potassium as well as micronutrient's effect on carinata growth, development, and productivity.

Carinata is sensitive to drought stress, evidenced by decreased leaf size, reduced dry weight of plant parts, stomatal conductance, and photosynthesis (Ashraf & Mehmood, 1990; Pan et al., 2011; Husen et al., 2014). Drought stress reduced the root length by 6%, shoot length by 9%, the number of leaves by 15% in a controlled environment study (Husen et al., 2014). To overcome drought stress for a short period and protect leaves against dehydration, there is increased wax deposition and partial stomatal closure in carinata leaves to limit water loss (Albert et al., 2012; Husen et al., 2014). Nitrogen and water limiting conditions also stimulate elongation of the main and lateral roots in carinata, which can increase root exploration for efficient nutrient and water uptake (Singh & Singh, 2018, Hossain et al., 2019; Qin et al., 2019). Drought stress lowers leaf water potential leading to reduced turgor, stomatal conductance, photosynthesis (Kumar and Singh, 1998), biomass production (Husen et al., 2014), and seed and oil yields (Gesch et al., 2019). In Fort Collins, Colorado, irrigation increased biomass production, height, and pod density by 103%, 94%, and 7%, respectively, over rainfed carinata (Enjalbert et al., 2013). Maximum seed yield (2057 kg ha^{-1}), quality, and water use efficiency were achieved when irrigation was applied at the seedling stage, 50% flowering and pod development stages in the semi-arid regions of India (Verma et al., 2018). Carinata is better suited as a rainfed crop for regions with adequate growing season rainfall than in arid or semi-arid regions. A significant intraspecific variation for drought resistance exists within the carinata species that can be exploited to improve the drought tolerance of carinata through selection and breeding (Ashraf and Sharif, 1998; Lohani et al., 2019).

In addition to N and drought stress, high-temperature stress is also detrimental to carinata's growth and yield, especially during flowering (Gan et al., 2004). Under high-temperature stress (35/15°C day/night temperatures), only early formed floral primordia develops into flowers and pods (Angadi et al., 2000). Improved carinata lines that can maintain pod production and seed development under high temperatures are needed to contribute to increased yield (Gan et al., 2004). Under extreme temperatures (heat and cold), brassicas tend to increase the production of antioxidant defenses, responding to an increase in reactive oxygen species (ROS; Soengas et al., 2018). In the absence of the antioxidant defense mechanism, there is an increased production of ROS in the chloroplasts, which decreases the chlorophyll content and elicits photoinhibition, thereby reducing CO_2 fixation and loss of dry weight (Soengas et al., 2018).

Sequential flowering in carinata produces a mixture of pods with different maturity (Seepaul et al., 2018). Delayed maturity of current varieties (Kumar et al., 2020) requires agronomic management tools to accelerate carinata uniform maturity to allow timely land preparation and planting of summer row crops (Seepaul et al., 2018). Carinata can be swathed in arid or semi-arid regions or chemically desiccated in the US southeast (Seepaul et al., 2018). For safe seed storage, carinata must be at 10% seed moisture or less. If moisture is greater than 10%, seeds can be dried with forced air at low temperatures or air-dried (Seepaul et al., 2018).

5.4 Pest management

Carinata is resistant to diseases that commonly affect other oilseed brassica species (Katiyar et al., 1986), including black rot caused by *Xanthomonas campestris* pv. Campestris (Sharma et al., 2016; Tonguc and Griffiths, 2004) and blackleg or stem canker caused by *Leptosphaeria maculans* (Gugel et al., 1990; Rimmer and Vandenberg, 1992). Disease reports include those for turnip mosaic virus (Babu et al., 2013), sclerotinia stem rot caused by *Sclerotinia sclerotiorum* (Young et al., 2012), alternaria black spot caused by *Alternaria alternata* (Dunbar et al., 2017), powdery mildew caused by *Erysiphe cruciferarum* (Gunasinghe et al., 2013), charcoal rot caused by *Macrophomina phaseolina* (Tande et al., 2015), and root rot caused by *Fusarium species* (Okello et al., 2018). Some of these pathogens are generalists which may affect the subsequent rotational crops (Okello et al., 2018). Like other brassicas, carinata is susceptible to insect pests, including cabbage looper *Trichoplusia ni*, diamond back moth *Plutella xylostella*, spotted cucumber beetle *Diabrotica undecimpunctata*, turnip aphid *Lipaphis pseudobrassicae*, yellow margined leaf beetle *Microtheca ochroloma* and *Pieris rapae* (Baldwin, 2019). There is a limited number of studies that quantified the effects of weeds on carinata seed yield. One study in North Dakota reported a 16% yield reduction when weeds are not controlled (Hossain et al., 2018). Although carinata forms a competitive canopy (Gesch et al., 2015) against weeds, an integrated weed management strategy that employs cultural, mechanical practices and the use of herbicides is prudent for profitable production. Managing plant populations by optimizing seeding rate and row spacing along with optimal fertilizer application can reduce the impact of weeds. Pendimethalin and S-metolachlor can be used for preemergence weed control, while broadleaf and grass control can be achieved using Clopyralid and Clethodim (Leon et al., 2017). Carinata is not invasive or likely to become a weed in subsequent crops. Flumioxazin, acifluorfen, bentazon, and

carfentrazone can be used to control volunteer carinata in rotational crops (Leon et al., 2017). The prevalence of wild radish (*Raphanus raphanistrum*) in the Southeast US can reduce yield and harvest quality through resource competition and contamination of harvested carinata seeds. Increasing cropping system diversity by planting carinata in a three-year crop rotation may reduce insect pests, diseases, and weed pressure in rotational crops like canola (Harker et al., 2015a; Harker et al., 2015b). Carinata's vigorous growth and broad leaves smother weeds. Weed biomass decreased by 67% as the seeding rate increased from 50 to 300 seeds m^{-2} (Hossain et al., 2018). Planting in narrow rows (no more than 36 cm and preferably 19 cm) and using high seeding rates (greater than 5.6 kg ha^{-1}) will favor rapid canopy closure and weed suppression (www.sparc-cap.org/resources/factsheetscarinata).

5.5 Seed Quality

Carinata has 39% and 61% long-chain (C14−C18) and very-long-chain (>C19) fatty acids, respectively. Of these, 6%, 31%, and 62% are saturated, polyunsaturated, and monounsaturated fatty acids, respectively (Seepaul et al., 2020). Oil concentration responds to nutrient management, particularly N application rates. An increase in N application rates resulted in a decrease in oil content, and an increase in protein content as oil and protein concentrations are inversely related (Pan et al., 2012; Johnson et al., 2013; Hossain et al., 2018; Verma et al., 2018; Hossain et al., 2019; Seepaul et al., 2019; Kumar et al., 2020; Seepaul et al., 2020). Oil concentration decreased at a rate of 0.26 g kg^{-1} for every kg increase in nitrogen application per hectare (Alberti et al., 2019). Nitrogen application rates, split management of N, or N source did not affect the concentration of fatty acids (Seepaul et al., 2020). Oil concentration increased with seeding rate in one study (Hossain et al., 2018) but did not differ in others (Pan et al., 2012; Mulvaney et al., 2019) and did not respond to row spacing (Mulvaney et al., 2019) or irrigation (Verma et al., 2018). Plants grown at lower seeding rates generally have greater nitrogen uptake than those from higher seeding rates, which may negatively affect oil concentration (Harker et al., 2012; Harker et al., 2015). Except for nitrogen management, carinata seed and oil yields can be optimized through agronomic manipulations with little or no effect on seed oil, protein, and fatty acid concentrations.

Glucosinolates (GSLs) are often concentrated in the leaves and roots during early stages of development and reallocated to the seeds at maturity (Bellostas et al, 2004; Jørgensen et al., 2015). Carinata shoots comprise mostly of aliphatic GSLs with roots containing aliphatic and aromatic GSLs at about a 1:1 ratio and ranging from 8 to 25 μmol g^{-1} of total GSLs (Kirkegaard & Sarwar, 1998). Seeds contain about 80 μmol g^{-1} of GSL comprising mainly of sinigrin, but can be as high as 160 μmol g^{-1} (Mnzava & Olsson, 1990; Marquez-Lema et al., 2008; Seepaul et al., 2019; Seepaul et al., 2020). After oil extraction, carinata seed meal can be used as supplemental protein for animal feed since it contains up to 53.5% crude protein, 76% of which are rumen degradable protein (Ban, Khan, & Yu, 2017; Paula et al., 2019). However, carinata meal must contain less than 2.0% erucic acid and less than 30 μmol g^{-1} of GSLs like sinigrin due to their detrimental effects on the health of animals by reducing palatability and interfering with iodine uptake (Tripathi & Mishra, 2007; Nega & Woldes,

2018). Therefore, carinata meal is restricted to 10% of their total diet or 0.3% of body weight per day (Paula et al., 2019; Schulmeister et al., 2019). The seed meal containing high GSLs levels (44-168 μmol g^{-1}) can be used as a soil amendment due to its biofumigant properties to suppress pest and disease activity (Pattison et al., 2006; Gimsing & Kirkegaard, 2009; Mazzola & Manici, 2012).

6. Current state of crop improvement

Successful oilseed crop improvement involves developing varieties with a higher yield, better oil quality, and resistance to various biotic and abiotic stresses. This requires access to phenotypically and genotypically diverse germplasm from existing germplasm resources and wild varieties, which can broaden the genetic base by identifying and incorporating desirable traits into the existing varieties.

6.1 Carinata as a desirable donor for inter-specific hybridization

Carinata possesses several desirable traits like tolerance to abiotic stress (heat, salt, and metal toxicity; Gugel et al., 1990; Irtelli and Navari-izzo, 2008; Mafakheri and Kordrostami, 2020), resistance to various biotic stresses (blackleg disease, stem rot, white rust, alternaria black spot, powdery mildew, and aphids; Gugel et al., 1990; Gebre-Medhin and Mulatu, 1992; Yitbarek, 1992; Tonguc and Griffiths, 2004; Navabi et al., 2010; Chavan and Kamble, 2014; Mehta, 2014; Sharma et al., 2017), pod shattering resistance and relatively large seed size (Getinet et al., 1996; Thakur et al., 2019) which makes it a desirable donor for various inter-specific hybridization programs for the improvement of related species. Traits like late maturity, long and profuse vegetative growth, tall plant stature, low oil content, high erucic acid content, low harvest index, and unattractive seed coat color are major constraints for its adoption as an oilseed crop for edible purposes (Thakur et al., 2019). Inter-specific crosses between carinata (female) and *B. juncea* (male) resulted in the successful production of F1 progeny in different studies (Rahman, 1976; Getinet et al., 1997; GhoshDastidar and Varma, 1999; La Mura et al., 2010). Similar reports of successful hybridization between different Brassicaceae members like carinata (female) and *B. napus* (male; Fernandez-Escobar et al., 1988; Getinet et al., 1997; La Mura et al., 2010; Niemann et al., 2014); carinata (female) and *B. nigra* (male; Mizushima, 1950; Chang et al., 2011); carinata (female) and *B. oleracea* (male; Rahman, 2001 and 2004; Chang et al., 2011); carinata (female) and *B. rapa* (male; Struss et al., 1991 and 1992;

Choudhary et al., 2000; Rahman, 2001, 2002 and 2004; Jiang et al., 2007; Liu et al., 2009; Lu Mura et al., 2010) are available.

In some cases, hybridization between allotetraploid (carinata, napus and *B. juncea*) and diploid (*B. nigra, B, rapa* and *B. oleracea*) brassica species have resulted in the formation of non-viable seeds due to pre- (Diederichsen and Sacristan, 1994) and post-fertilization barriers (Nishiyama et al., 1991). Doubled haploid methods, ovary and ovule culture, embryo culture, and protoplast fusion techniques have been utilized to overcome post-zygotic inter-specific incompatibility barriers for these crosses (Diederichsen and Sacristan, 1994). Ovary and ovule culture (Sabharwal and Dolezel, 1993) and protoplast fusion (Klima et al., 2009) technique resulted in the production of viable F1 seeds between carinata and napus. Protoplast fusion between black rot resistant carinata accession PI 199947 and susceptible rapid cycling *B. oleracea* breeding line followed by backcross to *B. oleracea* resulted in the generation of resistant varieties (Tonguc et al., 2003). Similarly, embryo culture between carinata and *B. oleracea* (Rahman, 2004; Tonguc and Griffiths, 2004; Sharma et al., 2017) and carinata and *B. rapa* (Quiros et al., 1985; Busso et al., 1987; Meng et al., 1998; Rahman, 2004) resulted into production of successful F1 hybrids.

6.2 Oil quality, quantity, and secondary metabolites

The development of genotypes with high erucic acid content in brassica species is an important research area due to its high demand for various industrial applications (Taylor et al., 1995). Velasco et al., (1998) used ethyl-methane sulfonate (EMS) to increase the erucic acid content in carinata line C-101. It led to an increase of the erucic acid content of M4 generation lines to 52.2-59.3% compared to 39.0-47.6% in parental lines. Velasco et al., (1995) also used EMS to develop low erucic acid (5-10%) containing carinata lines fit for edible purposes. Carinata breeding programs have also reported the development of varieties with very low erucic acid content ranging from 0% to 2% suitable for edible purposes and high erucic acid-containing varieties (up to 50%) suitable for industrial application (Fernandez- Escobar et al., 1988; Alonso et al., 1991; Getinet et al., 1996; Jadhav et al., 2005).

The development of transgenic lines in carinata is another strategic approach to increase erucic acid (C-22:1) and nervonic acid (C-24:1) content. Minimal efforts have been made to develop transgenic

lines with improved erucic and nervonic acid content in carinata. Jadhav et al., (2005) used two approaches i.e. co-suppression and anti-sense repression of *FAD2* gene in carinata, to decrease the production of polyunsaturated C-18 fatty acids and increase of erucic acid and VLCFA (very long-chain fatty acid) content. This study resulted in an increased proportion of erucic acid content by 12-27% for co-suppressed and 5-19% for antisense repression transgenic lines, while the VLCFA content increased by 6-15% and 5-19% for co-suppressed and antisense repression transgenic lines of carinata. Miethiewska et al., (2008) used 3'-UTR of the *FAD2* gene to form an intron-spliced hairpin RNA (ihp RNA) to silence *FAD2* gene which led to an increase of 16% and 10% in oleic acid and erucic acid content, respectively in carinata. They also used a second construct containing ihp RNA targeted to the endogenous *FAD2* gene of carinata along with heterologous *Crambe abyssinica FAE gene* with seed-specific napin promoter to increase erucic acid production by 16%. Reports of varieties with increased nervonic acid, 5,13- docosadienoic acid, 5-eicosenoic acid, eicosapentaenoic acid contents to meet various industrial, biofuel, and nutritional needs are also available in carinata (Jadhav et al., 2005; Chang et al., 2010; Taylor, 2010). The mean value of different oil quality traits of carinata has been provided in Table 4.

6.3 Breeding targets

The development of disease tolerant or resistant carinata lines is important for higher yield and oil content. Tonguc and Griffiths (2003) evaluated 54 carinata accessions from the USDA collection for resistance against race 4 of *Xanthomonas campestris* pv. *campestris* (Xcc) causing black rot disease in other brassica species. Out of the 54 accessions of carinata tested, two accessions (A 19182 and A 19183) did not exhibit any symptoms, while three accessions (PI 199947, PI 199949, and PI 194256) segregated for resistance to Xcc. The National Gene Bank of India (NBPGR) at New Delhi contains two registered varieties (IC 443624 and IC 544702) resistant to white rust disease. The gene bank also has one variety each, which is registered for tolerance to *Alternaria* blight (IC 305114), resistance to white rust and leaf and stag head stage (IC 523914), good yielding (IC 296346), and resistance to pod shattering and lodging (IC 555215). Efforts are being made to develop herbicide-tolerant lines of carinata against Group 2 and Group 4 herbicides like Dicamba at Agriculture and Agri-Food, Canada. They have developed two tolerant lines UF-S2 and UF-S3, which will be tested for their herbicide

tolerance at a field trial in Uruguay by Nuseed (https://westerngrains.com/projects/developing-unique-herbicide-tolerant-brassica-carinata-and-brassica-juncea-germplasm/). The development of early maturing varieties of carinata has been reported from some parts of the world. The National Gene Bank of India (NBPGR) at New Delhi has one registered early maturing variety (IC 467732) of carinata in their collection. The gene bank also contains three registered varieties of carinata (IC 555215, EC 223405, and IC 199711), which are yellow seeded. Evaluation of the agronomic performance of 11 genotypes in Florida has resulted in the identification of early maturing genotypes taking 7 to 14 days less to mature than the control genotype (Kumar et al., 2020).

7. Perspectives

Carinata is widely adaptable to diverse growing regions, cropping systems, and management regimes with demonstrated potential to be grown on the continents of Asia, Africa, North America, South America, Europe, and Australia either as a spring or winter crop in double-cropped systems. Carinata is a biomolecular platform for fuel, meal, and co-products (Mcvetty et al., 2016; Schulmeister et al., 2019; Taylor et al., 2010) shown to increase farmer incomes and provide ecosystem goods and services (Basili and Rossi, 2018; Christ et al., 2020). Producers need to connect the ancillary value of growing carinata in their rotation with their entire cropping system. Further, quantifying and valuing the ecosystem goods and services and providing economic incentives to grow a commodity crop with significant cover crop benefits can aid in adoption.

Adopting carinata in double-cropping systems would require continuing research to integrate crop biology with agronomy, to understand growth and development and its interaction with agricultural inputs and management. Such research results would enhance the productivity of carinata in diverse cropping systems when planted either as a spring or winter crop. Improving carinata productivity requires the genetic improvement of agronomic traits and the development of best management practices to optimize the crop in diverse rotations. Current carinata lines have low genetic diversity (Khedikar et al., 2020); therefore, research should focus on developing genomic resources to characterize genetic diversity to aid marker-trait associations for agronomic traits (Thakur et al., 2019). Reducing the growth cycle length by 2 to 3 weeks with minimal yield penalty and improving freeze tolerance at the vegetative and bolting stages can increase adoption as a winter crop. Improving

resource use efficiency (nutrients and water) of carinata may lead to sustainable yields over a broader range of environmental conditions. Production on marginal lands, especially in arid and semi-arid regions, can enhance the crop's ecological value as demonstrated in Italy (Basili and Rossi, 2018; Cardone et al., 2003; Cardone et al., 2002). With increasing acreages under cultivation, the probability of pests and diseases increases; therefore, identifying resistance to pests and diseases common within potential growing regions and developing integrated control methods are necessary. Improvements in oil profiles and very-long-chain fatty acid concentrations, such as nervonic acid, can lead to the development of novel biomolecules. Adaptable and yield-stable carinata varieties would require best management practices for sustainable production. These include refining recommendations for optimal rotation sequences, tillage practices, planting date, seed rate, row spacing, fertilizer application, pest control, and harvest management of spring and winter planted carinata in different growing regions.

Scaling up commercial production to produce advanced renewables in the US must fit within the contextual framework of the USDA Agriculture Innovation Agenda benchmarks to increase productivity by 40%, reduce carbon footprint by 50%, reduce nutrient loss by 30%, increase biofuel feedstock production and biofuel production efficiency by 2050 (https://www.usda.gov/aia). Fitting carinata into this framework requires developing a regional bioeconomy with infrastructure and logistics to grow, transport, crush, convert, distribute, and use oil, meal, and co-products close to where the crop is grown to minimize carbon footprint and to improve the environmental sustainability of biofuel production systems.

Acknowledgments

This research was funded by USDA-NIFA Bioenergy Coordinated Agricultural Projects Grant # 2016-11231 (Southeast Partnership for Advanced Renewables from Carinata). Our sincere thanks to Ms. Sapna Rawat, University of Delhi, for sketching the growth stages of carinata.

Author Contributions

R.S. and S.K. planned and designed the research. R.S., S.K., J.E.I, M.B. and T.S. wrote the manuscript. R.S. and S.K. made the figures and tables. R.S., S.K., R.K., K.J.B, M.J.M, I.M.S., S.G. and D.L.W., reviewed and edited the manuscript.

Data Sharing: Data sharing is not applicable to this article, as no new data were created or analyzed in this review of the literature.

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Table 1: List of germplasm/gene banks across the world with carinata germplasm collection (accessed on September 24, 2020).

Germplasm/gene Banks	Source (# of countries with accessions in the gene bank)	Total accessions (#)
Plant Gene Resources (PGRC), Canada	6	91
Svalbard Global Seed Vault	17	402
EURISCO[a]	9	386
U.S. National Plant Germplasm System, USDA, ARS, USA	12	77
NBPGR, New Delhi, India	NA	60
Australian Grains Genebank, Horsham, Australia (Raman et al., 2017)	NA	83
Ethiopian Biodiversity Institute[b]	1	402
AVRDC, Taiwan[b]	8	206

[a]comprises collections from different genebanks of Europe

[b]gene banks with carinata collection as leafy vegetable crop

Table 2: Comparative analysis of resources available in NCBI database for different brassica species (accessed from https://www.ncbi.nlm.nih.gov/ on September 24, 2020).

NCBI Database	**Carinata**	**Napus**	***B. juncea***	***B. rapa***
Nucleotide	2,819	933,899	161,744	150,430
Protein	673	198,437	2,345	153,772
Structure	-	11	11	3
Genome	1	1	1	1
PopSet	26	96	57	247
GEO Datasets	13	953	98	849
PubMed Central	240	7,684	1,959	4,055
Gene	89	128,793	230	60,999
SRA Experiments	12	10,145	705	4,101
Identical Protein Groups	323	151,342	1,076	122,266
Bio Project	9	878	41	1,280
Bio Sample	18	10,549	709	3,905
Bio Systems	-	271	-	260
Assembly	-	4	-	6
PubChem BioAssay	-	98	68	4

PopSet- Population sequence data; GEO datasets- Gene expression omnibus datasets; SRA- Sequence read archive;

Table 3: Seed and straw nutrient uptake of carinata yielding 2778 kg seed ha^{-1} and 7017 kg straw ha^{-1} at Quincy, FL.

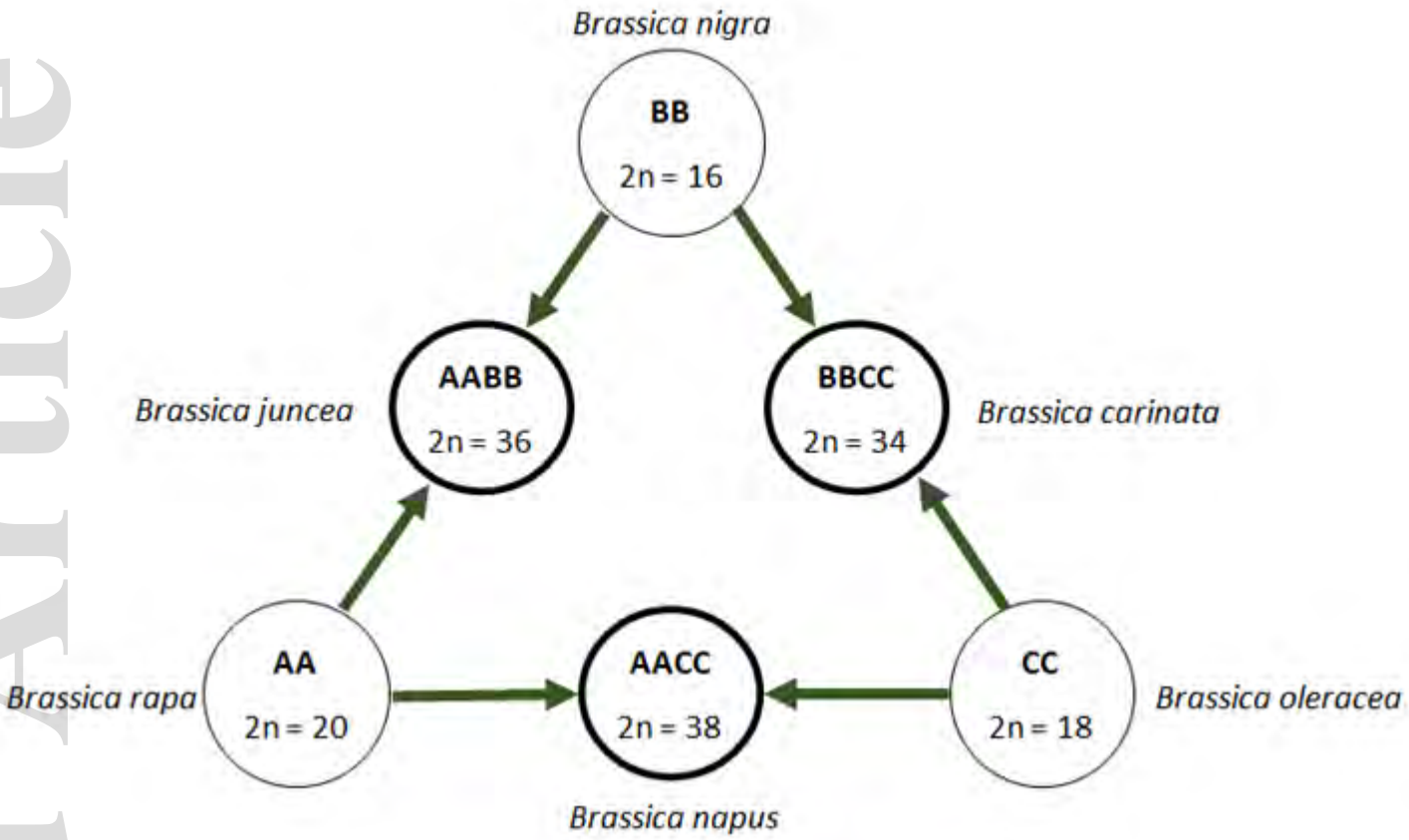
Uptake	Nitrogen(N)	Phosphorous (P)	Potassium (K)	Calcium (Ca)	Magnesium (Mg)	Sulfur (S)	Boron (B)	Manganese (Mn)	Iron (Fe)	Zinc (Zn)	Copper (Cu)
	kg ha^{-1}						g ha^{-1}				
Seed Uptake	100.1	22.3	29.1	11.3	11.1	28.9	40.8	87.2	233.5	151.9	14.6

Straw Uptake	46.8	3.6	60.7	51.7	8.2	17.1	99.5	92.9	199.7	174.6	19.9
Total Uptake	146.9	25.9	89.8	63.0	19.4	46	140.4	180.1	433.2	326.5	34.5

Table 4: Mean value of seed quality traits of commercial carinata variety Avanza 641 grown in north Florida (adapted and modified from Mulvaney et al., 2019). The mean represents the average of 128 to 304 samples from 4 site-years.

Trait	**Mean**	**SEM**
Oil concentration, %	39.7	0.2
Protein in seed, %	31.6	0.2
Glucosinolates, µmol g^{-1}	92.9	1
Saturated fatty acids, %	6.2	0
Monounsaturated fatty acids, %	57.2	0.1
Polyunsaturated fatty acids, %	35.9	0.1
Long-chain fatty acids, % with chain length 14–18 C	49.8	0.2
Very long-chain fatty acids, % with chain length > 19 C	52.7	0.2
Iodine value	113.7	0.1
Other fatty acids in seed, %	0.6	0
C16:0 (palmitic acid), %	3.4	0
C16:1 (palmitoleic acid), %	0.2	0
C18:1 (oleic acid), %	12.7	0.1
C18:0 (stearic acid), %	1.1	0
C18:2 (linoleic acid), %	18.3	0.1
C18:3 (linolenic acid), %	12.9	0.1
C20:0 (arachidic acid), %	0.8	0
C20:1 (eicosenoic acid), %	8.6	0.1
C16:0 (eicosadienoic acid), %	1.4	0.1
C22:0 (behenic acid), %	0.5	0
C22:1 (erucic acid), %	36.4	0.1
C22:2 (docosadienoic acid), %	0.5	0
C24:0 (lignoceric acid), %	0.3	0

C24:1 (nervonic acid), %	1.4	0



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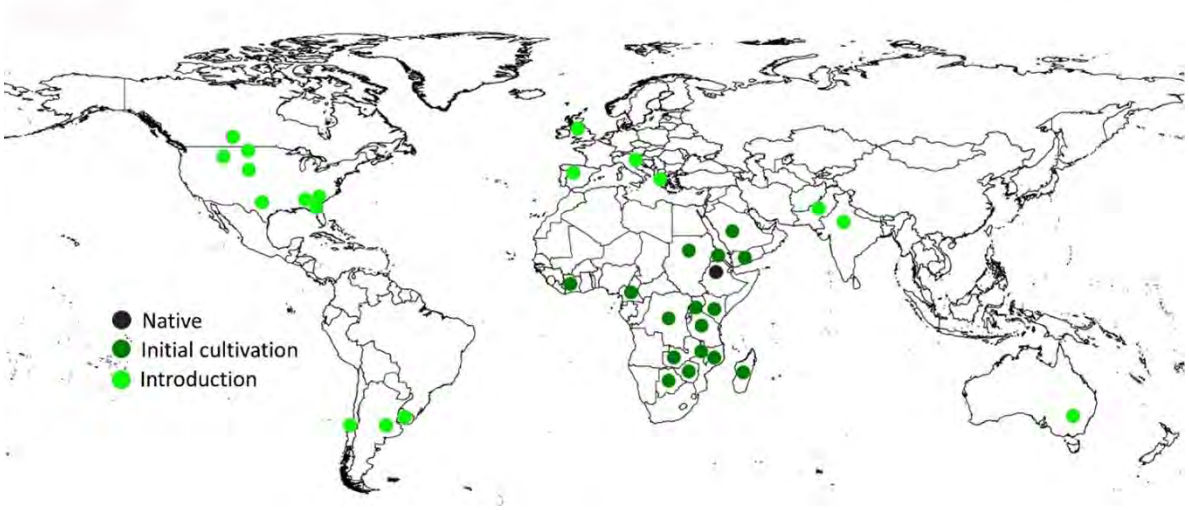

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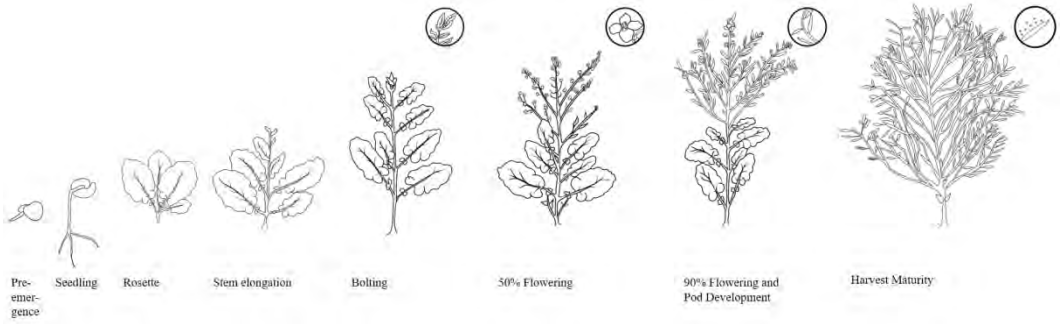

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