biology

MDPI

Article

Nitrogen Availability Modulates the Effect of *Transparent Testa 8* Knockout on Seed Oil Concentration in *Camelina sativa*

Demian Dlakić [1], Patrick Munson [1], Xiao-Hong Yu [2,3], Chaofu Lu [1], John Shanklin [2] and Andreas Fischer [1,*]

[1] Department of Plant Sciences and Plant Pathology, Montana State University, 119 Plant Bioscience Building, Bozeman, MT 59717, USA; ded95@cornell.edu (D.D.); clu@montana.edu (C.L.)
[2] Department of Biology, Brookhaven National Laboratory, 50 Bell Ave., Upton, NY 11973, USA; xhyu@bnl.gov (X.-H.Y.); shanklin@bnl.gov (J.S.)
[3] Department of Biochemistry and Cell Biology, Stony Brook University, 450 Life Sciences Building, Stony Brook, NY 11794, USA
* Correspondence: fischer@montana.edu

Simple Summary

Camelina is an ancient oilseed crop from the mustard family experiencing a resurgence due to its ability to grow under cold, dry conditions. Increasing seed oil concentration and oil yield are primary goals for camelina improvement. Previous research has shown that eliminating the function of *Transparent Testa 8* (*TT8*) genes redirects carbon from the outer shell of seeds (the testa) to lipid synthesis, increasing seed oil concentration. In this study, we analyzed the influence of plant nitrogen nutrition on camelina seed oil concentration and oil yield, comparing plants with (control) and without *TT8* function (modified). Interestingly, the expected effect of the loss of *TT8* function on seed oil concentration (28–30% vs. 25% of dry mass for controls) was observed only when plants were grown under sufficient nitrogen availability, but not under nitrogen deficiency. Additionally, we observed that modified camelina lines produced heavier seeds than control plants, particularly under nitrogen deficiency; they also showed reduced carbon concentration in plant residue (shoots and leaves) at harvest. Our study therefore demonstrates that the effect of a loss of *TT8* function on oil concentration is modulated by nitrogen availability, and suggests that *TT8* loss also affects carbon metabolism of vegetative plant tissues.

Abstract

Camelina is a promising intermediate oilseed crop for sustainable biofuel production. Knockout of *Transparent Testa 8* (*TT8*) genes increases seed oil concentration, but the influence of nitrogen deficiency on this trait remains unclear. To address this knowledge gap, two *tt8* knockout lines (26-6 and 26-7) and WT plants (var. 'Suneson') were grown on nutrient film tables using high nitrogen (HN, 6.5 mM nitrogen) and low nitrogen (LN, 0.65 mM nitrogen) nutrient solutions. At maturity, shoots, silicles (fruit pericarp), and seeds were analyzed for nitrogen and carbon content and yield traits. Compared to WT, both *tt8* lines showed increased seed oil concentrations under HN (28–30% vs. 25% of dry mass), but not under LN. A significant genotype × nitrogen regime interaction supported this differential response. Seed weight was slightly (but not significantly) higher in *tt8* lines under HN, and significantly higher than WT under LN. Residual shoot carbon concentration was lower than WT under HN, while carbon concentrations were higher than WT in silicles under LN. Our results indicate that the influence of *TT8* knockout on oil concentration is modulated by nitrogen availability, and suggest that this engineered trait also affects carbon metabolism in vegetative plant tissues.

Check for updates

Academic Editor: Dorothea Bartels

Received: 14 August 2026
Revised: 11 November 2026
Accepted: 13 November 2026
Published: 18 November 2026

Copyright: © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

Keywords: camelina; carbon use; nitrogen nutrition; seed oil concentration; *TRANSPARENT TESTA 8* (*TT8*)

1. Introduction

Camelina (*Camelina sativa* (L.) Crantz) is an oilseed crop in the Brassicaceae family that has emerged as an intermediate crop with the potential to complement a wide variety of cropping systems [1–3]. Camelina is both cold and drought-tolerant and has a relatively short life cycle [4–6]. These traits make it an ideal alternative crop for semi-arid environments, and it is often used as an alternative to fallow [7–9] or as part of an intercropping system [10,11]. Camelina oil has end uses as a feedstock for sustainable aviation fuel (SAF) [12,13] and as a dietary supplement for animals [14–16] and humans [17,18]. Camelina has also emerged as a model crop for plant biotechnology [19] due to an increasing number of genomic and transcriptomic resources and a robust transformation protocol [20–22]. Camelina improvement has largely focused on increasing its relatively low oil yields [23,24], which are strongly influenced by location and environmental effects [25,26]. Low oil yield is a significant limitation to agronomic viability and represents a major roadblock to wider camelina adoption [27,28]. To address the need for camelina improvement, both conventional breeding [29] and genetic engineering approaches have been used [23,30].

Genetic engineering studies in camelina have mostly focused on improving fatty acid (FA) concentration in seeds and (subsequently) oil yields, with varied results [31–35]. Early strategies have targeted genes involved in acyl lipid metabolism, because the relevant genes are highly conserved with those in Arabidopsis [36,37]. Knockouts of fatty acid desaturase (*FAD2*, *FAD3*) and fatty acid elongase (*FAE1*) genes have resulted in significant beneficial changes to oil composition and increases in oil concentration, but not increased oil yield [32,34,35]. Other approaches have focused on altering the seed coat composition, a long-recognized breeding objective in Brassicaceae family crops based on evidence that yellow-colored, thin-coated varieties produce higher oil concentrations and yields [38]. The proanthocyanin biosynthesis pathway plays an important role in determining seed coat characteristics, and yellow-seeded varieties of Arabidopsis and Brassicaceae crops with mutations in this pathway have been developed through traditional breeding and genetic engineering approaches [39–43]. Within the proanthocyanin pathway, the *Transparent Testa 8* (*TT8*) gene plays a key role in seed FA production and oil yield, and has recently been examined in camelina [44].

TT8 encodes a transcription factor that regulates two genes (*DIHYDROFLAVONOL 4-REDUCTASE* and *BANYULS*) and indirectly inhibits seed FA accumulation through regulation of other seed development transcription factors [45,46]. *TT8* was first identified in Arabidopsis and has since been linked to yellow-seeded phenotypes in multiple Brassicaceae species [41,46–48]. *TT8* knockouts in Arabidopsis and *Brassica napus* resulted in a lighter seed coat color and increased seed FA concentration [43,46,49]. Recently, Cai et al. [44] generated camelina lines with knockout mutations in the plant's three *CsTT8* homeologs. The homozygous mutant plants displayed similar changes to the seed coat as those seen in related species; the seeds were yellow in color, showed increased oil concentration, and reduced seed coat mucilage [44].

Camelina is often described as a low-input crop [2,50,51] with a high nitrogen use efficiency [52–54], but fertilizer is still the largest share of input costs in most cropping systems [28,55]. Camelina yields increase significantly in response to higher nitrogen availability, which has been demonstrated in a number of field studies [54,56–60]. Nitro-

gen and carbon metabolism are tightly linked in plants; nitrogen status controls carbon assimilation [61] because a large fraction of plant nitrogen is invested in the photosynthetic apparatus. Conversely, carbon supply affects nitrogen uptake and assimilation [61]. It is therefore likely that changes in seed carbon utilization, such as those associated with the loss of *TT8* function, both influence and are influenced by plant nitrogen nutrition. However, this potential interaction has not been investigated in camelina or other oilseed crops. To address this knowledge gap, we examined camelina lines carrying knockout mutations in three *CsTT8* homologs [44] under two strongly contrasting nitrogen regimes, assessing seed traits and carbon and nitrogen concentrations in residual shoot biomass to determine how nitrogen availability influences the phenotypic effects of *TT8* knockout.

2. Materials and Methods

2.1. Plant Material

The *tt8* lines 26-6 and 26-7 used in this study were originally generated in the *Camelina sativa* cultivar 'Suneson.' Both lines were isolated as distinct T1 progeny of the same T0 CRISPR/Cas9 transformation event (line 26), and both were homozygous for mutations affecting all three *CsTT8* homeologs. Line 26-6 contained +1 bp and −4 bp edits at the S1 and S2 target sites, respectively, in *CsTT8-2*; a −4 bp edit at S2 in *CsTT8-8*; and a homozygous −49 bp deletion in *CsTT8-13*. Line 26-7 contained a homozygous −48 bp deletion in *CsTT8-2*, a −4 bp edit at S2 in *CsTT8-8*, and a −1 bp edit at S2 in *CsTT8-13* [44]. Both lines were previously confirmed to be free of the Cas9/DsRed transgene. The plants used in the study presented here were T2 descendants of these homozygous lines.

2.2. Growing Conditions and Experimental Design

Camelina variety 'Suneson' (WT) and *tt8* lines 26-6 and 26-7 were grown as described by Dlakić et al. [52]. Seeds were germinated in flats with 200 rockwool cubes (2.5 × 2.5 cm; Grodan, Byhalia, MS, USA) in a growth chamber set at 12 °C under a 16/8 h day–night cycle and photosynthetically active radiation of ~100 µmol m^{-2} s^{-1} at the seedling level. All seedlings were supplied with 1 L of half-strength high-nitrogen nutrient solution per week during germination (Supplementary Table S1). The described conditions promote the development of vigorous, uniformly growing seedlings and have been previously used by our research group [52].

Two weeks after germination, seedlings were transferred to nutrient film tables (NFTs). The tables had eight channels; each channel was 2.5 cm deep and 10.2 cm wide, with 2.5 × 2.5 cm holes in the covers for each plant. Each channel contained wild-type, line 26-6 and line 26-7 plants arranged using a random pattern across the eight channels.

Two separate experiments were performed (Supplementary Figure S1); for the first experiment (high nitrogen, HN), the NFT was supplied with 100 L (from a single reservoir) of a modified Hoagland solution containing 6.5 mM nitrogen from 2.5 mM KNO_3, 1 mM $Ca(NO_3)_2$, and 1 mM NH_4NO_3 (i.e., 5.5 mM nitrate and 1 mM ammonium; Supplementary Table S1). For the second experiment (low nitrogen, LN), the NFT was supplied with 100 L of a nutrient solution containing 0.65 mM nitrogen from 0.25 mM KNO_3, 0.10 mM $Ca(NO_3)_2$, and 0.10 mM NH_4NO_3 (i.e., 0.55 mM nitrate and 0.10 mM ammonium; Supplementary Table S1). Under LN, 2.25 mM KCl and 0.90 mM $CaCl_2$ were added to keep potassium and calcium ion concentrations at the same level as in the HN experiment. Replacing nitrate with chloride ions is a common approach in experiments investigating nitrogen deprivation or limitation. Chloride was selected as the replacement anion because, unlike sulfate, it is not substantially incorporated into organic metabolites and will be compartmentalized into the vacuole [62,63]. Concentrations of additional chloride ions (4.05 mM) were below the threshold of potential toxicity [64,65]. All other

nutrients were kept constant across both experiments (Supplementary Table S1). In both experiments, nutrient solutions were cycled through the channels using a 1400 L h^{-1} pump (Ecoplus, Vancouver, WA, USA), and the reservoir was aerated by two air stones fed by a Tetra Whisper model 60 aquarium air pump (Spectrum Brands Pet, Blacksburg, VA, USA; 1100 L h^{-1}). Nutrient solutions were completely replaced once per week throughout the growing period. The growth room lights were Philips Greenpower toplighting 200–400 v LED rails providing 300–500 µmol m^{-2} s^{-1} photosynthetically active radiation (PAR) at leaf level, with 16 h/8 h day–night cycles, a constant temperature of 22 °C, and a relative humidity of ~60%.

In both experiments, plants were grown to maturity, defined as the time point when the entire shoot system, including all leaves and silicles, had fully senesced and dried. Due to the well-established role of nitrogen in delaying plant senescence [52,66,67], this developmental stage occurred much later under HN (14 weeks after the onset of flowering) than under LN (six weeks after the onset of flowering). The onset of flowering was defined as the time point when half the plants had at least one flower that had opened (45 days after germination for both wild-type and *tt8* lines, under both LN and HN). Data obtained for WT plants (variety 'Suneson') under HN have been previously published in a study analyzing camelina nitrogen use efficiency [52].

2.3. Tissue Collection and Analysis

In both LN and HN experiments, one mature plant was harvested from each channel to account for positional effects, including eight wild-type (var. 'Suneson'), eight 26-6 and eight 26-7 plants. Silicles with seeds were harvested from whole plants and dried at room temperature for one month before being separated by crushing. Silicles (fruit pericarp without seeds) and seeds were separately placed in envelopes. All remaining aboveground tissue (shoots, leaves, and fallen leaves) was defined as the shoot system and placed into paper bags. The shoot system and silicles (without seeds) were dried at 43 °C for one week, at which point the dry biomass was measured. The tissues were then separately ground with a Thomas Wiley Mill Model 4 (Thomas Scientific, Swedesboro, NJ, USA) and filtered through a 0.5 mm sieve. Nitrogen and carbon contents of shoot systems, silicles, and seeds were measured via combustion analysis using a Costech ECS 4010 elemental combustion analyzer (Costech Analytical Technologies, Inc., Valencia, CA, USA) at the Environmental Analytical Laboratory at Montana State University, Bozeman, MT, USA.

The instrument was calibrated using six acetanilide (C_8H_9NO) standards of increasing weight, with readings verified every 10–12 samples using a lab standard consisting of wheat flour of known N concentration. Seed oil contents were determined by a benchtop nuclear magnetic resonance seed analyzer (MQC23, Oxford Instruments, Concord, MA, USA) and are presented as percentages of seed mass. The results were validated by gas chromatography using heptadecanoic acid methyl ester (C17:0) as an internal standard. Composition of seed fatty acids (FAs) was determined by analyzing fatty acid methyl esters (FAMEs), which were prepared by heating 3 crushed seeds at 80 °C in 1 mL 2.5% H_2SO_4 (v/v) in methanol for 90 min, and extracted with 0.3 mL hexane and 1.5 mL 0.5% NaCl (w/v). Samples were injected into a Shimadzu 2010 gas chromatography GC system (Shimadzu, Kyoto, Japan) fitted with an Agilent HP-Innowax column (19091N–133; 30 m × 0.25 mm i.d. × 0.25 µm) (Agilent Technologies, Inc., Santa Clara, CA, USA) and helium (He) as the carrier gas. The oven temperature was programmed at 190 °C initially, followed by an increase of 20 °C min^{-1} to 250 °C and maintained for 3 min. Fatty acid type percentages are reported as a percentage of all identified fatty acids [68].

Thousand-seed weight was calculated from sub-samples of 200 seeds harvested from each plant. Oil yield was calculated as seed yield (g $plant^{-1}$) × oil concentration (percent of

dry mass). Harvest index (HI) for each separate plant was calculated as seed yield/(seed yield + shoot dry mass + silicle dry mass) (all measured in g plant^{-1}). Nitrogen masses were obtained by multiplying N concentrations (percent of dry mass) with shoot, silicle or seed dry mass (g plant^{-1}); nitrogen harvest index (NHI) was then determined as seed nitrogen yield/(seed nitrogen yield + shoot nitrogen mass + silicle nitrogen mass), with carbon harvest index (CHI) determined using the same approach as NHI.

2.4. Statistical Analysis

Statistical analyses and graphs were completed in R using data from eight plants sampled per genotype and nitrogen regime (Supplementary Figure S1). Two-factor analysis of variance (ANOVA) was used to evaluate the effects of nitrogen regime (LN vs. HN), genotype (WT, 26-6, and 26-7), and their interaction, with statistical significance defined as $p < 0.05$. Seed oil fatty acid composition was analyzed using a three-factor ANOVA with fatty acid type, nitrogen regime, and genotype as factors, with statistical significance defined as $p < 0.05$. Because the eight plants within each nitrogen regime shared a common nutrient reservoir, they were not fully independent experimental units (Supplementary Figure S1). In addition, the HN and LN treatments were conducted as separate experiments; therefore, nitrogen regime and nitrogen regime × genotype interaction effects should be interpreted cautiously. Two-sided 95% confidence intervals were calculated using treatment means and are shown in the figures. Post hoc comparisons of all measured traits were conducted with the emmeans package and included a Bonferroni correction to account for multiple comparisons [69]. Compact letter displays were also generated with the emmeans package. Post hoc comparisons of all measured traits except fatty acid composition were stratified by nitrogen regime to make comparisons between lines within each treatment. Post hoc comparisons of fatty acid composition were stratified by nitrogen regime to compare fatty acid type across lines. Fatty acid composition post hoc comparisons were also stratified by genotype to compare fatty acid type across nitrogen treatments. All graphs were constructed using the ggplot2 package [70].

3. Results

3.1. Influence of Nitrogen Regime and TT8 Function on Seed Composition and Yield

Camelina seed oil concentrations were substantially higher (>40%) for all tested genotypes under low nitrogen (LN) as compared to high nitrogen (HN) conditions (25–30%) (Figure 1A), with ANOVA analysis reporting a significant treatment effect ($p < 0.001$; Supplementary Table S2). This difference has been previously noted by our research group in camelina plants grown using the same approach and nitrogen concentrations as in the present study [52]; it reflects the inverse relationship between nitrogen availability and seed oil concentration, which has also been observed by other authors [58,59,71]. Under HN, seed oil concentrations were significantly higher in both *tt8* lines (29.6% in 26-6 and 27.6% in 26-7) than in the WT plants (25.4%) (Figure 1A), confirming results from previous studies analyzing such knockouts in camelina and related Brassicaceae species [44,46,49]. The largest difference in oil concentration between the LN and HN experiments was observed in the WT (14.9 percentage points difference), while line 26-6 was the most similar (9.9 percentage points difference) (Figure 1A). In contrast to the HN treatment, no significant differences in oil concentration were observed between *tt8* and WT plants grown under LN (Figure 1A), resulting in a significant N treatment and genotype interaction ($p < 0.001$; Supplementary Table S2).

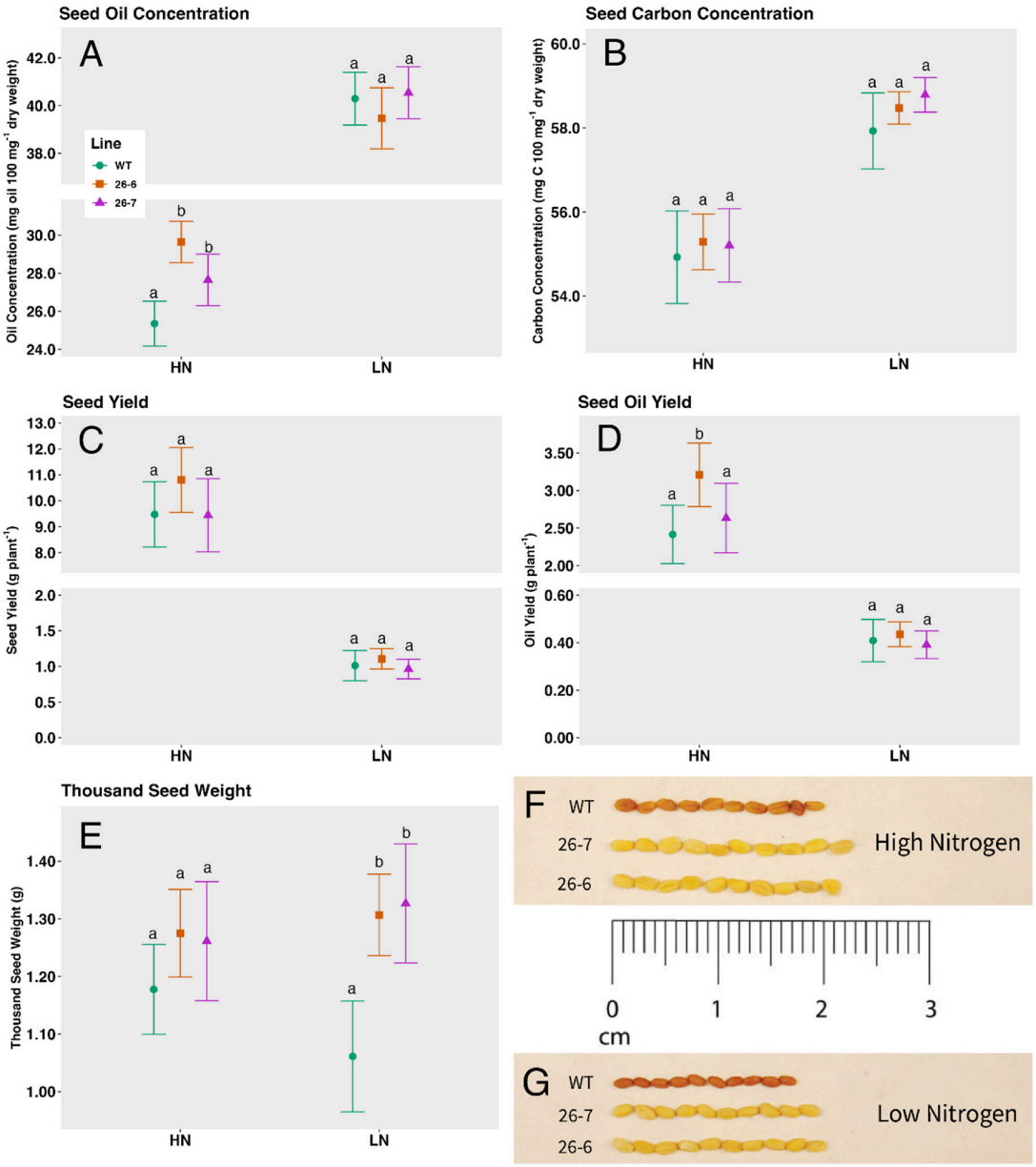


Figure 1. Comparisons of seed traits and oil yield between *tt8* lines 26-6, 26-7, and WT under HN and LN treatments for plants harvested at maturity. (**A**), seed oil concentration; (**B**), seed carbon concentration; (**C**), seed yield; (**D**), seed oil yield; (**E**), thousand-seed weight; (**F**), seed size under HN treatment; (**G**), seed size under LN treatment. Mean values and error bars representing a 95% confidence interval are shown. Compact letter displays indicate significant differences ($p < 0.05$; determined by Bonferroni-corrected estimated marginal means) between lines under the same nitrogen treatment. Values were calculated using eight plants per genotype/line and nitrogen treatment.

Similar to seed oil concentrations, total seed carbon concentrations were substantially higher in plants grown under LN (>58%) than under HN (<56%) (Figure 1B), and seed yield was ~10-fold higher under HN, reflecting the 10-fold difference in nitrogen availability between the treatments (Figure 1C). No significant differences between WT and *tt8* lines were detected for either trait under either N regime (Supplementary Table S2). Although the HN treatment resulted in lower oil concentrations across all lines than the LN treatment, oil yields were much higher under HN, with one *tt8* line (26-6) producing a significantly higher oil yield than WT plants (Figure 1A,D), and ANOVA reporting a significant genotype effect on oil yield ($p = 0.031$; Supplementary Table S2). Thousand-seed weight trended higher in *tt8* lines than WT under HN, and was significantly higher in *tt8* lines than WT

under LN (Figure 1E), with a significant effect of genotype on seed weight ($p < 0.001$; Supplementary Table S2). Visual comparison showed slightly larger seeds for *tt8* lines than WT under both HN and LN (Figure 1F,G).

Although significant differences in oil concentration, oil yield, and thousand-seed weight were observed between *tt8* lines and the WT (Figure 1, Supplementary Table S2), several other seed traits did not vary between lines. Seed nitrogen concentration and seed carbon-to-nitrogen (C/N) ratio were similar between all lines under both nitrogen treatments; as expected, the seed nitrogen concentration was higher and the C/N ratio was lower under HN (Supplementary Figure S2A,B and Table S2). Seed carbon and nitrogen masses also did not vary between lines under either treatment, but there was an approximately 10-fold difference in masses between LN and HN (Figure S2C,D). Harvest index (HI) was also similar between all lines under both LN and HN, with a higher average across all lines under LN (Figure S2E, Supplementary Table S2). Carbon harvest index (CHI) was also higher under LN across all lines, and trended higher than WT in *tt8* lines under HN, but lower in *tt8* lines under LN (Figure S2F). However, genotype effects on this parameter were not significant (Supplementary Table S2). Nitrogen harvest index (NHI) trended lower in *tt8* lines under both nitrogen treatments, and was also lower in all lines under HN (Figure S2G), but no significant genotype effects on NHI were identified (Supplementary Table S2).

Results from the two-way ANOVA indicated that genotype effects were concentrated in selected seed traits. Significant genotype effects were detected for seed oil concentration ($p = 0.019$), oil yield ($p = 0.031$), and thousand-seed weight ($p < 0.001$), whereas seed carbon and nitrogen concentrations and masses, as well as seed C/N ratio, did not differ significantly among genotypes (Supplementary Table S2). The genotype × nitrogen regime interaction was significant for seed oil concentration ($p < 0.001$), consistent with the higher oil concentration of both *tt8* lines than WT under HN but not LN; however, this interaction should be interpreted cautiously given the experimental design. In contrast, the interactions for oil yield ($p = 0.051$) and thousand-seed weight ($p = 0.116$) were not significant.

3.2. Seed Oil Composition

Seed oil composition was largely similar between *tt8* lines and WT within each nitrogen treatment (Table 1), although significant differences were observed for several individual fatty acids. Consistent with these differences, the three-way ANOVA revealed a significant fatty acid type × genotype interaction ($p < 0.001$; Supplementary Table S3), suggesting that genotype-associated differences varied among fatty acid types. Under HN, the relative proportion of linoleic acid (18:2) trended higher in line 26-6 and was significantly higher in line 26-7 than in WT (Table 1). In both *tt8* lines, this difference coincided with significantly lower relative proportions of linolenic acid (18:3). Under LN, the relative proportion of linoleic acid was also higher in both *tt8* lines, although the difference was significant only in line 26-7 (Table 1). In addition, both *tt8* lines had significantly higher relative proportions of eicosenoic acid (20:1) under LN, whereas the relative proportions of the minor fatty acids eicosatrienoic acid (20:3) and behenic acid (22:0) were lower, with significant differences observed in line 26-7 (Table 1).

The three-way ANOVA also revealed a significant fatty acid type × nitrogen treatment interaction ($p < 0.001$; Supplementary Table S3), suggesting that the effects of nitrogen treatment on relative fatty acid proportions varied among fatty acid types. Treatment comparisons within individual genotypes (Supplementary Table S4) showed that the relative proportion of stearic acid (18:0) was significantly higher under HN than LN in both *tt8* lines, but not in WT plants. In contrast, oleic acid (18:1) was significantly lower under HN in WT and both *tt8* lines. The relative proportions of linolenic acid (18:3)

and palmitoleic acid (16:1) were significantly higher under HN in WT but did not differ significantly between nitrogen treatments in *tt8* lines except for linolenic acid in line 26-7, which was significantly lower under HN. Several other fatty acids (16:1, 20:0, 20:1, 20:2, 22:0, and 22:1) also showed treatment-associated differences, with the patterns varying among genotypes (Supplementary Table S4).

Table 1. Comparison of seed oil composition between *tt8* lines 26-6, 26-7, and WT under HN and LN treatments at maturity. Values represent the relative contribution of individual fatty acids, with the fatty acid composition for each genotype/line summing to 100%. Compact letter displays indicate significant differences ($p < 0.05$; determined by Bonferroni-corrected estimated marginal means) between lines under the same nitrogen treatment for each fatty acid type. Values were calculated using eight plants per genotype/line and nitrogen treatment.

Fatty Acid	16:0	16:1	18:0	18:1	18:2	18:3	20:0	20:1	20:2	20:3	22:0	22:1
HN–WT	6.88 ^{a}	1.78 ^{a}	2.74 ^{a}	12.09 ^{a}	17.56 ^{a}	39.52 ^{a}	1.69 ^{a}	12.14 ^{a}	1.68 ^{a}	1.42 ^{a}	0.43 ^{a}	2.15 ^{a}
HN–26-6	6.89 ^{a}	1.92 ^{a}	3.18 ^{a}	12.38 ^{a}	18.14 ^{a}	38.32 ^{b}	1.71 ^{a}	11.83 ^{a}	1.78 ^{a}	1.49 ^{a}	0.42 ^{a}	2.18 ^{a}
HN–26-7	6.86 ^{a}	2.08 ^{a}	2.93 ^{a}	12.14 ^{a}	18.86 ^{b}	37.29 ^{c}	1.48 ^{a}	12.09 ^{a}	1.85 ^{a}	1.40 ^{a}	0.52 ^{a}	2.33 ^{a}
LN–WT	6.60 ^{a}	1.14 ^{a}	2.40 ^{a}	14.08 ^{ab}	18.00 ^{a}	38.03 ^{a}	0.65 ^{a}	10.94 ^{a}	2.96 ^{a}	1.79 ^{a}	1.28 ^{a}	1.64 ^{a}
LN–26-6	6.81 ^{a}	1.50 ^{a}	2.17 ^{a}	13.71 ^{a}	18.54 ^{a}	37.81 ^{a}	0.51 ^{a}	11.88 ^{b}	2.69 ^{a}	1.64 ^{ab}	0.78 ^{ab}	1.76 ^{a}
LN–26-7	7.00 ^{a}	1.58 ^{a}	2.06 ^{a}	14.42 ^{b}	19.22 ^{b}	38.04 ^{a}	1.17 ^{a}	11.92 ^{b}	1.67 ^{b}	1.11 ^{b}	0.23 ^{b}	1.57 ^{a}

3.3. Influence of Nitrogen Regime and TT8 Function on Shoot and Silicle Traits

The *tt8* plants displayed a lower residual shoot system carbon concentration than WT under both nitrogen treatments, with significant differences under HN (Figure 2A), and ANOVA analysis showed a significant genotype effect ($p < 0.001$; Supplementary Table S2). In contrast, residual shoot nitrogen concentrations trended higher in *tt8* plants than the WT under HN (Figure 2B). Residual shoot nitrogen concentrations were <1% in WT and >1% in *tt8* plants under HN, while all lines produced <1% shoot nitrogen under LN (Figure 2B); however, the genotype effect for this parameter was not significant ($p = 0.158$; Supplementary Table S2).

Shoot system biomass was highest in line 26-6 under both nitrogen treatments, and significantly higher than the WT under HN (Supplementary Figure S3A), with ANOVA analysis showing a significant genotype effect ($p = 0.006$; Supplementary Table S2). Shoot system biomass was >10-fold higher in HN- than in LN-grown plants, with the largest difference seen in WT (15.6-fold) (Supplementary Figure S3A). Line 26-6 also produced the highest average shoot carbon mass under both nitrogen treatments (Supplementary Figure S3B) but, unlike carbon concentration, the genotype effect on shoot carbon mass was not significant ($p = 0.068$; Supplementary Table S2). Residual shoot nitrogen mass was significantly higher than WT in line 26-6 under HN (Supplementary Figure S3C), reflected by a significant genotype effect ($p = 0.042$; Supplementary Table S2). The shoot C/N ratio was higher in the WT than *tt8* lines under HN, although the difference was not significant (Figure S3D).

Carbon concentration in silicles (fruit pericarp) was similar to the shoot system under HN, and the WT produced the highest carbon concentration, significantly higher than line 26-7 (Figure 2C). Interestingly, this trend reversed under the LN treatment, where both *tt8* lines produced significantly higher carbon concentrations in silicles, with significant nitrogen treatment, genotype, and interaction effects (Figure 2C, Supplementary Table S2). Nitrogen concentrations in silicles did not differ significantly across all lines; the HN treatment produced nitrogen concentrations >1%, while concentrations were ~0.8% under LN (Figure 2D). Silicle biomass, silicle carbon mass, and silicle nitrogen mass were highest in line 26-6 under HN, though not significantly different from the WT (Supplementary Figure S3E–G). All lines were similar under LN, and values were ~10-fold lower than under HN (Supplementary Figure S3E–G). All lines also produced similar silicle C/N ratios under HN and LN but, in contrast to shoot C/N ratios, silicle C/N ratios were

lower under HN than LN (Supplementary Figure S3H), reflected by a significant treatment effect ($p < 0.001$; Supplementary Table S2).

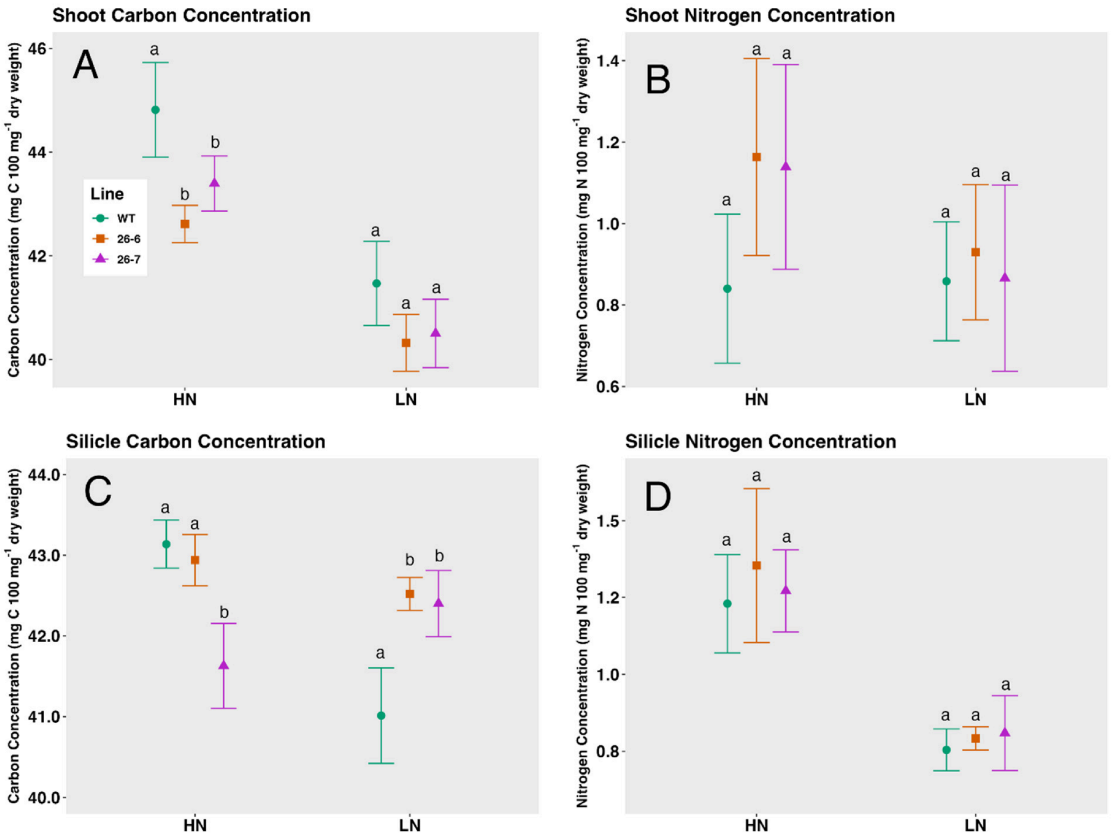


Figure 2. Comparisons of carbon and nitrogen concentrations in shoots and silicles between *tt8* lines 26-6, 26-7, and WT under HN and LN treatments for plants harvested at maturity. (**A**), shoot carbon concentration; (**B**), shoot nitrogen concentration; (**C**), silicle carbon concentration; (**D**), silicle nitrogen concentration. Mean values and error bars representing a 95% confidence interval are shown. Compact letter displays indicate significant differences ($p < 0.05$; determined by Bonferroni-corrected estimated marginal means) between lines under the same nitrogen treatment. Values were calculated using eight plants per genotype/line and nitrogen treatment.

The two-way ANOVA analysis indicated that genotype effects on residual maternal tissues at harvest were primarily associated with carbon-related traits. Genotype significantly affected shoot biomass ($p = 0.006$) and shoot carbon concentration ($p < 0.001$), while shoot carbon mass only showed a trend ($p = 0.068$) and shoot nitrogen concentration did not differ significantly among genotypes ($p = 0.158$; Supplementary Table S2). For silicles, genotype significantly affected carbon concentration ($p = 0.002$) and carbon mass ($p = 0.029$), but not nitrogen concentration or nitrogen mass. A significant genotype × nitrogen regime interaction was observed for silicle carbon concentration ($p < 0.001$) and silicle carbon mass ($p = 0.039$), although these interaction results should be interpreted cautiously given the experimental design.

4. Discussion

The effects of knocking out *TT8* on seed traits in oilseed crops are well-established, but how these effects are influenced by nitrogen availability has not been investigated previously [44,46,49]. Carbon and nitrogen metabolism are tightly linked, and, as in other oilseed crops, camelina seed oil concentration decreases with increasing nitrogen availability [59]. However, the accompanying increase in seed yield more than offsets this reduction,

resulting in higher oil yields [52]. For this reason, camelina is often grown under high nitrogen fertilization, but the resulting low seed oil concentrations leave substantial room for improvement [23]. Therefore, the potential agricultural value of *TT8* knockout lines depends on their ability to increase seed oil concentrations under high nitrogen availability, thereby further increasing total oil yield. Several field studies have established the effect of nitrogen availability on camelina oil yield [56,58,59], and further characterization of *tt8* camelina lines across a range of nitrogen treatments will be needed to assess the full potential of oil yield increases.

Our study confirms previous work demonstrating that *TT8* knockout leads to higher seed oil concentrations, but the effect was only observed under HN conditions (Figure 1A). While the LN treatment did not exhibit any difference in oil concentration between *tt8* lines and the WT, oil concentrations were much higher than under HN (>40% vs. <30% under LN and HN, respectively). The significant genotype × nitrogen regime interaction for oil concentration is consistent with this pattern, although the interaction should be regarded as exploratory given the experimental design. The positive effect of *TT8* knockouts on oil concentration under HN also resulted in higher oil yields per plant, with a significant increase in line 26-6 (Figure 1D) and a significant effect of genotype on oil yield (Supplementary Table S2). Line 26-6 had a higher seed yield (although the difference was not significant; Figure 1C), which likely contributed to the higher total oil yield per plant (Figure 1D).

Differences in seed oil fatty acid composition between WT and *tt8* lines were comparatively modest and were concentrated in specific fatty acids rather than reflecting consistent shifts across the overall fatty acid profile (Table 1 and Supplementary Table S4). Three-way analysis (Supplementary Table S3) showed significant interactions among fatty acid type, nitrogen treatment, and genotype, suggesting that genotype-associated differences in fatty acid composition depended on nitrogen availability. Comparisons of individual fatty acids within nitrogen treatments identified differences between *tt8* lines and WT in several fatty acids, particularly linoleic and α-linolenic acids under HN and eicosenoic acid under LN. The HN results are consistent with those reported by Cai et al. [44], who also observed proportional increases in linoleic acid and decreases in α-linolenic acid in *tt8* lines. To our knowledge, the proportional increase in eicosenoic acid in *tt8* lines under LN has not previously been reported and raises the possibility that nitrogen availability modifies the effects of *TT8* knockout on specific fatty acids. Comparisons between nitrogen treatments also revealed significantly higher stearic acid proportions under HN than under LN in both *tt8* lines, whereas this difference was not significant in WT, again suggesting genotype-dependent responses to nitrogen treatment. The decrease in oleic acid under HN relative to LN previously reported in WT [52] was also observed in both *tt8* lines, suggesting that this response to nitrogen availability is retained following *TT8* knockout. Changes in the proportions of linoleic and α-linolenic acids between WT and *tt8* lines (Table 1), and the higher proportions of stearic acid in *tt8* lines under HN as compared to LN (Supplementary Table S4) may be relevant to nutritional oil quality [16–18]. However, the magnitude of these compositional differences was relatively modest compared with the differences in total seed oil concentration associated with *TT8* knockout under HN.

The present study also reports significant effects of genotype on thousand-seed weight. Seeds of *tt8* lines were significantly heavier than WT under LN and showed a smaller (not significant) increase under HN, while the genotype × nitrogen regime interaction was not significant. Thus, our data support a (desirable) genotype-associated increase in seed weight, but do not indicate that this effect is specifically dependent on nitrogen regime. Similarly, the lack of a significant influence of genotype on seed yield and harvest indices suggests that the observed seed phenotype did not result from a broad change in

resource partitioning to developing seeds. However, our results contrast with the previously observed negative correlation between thousand-seed weight and oil concentration in camelina breeding cultivars [72], suggesting that introgression of *TT8* knockouts into breeding germplasm may provide an opportunity to increase seed oil concentration without compromising seed weight.

Previous investigations of *TT8* knockouts in camelina, canola, and Arabidopsis have primarily focused on changes to seed traits [44,46,49]. This study also reports an association of the *tt8* phenotype with changes in maternal tissues at plant maturity. Shoots of both *tt8* lines contained lower carbon concentrations than WT under HN, with smaller (not significant) differences also seen under LN (Figure 2A), while silicles (fruit pericarp) showed the opposite pattern under LN, with both *tt8* lines having higher residual carbon concentration than WT (Figure 2C). ANOVA analysis identified genotype effects for several carbon-related traits and significant genotype x nitrogen-regime interactions for silicle carbon concentration and carbon mass. In contrast, most nitrogen concentrations and nitrogen masses were not genotype-dependent. Importantly, differences in carbon concentration were not consistently accompanied by differences in carbon mass. This distinction suggests that loss of *TT8* may alter tissue composition without changing the total amount of carbon retained in those tissues at maturity. The mechanism underlying these differences cannot be resolved from endpoint measurements, but the results indicate that the consequences of *TT8* loss extend beyond seed oil accumulation. While *TT8* expression was found primarily in camelina seeds [44], *transparent testa* mutants including *tt8* have previously been shown to influence Arabidopsis root and shoot development traits [73], raising the possibility that knockouts studied here reduce utilization of carbon for the flavonoid pathway in shoots as well. Under LN, silicle (fruit pericarp) carbon concentrations were significantly elevated in both *tt8* lines (Figure 2C), but silicle carbon masses were not different from the WT (Figure S3F). Similar to shoot tissues, this result suggests compositional changes to the silicle tissue, but an increase in carbon concentration cannot be explained by reduced synthesis of flavonoids. Metabolomic analyses of camelina shoots and fruit pericarp during seed maturation could help elucidate the mechanisms underlying the compositional changes suggested by this study, and provide a more comprehensive understanding of how *TT8* knockout affects carbon metabolism beyond the seed.

5. Conclusions

Our results indicate that nitrogen availability modulates the effect of *TT8* disruption on seed oil concentration in camelina. Under high nitrogen, both *tt8* lines exhibited increased seed oil concentration relative to the WT, while this difference was not observed under low nitrogen. Our data also indicate that increased seed oil concentration was not associated with a reduction in seed weight. Differences in carbon concentrations of mature shoot and silicle (fruit pericarp) tissues suggest that *TT8* disruption influences plant carbon use beyond the seed. Together, these findings highlight nitrogen availability as an important consideration for deploying the *TT8* knockout trait in camelina breeding, suggesting a need for evaluating the stability of the increased-oil phenotype across a range of nitrogen availability and production environments.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/biology15222116/s1, Figure S1: Experimental design; Figure S2: Comparisons of seed traits and harvest indices between *tt8* lines 26-6, 26-7, and WT under HN and LN treatments for plants harvested at maturity; Figure S3: Comparisons of shoot system and silicle traits between *tt8* lines 26-6, 26-7, and WT under HN and LN treatments for plants harvested at maturity; Table S1: Recipes for high nitrogen (HN) (6.5 mM nitrogen) and low nitrogen (LN) (0.65 mM nitrogen) nutrient solutions; Table S2: Significance *p*-values for all measured traits; Table S3:

Significance *p*-values from three-way ANOVA of seed oil fatty acid composition; Table S4: Comparison of seed oil composition between *tt8* lines 26-6, 26-7, and WT under HN and LN treatments at maturity; Table S5: Raw data for all measured traits except individual fatty acid types; Table S6: Raw data for seed oil fatty acid composition.

Author Contributions: D.D. helped conceive the study, grew the plants, collected samples and photographs, processed samples for elemental analysis, analyzed data, wrote the initial draft, and edited the manuscript. P.M. processed samples for elemental analysis, analyzed data, and edited the manuscript. X.-H.Y. helped D.D. conceive the study, created the knockout lines, and edited the manuscript. C.L. helped D.D. conceive the study and performed the fatty acid analysis on harvested seeds. J.S. helped D.D. conceive the study and edited the manuscript. A.F. helped D.D. conceive the study, oversaw the experiments, and edited the manuscript. C.L., J.S. and A.F. acquired funding. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the Department of Energy (DOE), Office of Science, Biological and Environmental Research (BER) grant no. DE-SC0021369, and by the Montana Agricultural Experimental Station (MAES), project MONB00248.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Raw data used for the generation of Figures 1 and 2, Table 1, Supplementary Figures S2 and S3 and Tables S2–S4 are available as Supplementary Tables S5 and S6.

Acknowledgments: The authors would like to acknowledge the Environmental Analytical Laboratory at Montana State University for their services of elemental analysis, and David Baumbauer for help with greenhouse and hydroponic systems.

Conflicts of Interest: John Shanklin has a financial interest in AtTAG Bio. Inc. The other authors have no relevant financial or non-financial interests to disclose.

References

1. Vollmann, J.; Eynck, C. Camelina as a Sustainable Oilseed Crop: Contributions of Plant Breeding and Genetic Engineering. *Biotechnol. J.* **2015**, *10*, 525–535. [CrossRef] [PubMed]
2. Berti, M.; Gesch, R.; Eynck, C.; Anderson, J.; Cermak, S. Camelina Uses, Genetics, Genomics, Production, and Management. *Ind. Crops Prod.* **2016**, *94*, 690–710. [CrossRef]
3. Weiss, R.M.; Zanetti, F.; Alberghini, B.; Puttick, D.; Vankosky, M.A.; Monti, A.; Eynck, C. Bioclimatic Analysis of Potential Worldwide Production of Spring-Type Camelina [*Camelina sativa* (L.) Crantz] Seeded in the Spring. *GCB Bioenergy* **2024**, *16*, e13126. [CrossRef]
4. Hunsaker, D.J.; French, A.N.; Thorp, K.R. Camelina Water Use and Seed Yield Response to Irrigation Scheduling in an Arid Environment. *Irrig. Sci.* **2013**, *31*, 911–929. [CrossRef]
5. Gesch, R.W.; Cermak, S.C. Sowing Date and Tillage Effects on Fall-Seeded Camelina in the Northern Corn Belt. *Agron. J.* **2011**, *103*, 980–987. [CrossRef]
6. Alberghini, B.; Vicino, M.; Zanetti, F.; Silvestre, S.; Haslam, R.; Zegada-Lizarazu, W.; Monti, A. Assessing Different Physiological, Seed Yield and Quality Responses of Camelina Lines to Drought. *Ind. Crops Prod.* **2025**, *234*, 121528. [CrossRef]
7. Obour, A.K.; Chen, C.; Sintim, H.Y.; McVay, K.; Lamb, P.; Obeng, E.; Mohammed, Y.A.; Khan, Q.; Afshar, R.K.; Zheljazkov, V.D. *Camelina sativa* as a Fallow Replacement Crop in Wheat-Based Crop Production Systems in the US Great Plains. *Ind. Crops Prod.* **2018**, *111*, 22–29. [CrossRef]
8. Blackshaw, R.E.; Johnson, E.N.; Gan, Y.; May, W.E.; McAndrew, D.W.; Barthet, V.; McDonald, T.; Wispinski, D. Alternative Oilseed Crops for Biodiesel Feedstock on the Canadian Prairies. *Can. J. Plant Sci.* **2011**, *91*, 889–896. [CrossRef]
9. Eberle, C.A.; Thom, M.D.; Nemec, K.T.; Forcella, F.; Lundgren, J.G.; Gesch, R.W.; Riedell, W.E.; Papiernik, S.K.; Wagner, A.; Peterson, D.H.; et al. Using Pennycress, Camelina, and Canola Cash Cover Crops to Provision Pollinators. *Ind. Crops Prod.* **2015**, *75*, 20–25. [CrossRef]
10. Berti, M.; Gesch, R.; Johnson, B.; Ji, Y.; Seames, W.; Aponte, A. Double- and Relay-Cropping of Energy Crops in the Northern Great Plains, USA. *Ind. Crops Prod.* **2015**, *75*, 26–34. [CrossRef]
11. Berti, M.; Samarappuli, D.; Johnson, B.L.; Gesch, R.W. Integrating Winter Camelina into Maize and Soybean Cropping Systems. *Ind. Crops Prod.* **2017**, *107*, 595–601. [CrossRef]

12. Wang, Z.; Feser, J.S.; Lei, T.; Gupta, A.K. Performance and Emissions of Camelina Oil Derived Jet Fuel Blends under Distributed Combustion Condition. *Fuel* **2020**, *271*, 117685. [CrossRef]
13. Moore, R.H.; Thornhill, K.L.; Weinzierl, B.; Sauer, D.; D'Ascoli, E.; Kim, J.; Lichtenstern, M.; Scheibe, M.; Beaton, B.; Beyersdorf, A.J.; et al. Biofuel Blending Reduces Particle Emissions from Aircraft Engines at Cruise Conditions. *Nature* **2017**, *543*, 411–415. [CrossRef] [PubMed]
14. Burron, S.; Richards, T.; Patterson, K.; Grant, C.; Akhtar, N.; Trevizan, L.; Pearson, W.; Shoveller, A.K. Safety of Dietary Camelina Oil Supplementation in Healthy, Adult Dogs. *Animals* **2021**, *11*, 2603. [CrossRef] [PubMed]
15. Richards, T.; Burron, S.; McCorkell, T.C.; Trevizan, L.; Patterson, K.; Minikhiem, D.; Ma, D.W.L.; Pearson, W.; Shoveller, A.K. Effects of Dietary Camelina, Flaxseed, and Canola Oil Supplementation on Transepidermal Water Loss, Skin and Coat Health Parameters, and Plasma Prostaglandin E2, Glycosaminoglycan, and Nitric Oxide Concentrations in Healthy Adult Horses. *J. Anim. Sci.* **2023**, *101*, skad373. [CrossRef] [PubMed]
16. Tocher, D.R.; Sprague, M.; Han, L.; Sayanova, O.; Norambuena, F.; Napier, J.A.; Betancor, M.B. Inclusion of Oil from Transgenic *Camelina sativa* in Feed Effectively Supplies EPA and DHA to Atlantic Salmon (*Salmo salar*) Grown to Market Size in Seawater Pens. *Food Chem.* **2024**, *456*, 139414. [CrossRef] [PubMed]
17. Jalili, C.; Talebi, S.; Mehrabani, S.; Bagheri, R.; Wong, A.; Amirian, P.; Zarpoosh, M.; Ghoreishy, S.M.; Kermani, M.A.H.; Moradi, S. Effects of Camelina Oil Supplementation on Lipid Profile and Glycemic Control: A Systematic Review and Dose–response Meta-Analysis of Randomized Clinical Trials. *Lipids Health Dis.* **2022**, *21*, 132. [CrossRef] [PubMed]
18. Dobrzyńska, M.A.; Przysławski, J. The Effect of Camelina Oil (α-Linolenic Acid) and Canola Oil (Oleic Acid) on Lipid Profile, Blood Pressure, and Anthropometric Parameters in Postmenopausal Women. *Arch. Med. Sci.* **2021**, *17*, 1566–1574. [CrossRef] [PubMed]
19. Haslam, R.P.; Michaelson, L.V.; Eastmond, P.J.; Napier, J.A. Born of Frustration: The Emergence of *Camelina sativa* as a Platform for Lipid Biotechnology. *Plant Physiol.* **2025**, *197*, kiaf009. [CrossRef] [PubMed]
20. Bird, K.A.; Brock, J.R.; Grabowski, P.P.; Harder, A.M.; Healy, A.L.; Shu, S.; Barry, K.; Boston, L.; Daum, C.; Guo, J.; et al. Allopolyploidy Expanded Gene Content but Not Pangenomic Variation in the Hexaploid Oilseed *Camelina sativa*. *Genetics* **2025**, *229*, iyae183. [CrossRef] [PubMed]
21. Kagale, S.; Nixon, J.; Khedikar, Y.; Pasha, A.; Provart, N.J.; Clarke, W.E.; Bollina, V.; Robinson, S.J.; Coutu, C.; Hegedus, D.D.; et al. The Developmental Transcriptome Atlas of the Biofuel Crop *Camelina sativa*. *Plant J.* **2016**, *88*, 879–894. [CrossRef] [PubMed]
22. Lu, C.; Kang, J. Generation of Transgenic Plants of a Potential Oilseed Crop *Camelina sativa* by *Agrobacterium*-Mediated Transformation. *Plant Cell Rep.* **2008**, *27*, 273–278. [CrossRef] [PubMed]
23. Ghidoli, M.; Ponzoni, E.; Araniti, F.; Miglio, D.; Pilu, R. Genetic Improvement of *Camelina sativa* (L.) Crantz: Opportunities and Challenges. *Plants* **2023**, *12*, 570. [CrossRef] [PubMed]
24. Yuan, L.; Li, R. Metabolic Engineering a Model Oilseed *Camelina sativa* for the Sustainable Production of High-Value Designed Oils. *Front. Plant Sci.* **2020**, *11*, 11. [CrossRef] [PubMed]
25. Obour, A.K.; Obeng, E.; Mohammed, Y.A.; Ciampitti, I.A.; Durrett, T.P.; Aznar-Moreno, J.A.; Chen, C. Camelina Seed Yield and Fatty Acids as Influenced by Genotype and Environment. *Agron. J.* **2017**, *109*, 947–956. [CrossRef]
26. Campbell, M.C.; Rossi, A.F.; Erskine, W. Camelina (*Camelina sativa* (L.) Crantz): Agronomic Potential in Mediterranean Environments and Diversity for Biofuel and Food Uses. *Crop Pasture Sci.* **2013**, *64*, 388–398. [CrossRef]
27. Angelini, L.G.; Abou Chehade, L.; Foschi, L.; Tavarini, S. Performance and Potentiality of Camelina (*Camelina sativa* L. Crantz) Genotypes in Response to Sowing Date under Mediterranean Environment. *Agronomy* **2020**, *10*, 1929. [CrossRef]
28. Gesch, R.W.; Long, D.S.; Palmquist, D.; Allen, B.L.; Archer, D.W.; Brown, J.; Davis, J.B.; Hatfield, J.L.; Jabro, J.D.; Kiniry, J.R.; et al. Agronomic Performance of Brassicaceae Oilseeds in Multiple Environments Across the Western USA. *BioEnergy Res.* **2019**, *12*, 509–523. [CrossRef]
29. Ghidoli, M.; Geuna, F.; De Benedetti, S.; Frazzini, S.; Landoni, M.; Cassani, E.; Scarafoni, A.; Rossi, L.; Pilu, S.R. Genetic Study of *Camelina sativa* Oilseed Crop and Selection of a New Variety by the Bulk Method. *Front. Plant Sci.* **2024**, *15*, 1385332. [CrossRef] [PubMed]
30. Sainger, M.; Jaiwal, A.; Sainger, P.A.; Chaudhary, D.; Jaiwal, R.; Jaiwal, P.K. Advances in Genetic Improvement of *Camelina sativa* for Biofuel and Industrial Bio-Products. *Renew. Sustain. Energy Rev.* **2017**, *68*, 623–637. [CrossRef]
31. Ruiz-Lopez, N.; Haslam, R.P.; Napier, J.A.; Sayanova, O. Successful High-Level Accumulation of Fish Oil Omega-3 Long-Chain Polyunsaturated Fatty Acids in a Transgenic Oilseed Crop. *Plant J.* **2014**, *77*, 198–208. [CrossRef] [PubMed]
32. Ozseyhan, M.E.; Kang, J.; Mu, X.; Lu, C. Mutagenesis of the *FAE1* Genes Significantly Changes Fatty Acid Composition in Seeds of *Camelina sativa*. *Plant Physiol. Biochem.* **2018**, *123*, 1–7. [CrossRef] [PubMed]
33. Abdullah, H.M.; Pang, N.; Chilcoat, B.; Shachar-Hill, Y.; Schnell, D.J.; Dhankher, O.P. Overexpression of the *Phosphatidylcholine: Diacylglycerol Cholinephosphotransferase* (*PDCT*) Gene Increases Carbon Flux toward Triacylglycerol (TAG) Synthesis in *Camelina sativa* Seeds. *Plant Physiol. Biochem.* **2024**, *208*, 108470. [CrossRef] [PubMed]

34. Alkotami, L.; White, D.J.; Schuler, K.M.; Esfahanian, M.; Jarvis, B.A.; Paulson, A.E.; Koley, S.; Kang, J.; Lu, C.; Allen, D.K.; et al. Targeted Engineering of Camelina and Pennycress Seeds for Ultrahigh Accumulation of Acetyl-TAG. *Proc. Natl. Acad. Sci. USA* **2024**, *121*, e2412542121. [CrossRef] [PubMed]
35. Jiang, W.Z.; Henry, I.M.; Lynagh, P.G.; Comai, L.; Cahoon, E.B.; Weeks, D.P. Significant Enhancement of Fatty Acid Composition in Seeds of the Allohexaploid, *Camelina sativa*, Using CRISPR/Cas9 Gene Editing. *Plant Biotechnol. J.* **2017**, *15*, 648–657. [CrossRef] [PubMed]
36. Blume, R.Y.; Hotsuliak, V.Y.; Nazarenus, T.J.; Cahoon, E.B.; Blume, Y.B. Genome-Wide Identification and Diversity of *FAD2*, *FAD3* and *FAE1* Genes in Terms of Biotechnological Importance in *Camelina* species. *BMC Biotechnol.* **2024**, *24*, 107. [CrossRef] [PubMed]
37. Kagale, S.; Koh, C.; Nixon, J.; Bollina, V.; Clarke, W.E.; Tuteja, R.; Spillane, C.; Robinson, S.J.; Links, M.G.; Clarke, C.; et al. The Emerging Biofuel Crop *Camelina sativa* Retains a Highly Undifferentiated Hexaploid Genome Structure. *Nat. Commun.* **2014**, *5*, 3706. [CrossRef] [PubMed]
38. Rakow, G.; Raney, J.P. Present Status and Future Perspectives of Breeding for Seed Quality in Brassica Oilseed Crops. In *Proceedings of the 11th International Rapeseed Congress*; The Royal Veterinary and Agricultural University: Copenhagen, Denmark, 2003; pp. 181–185.
39. Akhov, L.; Ashe, P.; Tan, Y.; Datla, R.; Selvaraj, G. Proanthocyanidin Biosynthesis in the Seed Coat of Yellow-Seeded, Canola Quality *Brassica napus* YN01-429 Is Constrained at the Committed Step Catalyzed by Dihydroflavonol 4-Reductase. *Botany* **2009**, *87*, 616–625. [CrossRef]
40. Chen, M.; Wang, Z.; Zhu, Y.; Li, Z.; Hussain, N.; Xuan, L.; Guo, W.; Zhang, G.; Jiang, L. The Effect of *TRANSPARENT TESTA2* on Seed Fatty Acid Biosynthesis and Tolerance to Environmental Stresses during Young Seedling Establishment in Arabidopsis. *Plant Physiol.* **2012**, *160*, 1023–1036. [CrossRef] [PubMed]
41. Qian, L.; Yang, L.; Liu, X.; Wang, T.; Kang, L.; Chen, H.; Lu, Y.; Zhang, Y.; Yang, S.; You, L.; et al. Natural Variations in *TT8* and Its Neighboring *STK* Confer Yellow Seed with Elevated Oil Content in *Brassica juncea*. *Proc. Natl. Acad. Sci. USA* **2025**, *122*, e2417264122. [CrossRef] [PubMed]
42. Cheng, H.; Cai, S.; Hao, M.; Cai, Y.; Wen, Y.; Huang, W.; Mei, D.; Hu, Q. Targeted Mutagenesis of *BnTTG1* Homologues Generated Yellow-Seeded Rapeseed with Increased Oil Content and Seed Germination under Abiotic Stress. *Plant Physiol. Biochem.* **2024**, *206*, 108302. [CrossRef] [PubMed]
43. Li, H.; Yu, K.; Zhang, Z.; Yu, Y.; Wan, J.; He, H.; Fan, C. Targeted Mutagenesis of Flavonoid Biosynthesis Pathway Genes Reveals Functional Divergence in Seed Coat Colour, Oil Content and Fatty Acid Composition in *Brassica napus* L. *Plant Biotechnol. J.* **2024**, *22*, 445–459. [CrossRef] [PubMed]
44. Cai, Y.; Liang, Y.; Shi, H.; Cui, J.; Prakash, S.; Zhang, J.; Anaokar, S.; Chai, J.; Schwender, J.; Lu, C.; et al. Creating Yellow Seed *Camelina sativa* with Enhanced Oil Accumulation by CRISPR-Mediated Disruption of *Transparent Testa 8*. *Plant Biotechnol. J.* **2024**, *22*, 2773–2784. [CrossRef] [PubMed]
45. Nesi, N.; Debeaujon, I.; Jond, C.; Pelletier, G.; Caboche, M.; Lepiniec, L. The *TT8* Gene Encodes a Basic Helix-Loop-Helix Domain Protein Required for Expression of *DFR* and *BAN* Genes in Arabidopsis Siliques. *Plant Cell* **2000**, *12*, 1863–1878. [CrossRef] [PubMed]
46. Chen, M.; Xuan, L.; Wang, Z.; Zhou, L.; Li, Z.; Du, X.; Ali, E.; Zhang, G.; Jiang, L. TRANSPARENT TESTA8 Inhibits Seed Fatty Acid Accumulation by Targeting Several Seed Development Regulators in Arabidopsis. *Plant Physiol.* **2014**, *165*, 905–916. [CrossRef] [PubMed]
47. Shen, S.; Tang, Y.; Zhang, C.; Yin, N.; Mao, Y.; Sun, F.; Chen, S.; Hu, R.; Liu, X.; Shang, G.; et al. Metabolite Profiling and Transcriptome Analysis Provide Insight into Seed Coat Color in *Brassica juncea*. *Int. J. Mol. Sci.* **2021**, *22*, 7215. [CrossRef] [PubMed]
48. Ding, X.; Duckworth, S.; Southworth, M.; Lipton, A.; Clendinen, C.S.; Gautam, B.; Esfahanian, M.; Velickovic, D.; Sedbrook, J.C.; Handakumbura, P. A Pennycress *transparent testa 8* Knockout Mutant Has Drastic Changes in Seed Coat Anatomy and Chemical Compositions. *J. Exp. Bot.* **2026**, *77*, 2106–2134. [CrossRef] [PubMed]
49. Zhai, Y.; Yu, K.; Cai, S.; Hu, L.; Amoo, O.; Xu, L.; Yang, Y.; Ma, B.; Jiao, Y.; Zhang, C.; et al. Targeted Mutagenesis of *BnTT8* Homologs Controls Yellow Seed Coat Development for Effective Oil Production in *Brassica napus* L. *Plant Biotechnol. J.* **2020**, *18*, 1153–1168. [CrossRef] [PubMed]
50. Jiang, Y.; Caldwell, C.D. Effect of Nitrogen Fertilization on Camelina Seed Yield, Yield Components, and Downy Mildew Infection. *Can. J. Plant Sci.* **2016**, *96*, 17–26. [CrossRef]
51. Zanetti, F.; Alberghini, B.; Marjanović Jeromela, A.; Grahovac, N.; Rajković, D.; Kiprovski, B.; Monti, A. Camelina, an Ancient Oilseed Crop Actively Contributing to the Rural Renaissance in Europe. A Review. *Agron. Sustain. Dev.* **2021**, *41*, 2. [CrossRef]
52. Dlakić, D.; Munson, P.; Lu, C.; Fischer, A. Efficient Nitrogen Remobilization and Nitrogen Partitioning to Seeds in *Camelina sativa* Grown under Contrasting Nitrogen Regimes. *BMC Plant Biol.* **2025**, *25*, 1740. [CrossRef] [PubMed]

53. Gregg, S.; Strock, J.S.; Gesch, R.W.; Coulter, J.A.; Garcia y Garcia, A. Rate and Time of Nitrogen Fertilizer Application for Winter Camelina. *Agron. J.* **2024**, *116*, 1804–1816. [CrossRef]
54. Wysocki, D.J.; Chastain, T.G.; Schillinger, W.F.; Guy, S.O.; Karow, R.S. Camelina: Seed Yield Response to Applied Nitrogen and Sulfur. *Field Crops Res.* **2013**, *145*, 60–66. [CrossRef]
55. Chen, C.; Bekkerman, A.; Afshar, R.K.; Neill, K. Intensification of Dryland Cropping Systems for Bio-Feedstock Production: Evaluation of Agronomic and Economic Benefits of *Camelina sativa*. *Ind. Crops Prod.* **2015**, *71*, 114–121. [CrossRef]
56. Johnson, G.A.; Wells, M.S.; Anderson, K.; Gesch, R.W.; Forcella, F.; Wyse, D.L. Yield Tradeoffs and Nitrogen between Pennycress, Camelina, and Soybean in Relay- and Double-Crop Systems. *Agron. J.* **2017**, *109*, 2128–2135. [CrossRef]
57. Johnson, J.M.F.; Gesch, R.W. Calendula and Camelina Response to Nitrogen Fertility. *Ind. Crops Prod.* **2013**, *43*, 684–691. [CrossRef]
58. Berti, M.T.; Morocho-Lema, M.; Anderson, J.V.; Lizarazo-Torres, C. Nitrogen Rates Affect Seed Yield and Carbon Intensity in Spring and Winter Camelina. *Ind. Crops Prod.* **2025**, *233*, 121473. [CrossRef]
59. Malhi, S.S.; Johnson, E.N.; Hall, L.M.; May, W.E.; Phelps, S.; Nybo, B. Effect of Nitrogen Fertilizer Application on Seed Yield, N Uptake, and Seed Quality of *Camelina sativa*. *Can. J. Soil Sci.* **2014**, *94*, 35–47. [CrossRef]
60. Bronson, K.F.; Hunsaker, D.J.; Thorp, K.R. Nitrogen Fertilizer and Irrigation Effects on Seed Yield and Oil in Camelina. *Agron. J.* **2019**, *111*, 1712–1719. [CrossRef]
61. Fañanás-Pueyo, I.; Carrera-Castaño, G.; Pernas, M.; Oñate-Sánchez, L. Signalling and Regulation of Plant Development by Carbon/Nitrogen Balance. *Physiol. Plant.* **2025**, *177*, e70228. [CrossRef] [PubMed]
62. Dodd, I.C.; Tan, L.P.; He, J. Do Increases in Xylem Sap ph and/or ABA Concentration Mediate Stomatal Closure Following Nitrogen Deprivation? *J. Exp. Bot.* **2003**, *54*, 1281–1288. [CrossRef] [PubMed]
63. Lee, J.M.; Goh, J.C.; Koh, E.; Mutwil-Anderwald, D.; Mutwil, M. Cross-Species Analysis of Abiotic Stress in Hydroponic Leafy Crops Reveals Conserved Regulatory Networks and Key Divergences. *Front. Plant Sci.* **2025**, *16*, 1613016. [CrossRef] [PubMed]
64. Colmenero-Flores, J.M.; Franco-Navarro, J.D.; Cubero-Font, P.; Peinado-Torrubia, P.; Rosales, M.A. Chloride as a Beneficial Macronutrient in Higher Plants: New Roles and Regulation. *Int. J. Mol. Sci.* **2019**, *20*, 4686. [CrossRef] [PubMed]
65. Teakle, N.L.; Tyerman, S.D. Mechanisms of Cl^- Transport Contributing to Salt Tolerance. *Plant Cell Environ.* **2010**, *33*, 566–589. [CrossRef] [PubMed]
66. Fan, H.; Quan, S.; Ye, Q.; Zhang, L.; Liu, W.; Zhu, N.; Zhang, X.; Ruan, W.; Yi, K.; Crawford, N.M.; et al. A Molecular Framework Underlying Low-Nitrogen-Induced Early Leaf Senescence in *Arabidopsis thaliana*. *Mol. Plant* **2023**, *16*, 756–774. [CrossRef] [PubMed]
67. Havé, M.; Marmagne, A.; Chardon, F.; Masclaux-Daubresse, C. Nitrogen Remobilization during Leaf Senescence: Lessons from Arabidopsis to Crops. *J. Exp. Bot.* **2017**, *68*, 2513–2529. [CrossRef] [PubMed]
68. Decker, S.; Craine, W.; Paulitz, T.; Chen, C.; Lu, C. Genomic Analysis of the Natural Variation of Fatty Acid Composition in Seed Oils of *Camelina sativa*. *Biology* **2025**, *14*, 1199. [CrossRef] [PubMed]
69. Lenth, R.V.; Piaskowski, J.; Banfai, B.; Bolker, B.; Buerkner, P.; Giné-Vázquez, I.; Hervé, M.; Jung, M.; Love, J.; Miguez, F.; et al. Emmeans: Estimated Marginal Means, Aka Least-Squares Means. R Package Version 2.0.5. 2026. Available online: https://rvlenth.github.io/emmeans/ (accessed on 31 October 2026).
70. Wickham, H. *Ggplot2: Elegant Graphics for Data Analysis*; Use R!; Springer: New York, NY, USA, 2016; 260p.
71. Clemente, C.; Ercolini, L.; Rossi, A.; Foschi, L.; Grossi, N.; Angelini, L.G.; Tavarini, S.; Silvestri, N. Spectral Response of Camelina (*Camelina sativa* (L.) Crantz) to Different Nitrogen Fertilization Regimes under Mediterranean Conditions. *Agronomy* **2023**, *13*, 1539. [CrossRef]
72. Vollmann, J.; Moritz, T.; Kargl, C.; Baumgartner, S.; Wagentristl, H. Agronomic Evaluation of Camelina Genotypes Selected for Seed Quality Characteristics. *Ind. Crops Prod.* **2007**, *26*, 270–277. [CrossRef]
73. Buer, C.S.; Djordjevic, M.A. Architectural Phenotypes in the *transparent testa* Mutants of *Arabidopsis thaliana*. *J. Exp. Bot.* **2009**, *60*, 751–763. [CrossRef] [PubMed]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.