

Agronomic evaluation of a Chinese camelina [*Camelina sativa* (L.) Crantz] cultivar in multiple semi-arid locations of northern China

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Highlights

- Agronomic performance of spring- and fall-seeded camelina cv 'Xiaoguo' was evaluated in semi-arid regions of China.
- Camelina cv 'Xiaoguo' showed good adaptability to the different growing environments in central and northern China.
- Spring camelina seedling transplanting provides an alternative approach to increase yield.
- Bioactive compounds (i.e., flavonoids, β -sitosterol) were detected in camelina seed.
- Camelina is promising as edible oil, biodiesel, and pharmaceutical source in semi-arid regions of China.

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See online Appendix for Supplementary Tables.

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Abstract

Agronomic performance evaluations of spring- and fall-seeded camelina [*Camelina sativa* (L.) Crantz] genotypes in China are limited despite a long tradition of growing this crop in the northern parts of the country (i.e., Gansu and Xinjiang provinces). A field experiment (2015-2019) was conducted to determine the seed yield and seed quality of spring- and fall-seeded Chinese camelina cv 'Xiaoguo' across five locations in semi-arid regions of China. The results showed that spring- and fall-seeded camelina 'Xiaoguo' has highly adapted to the different growing environments in central and northern China. Location and season were the key determinants for camelina seed yield, but not the year. Across locations and years, the mean seed and oil yields for fall-seeded camelina were more significant than those of spring-seeded camelina (seed yield: 2115 vs 1751 kg ha⁻¹; oil yield: 672 vs 547 kg ha⁻¹). Fall-seeded camelina at Huangzhong had the highest mean seed (2497 kg ha⁻¹) and oil yield (784 kg ha⁻¹), followed by fall-seeded in Anyang (2219 and 714 kg ha⁻¹), compared to other locations (range of mean: 1566-2033 and 489-639 kg ha⁻¹). The contents of saturated, monounsaturated, and polyunsaturated fatty acids in camelina seed oil varied from 11-14% (mean: 12%), 31-34% (mean: 32%), and 54-57% (mean: 56%), respectively. The mean β -sitosterol and total flavonoid contents across locations and years were 1723 μ g g⁻¹ (range: 1680-1778 μ g g⁻¹) and 3.4 mg g⁻¹ (range: 2.7-3.8 mg g⁻¹), respectively. In summary, its extensive environmental adaptability to drought and low temperature indicates that camelina is well-suited as an alternative oilseed crop or dual cropped with other crops in central and northern China or other countries with similar agricultural conditions. Camelina also showed great potential as a source of bioactive compounds (i.e., flavonoids, β -sitosterol) for pharmaceutical applications.

Introduction

Camelina [*Camelina sativa* (L.) Crantz], an oilseed of the Brassicaceae family has been grown for decades in different parts of the world (Putnam *et al.*, 1993; Zubr, 1997). Camelina seed oil and meal have a set of applications such as human food, animal feed, chemicals, and pharmaceuticals (Betancor *et al.*, 2015; Bansal and Durrett, 2016; Berti *et al.*, 2016, 2017a and 2017b). In China, camelina is a re-emerging oilseed crop with a long history as a traditional crop in northern China (*i.e.*, Gansu and Xinjiang provinces). Currently, it is mainly grown in northern and northwestern China as an alternative edible oil with an acreage of about 500 ha (Zhang, 2012; Du *et al.*, 2018). Due to its broad environmental adaptability (*i.e.*, to drought and low temperature), there is great potential for large-scale camelina cultivation in northern and northwestern China on marginal land suitable for this crop. However, information about the agronomic evaluation of field seeding camelina is limited in China.

Camelina seed oil consists of high amounts of unsaturated fatty acids, mainly linoleic and α -linolenic (Berti *et al.*, 2016). As such, camelina oil has been demonstrated to decrease significantly triacylglycerol and cholesterol in pig serum (Eidhin *et al.*, 2003). In addition to fatty acids, camelina also contains other important bioactive compounds such as tocopherols and phenols (Falk and Gugel, 2006; Salminen *et al.*, 2006). Flavonoids and β -sitosterol exhibit many bioactive functions, including antioxidation and reducing anxiety, with great potential as a source of compounds for pharmaceutical applications (Aziza *et al.*, 2010; Panayotis *et al.*, 2021). In general, camelina has both spring and winter biotypes (Berti *et al.*, 2016; Kurasiak-Popowska *et al.*, 2018; Chao *et al.*, 2019; Wittenberg *et al.*, 2019). The average seed yield of spring-seeded camelina ranged from 320 to 3300 kg ha⁻¹ in Europe and North America (Urbaniak *et al.*, 2008; Moser, 2010; Aiken *et al.*, 2015; Pavlista *et al.*, 2016; Bacenetti *et al.*, 2017; Obour *et al.*, 2017). Seed yields of winter camelina cv 'Joelle' varied from 99 to 1317 kg ha⁻¹ in North Dakota (Wittenberg *et al.*, 2020). Recently, Walia *et al.* (2021) reported 'Joelle' seed yields ranging from 704 kg ha⁻¹ in Leida, Spain, to 2095 kg ha⁻¹ in Morden, Canada. In a 5-year study in Poland, Kurasiak-Popowska *et al.* (2018) observed that the mean seed yield of eleven winter camelina genotypes was significantly higher than that of nine spring genotypes (1900 *versus* 1300 kg ha⁻¹).

A recent camelina study published by our team reported the results of a 5-year agronomic evaluation of spring-seeded camelina 'Xiaoguo' across 3 different environments in northern China (Zhang *et al.*, 2021). The results have shown that this cultivar has extensive environmental adaptability and consistently higher seed yields in different locations in northern China. It is worth noting that camelina 'Xiaoguo' has been widely grown as a spring- or fall-seeded local cultivar in Xinjiang province (between 34°25'–48°10'N and 73°40'–96°18'E) due to its superior winter hardiness and relatively high yield characteristics. In the present study, the agronomic performance of 'Xiaoguo' was evaluated further at a greater number of locations within the semi-arid region, both as a spring- and fall-seeded crop. The objectives of this study were to evaluate the seed yield and seed quality of spring- and fall-seeded 'Xiaoguo' (including seed oil content, major fatty acid groups and content, crude protein content, β -sitosterol, and total flavonoid content) in multi-locations of the semi-arid region of northern China. The experiment was conducted at five locations, including Anyang, Fuping, Huangzhong, Qingyang, and Tongliao, from 2015 to 2019.

Materials and methods

Camelina cultivar and experimental site characterisation

Camelina cv 'Xiaoguo' was used throughout the study. Camelina cv 'Xiaoguo' (origin of Xinjiang province and obtained from the Xinjiang Production and Construction Corps., China) is a local variety which could be grown both as a spring- and fall-seeded oilseed crop in northwest China due to its superior winter hardiness and relatively high seed yields (Zhang *et al.*, 2021). Seeds of camelina 'Xiaoguo' used at all experimental locations in the present study were initially produced and provided by Anyang *Camelina sativa* planting and engineering technology centre Henan province, China.

Field trials were carried out at five locations in the semi-arid region of northern China, including Anyang, Henan province; Fuping, Shanxi province; Huangzhong, Qinghai province; Qingyang, Gansu province; Tongliao, Inner Mongolia autonomous region, covering the geographical area between 35–45°N and 100–125°E (Figure 1 and Table 1). Before field seeding, soil fertility for each experimental location was determined from composite soil samples analysed at Anyang *Camelina sativa* planting and engineering technology center, Henan province, China. The soil type was determined accordingly. Briefly, the experimental locations were characterised by different soil types being fluvo-aquic soil with a pH of 6.8–7.3 at Anyang; yellow-cinnamon soil with a pH of 7.2–7.8 at Fuping; chernozem with a pH of 6.9–7.2 at Huangzhong; loessial soil with a pH of 7.3–7.9 at Qingyang; and sandy loam with a pH of 6.2–6.7 at Tongliao.

Field experiments

Agronomic evaluation of seed yield and seed quality of camelina 'Xiaoguo' was conducted at the locations mentioned above throughout 2015–2019. Detailed information on sowing season, sowing rate, fertilizer application, and sowing and harvest dates are described in Table 1. As spring-seeded camelina 'Xiaoguo' has previously been evaluated at Anyang, Huangzhong, and Qingyang between 2010–2014 (Zhang *et al.*, 2021), in the present study, only field evaluation of fall-seeded 'Xiaoguo' was conducted at these three locations (2015–2019). At the Fuping location, the treatments were spring seeding, spring field camelina seedling transplanting, and fall seeding. Spring field seedling transplanting was conducted to compare the yield potential with spring and fall seeding. Considering the high labour cost, this technique was only evaluated at the Fuping location. At the Tongliao location, agronomic evaluation of fall-seeded camelina was only conducted for two years (2015–2016 and 2016–2017) due to crop failure resulting from fall and winter sandstorms and bird damage. Therefore, the spring-seeded camelina was evaluated at this location throughout all experimental years.

Prior to seeding, weeds were controlled through an application of glyphosate at 675 g a.i. ha⁻¹ (Roundup®, AS, Monsanto Company, Shanghai, China), and all field plots were chisel ploughed and fertilised (N-P-K, 15-15-15, at the rate of 615–733 kg ha⁻¹) (Table 1). Camelina seeds (>90% germination rate) were seeded at a depth of approximately 1–1.5 cm with 15 cm row spacing using a seed drill (planting rate: 6.5–7.0 kg ha⁻¹) at all locations. At the Fuping and Tongliao locations, pre-grown camelina seedlings at the 2-leaf stage were manually transplanted into the field plots with 15–20 cm row spacing at a density of 3~5×10⁶ plants ha⁻¹ in the spring. Based on the preliminary camelina seedling trials, fall seeding took place between the first and second

week of October each year at all locations, and spring seeding occurred in early April at the Fuping and Tongliao. Depending on location, harvest occurred approximately three to five months after spring seeding and up to eight months after fall seeding (Table 1). Camelina seeding area across all experimental locations ranged from 0.7-2.8 ha in each experimental year. Camelina was the only crop at each experimental site. All trials were rain-fed and irrigation was only conducted just before or after camelina seeding based on the degree of drought. Field management followed standard agricultural practices. During the growing season, no herbicides or insecticides were applied.

Plant sampling, seed yield determination, and meteorological data collection

When camelina plants at each location reached full maturity (>90% of silicles dried up and most seeds were brown in colour), the plants were harvested (Table 1). Seed yield at all site years was determined from four randomly selected 10×10 m plots (each plot as one replication, n=4) where the plants were cut off at the stem base using a hand-held sickle and collected into cloth bags. Silicles dehiscence was not observed at the time of camelina plant harvesting. Seed yield samples from each plot were dried in mesh bags in a forced-air oven at 40°C for 60 h. Seed moisture content was determined by drying a subsample (500 g as one replication, n=3) at 60°C for 24 h in a drying oven, and seed yield was adjusted to moisture content varying from 6.3 to 9.8% depending on different sowing seasons and locations. Seed yield was reported as kg ha⁻¹ dry matter (d.m.). The remaining plants at each location were harvested with a plot combine, and seeds were prepared and stored for the following season of field sowing.

Throughout the experimental years, the daily air temperature and precipitation were recorded by a weather station located on-site or a local meteorological administration nearby. The accumulated growing days (GDD; °C d) from camelina sowing to harvest were calculated for each experimental site following Eq. (1):

$$GDD = \sum \left(\frac{T_{max} + T_{min}}{2} - T_{base} \right) \quad (1)$$

where T_{max} and T_{min} are the daily maximum and minimum air temperature, respectively, and T_{base} is a base temperature of 4°C used for the entire camelina growing cycle (Gesch and Cermak, 2011).

Seed quality evaluations

Camelina seed oil content was determined by nuclear magnetic resonance (NMR) (CNMR-1000, Wuhan Chenmu Technology Co., Ltd., China) using 10 g of seed from each replicated plot (n=3) at Anyang *Camelina sativa* planting and engineering technology center, Anyang, Henan province, China. Prior to measurement, seeds were dried for 1.5 h at 130°C in a forced-air oven and cooled for 30 min in a desiccator. The NMR was calibrated with 25 mL of pure camelina oil, and values of oil contents were reported as a percentage (%).

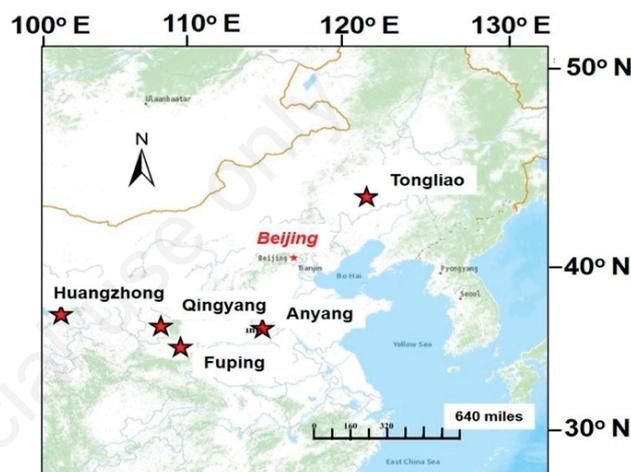


Figure 1. Agronomic performance evaluations of spring- and fall-seeded camelina cv 'Xiaoguo' in central and northern China, including Anyang (Henan province), Fuping (Shanxi province), Huangzhong (Qinghai province), Qingyang (Gansu province), and Tongliao (Inner Mongolia autonomous region) during the period of 2015-2019.

Table 1. Locations, sowing season, sowing rate/plant density, fertiliser application, sowing and harvest dates, and growing degree days for camelina cv 'Xiaoguo' during the study period of 2015-2019 across five different growing environments in central and northern China.

Location (city, province)	Coordinates	Sowing season	Sowing rate/plant density	NPK (15-15-15) (kg ha ⁻¹)	2015-2016			2016-2017			2017-2018			2018-2019		
					Sowing date	Harvest date	GDD									
Anyang, Henan	35°51'N, 114°23'E	Fall	6.5 kg ha ⁻¹	615	15 Oct.	10 Jun.	803	14 Oct.	12 Jun.	847	12 Oct.	5 Jun.	725	20 Oct.	11 Jun.	781
Fuping, Shanxi	34°44'N, 109°9'E	Fall*	6.5 kg ha ⁻¹	675	13 Oct.	15 Jun.	834	15 Oct.	13 Jun.	792	15 Oct.	18 Jun.	909	16 Oct.	13 Jun.	842
		Spring ^o	6.5 kg ha ⁻¹	680	7 Apr.	20 Jul.	1399	5 Apr.	17 Jul.	1443	1 Apr.	22 Jul.	1423	3 Apr.	15 Jul.	1356
		Spring [#]	3-5×10 ⁶ plants ha ⁻¹	680	22 Apr.	18 Jul.	1289	19 Apr.	15 Jul.	1317	15 Apr.	20 Jul.	1317	17 Apr.	11 Jul.	1293
Huangzhong, Qinghai	36°40'N, 101°31'E	Fall	6.8 kg ha ⁻¹	680	10 Oct.	8 Jun.	382	8 Oct.	9 Jun.	393	8 Oct.	12 Jun.	451	6 Oct.	7 Jun.	355
Qingyang, Gansu	35°59'N, 107°54'E	Fall	6.8 kg ha ⁻¹	733	12 Oct.	7 Jun.	1159	10 Oct.	7 Jun.	1166	12 Oct.	8 Jun.	1248	10 Oct.	10 Jun.	1039
Tongliao, Inner Mongolia	43°12'N, 121°2'E	Fall	6.7 kg ha ⁻¹	725	5 Oct.	-	-	10 Oct.	-	-	-	-	-	-	-	-
		Spring	6.7 kg ha ⁻¹	725	10 Apr.	15 Aug.	1546	10 Apr.	11 Aug.	1468	8 Apr.	13 Aug.	1444	6 Apr.	16 Aug.	1594

GDD, growing degree days. *Fall camelina seeding; ^ospring camelina seeding; [#]spring camelina seedling transplanting at Fuping during the study period of 2015-2019.

Seed protein content, seed oil fatty acid composition, seed total flavonoid, and seed β -sitosterol contents were determined at Henan Natural Product Biotechnology Co., Ltd., Zhengzhou, Henan province, China. To determine seed protein content, the total N was firstly determined from 5 g camelina seed from each replicated plot ($n=4$) using an automatic Kjeldahl apparatus (Kjeltec 8400 Analyzer Unit, FOSS Analytical AB, Hoganas, Sweden). Then, seed crude protein was calculated by multiplying percent N by 6.25.

Fatty acid methyl ester (FAME) profiles of camelina oil resulting from the cold press were analysed by gas chromatography (GC) (Shimadzu GC-2010 Plus, Shimadzu Corp. Kyoto, Japan) using the method described by Zhang *et al.* (2021). The quantity of each FAME was determined following Zhang *et al.* (2021), and the concentrations of individual fatty acids were expressed as a percentage of total fatty acid content.

Prior to determining total flavonoids, ethanolic extracts of camelina seed were prepared. Briefly, one gram of camelina seed was ground in a mortar followed by ethanolic extraction (10 mL of 80% ethanol) with continuous stirring at room temperature for 24 h. The suspension was filtered twice, and the supernatant was then concentrated in a rotary evaporator under reduced pressure at 40°C. The residue was re-dissolved in 10 mL of 80% ethanol and kept in the dark to determine the total flavonoids. The total flavonoid content in camelina seed, expressed as mg of rutin equivalents, was determined by a colourimetric method as described by Ouyang *et al.* (2021), with the following modifications: 1 mL of sample solution was added to test tubes containing 1 mL of 5% sodium nitrite and 1 mL of 10% aluminum nitrate. The mixture was allowed to stand for 6 min, and then 10 mL of 4% sodium hydroxide was added. After 30 min at room temperature, the absorbance was determined against a blank at 510 nm using a UV-visible spectrophotometer.

To determine the β -sitosterol content, five grams of camelina oil sample was transferred into a borosilicate glass test tube containing 40 mL 95% of ethanol and 8 mL 50% of potassium hydroxide water solution. The samples were mixed for 10 min using a rotator and then saponified in a water base at 60°C for 1 h. After cooling at room temperature, 80 mL of diethyl ether and 80 mL 1 M potassium hydroxide were added and mixed by rotator before centrifugation for 5 min. The diethyl ether phase was transferred to a screw borosilicate glass flask and dried under reduced pressure at 40°C. The residue was re-dissolved in 3 mL of heptane. The GC analyses were carried out using a Shimadzu GC-2010 Plus with an FID detector using an RTX-5 fused silica capillary column (30 m \times 0.25 mm \times 0.25 μ m, Restek Corp., PA, USA). The GC split valve was opened with a split ratio of 5:1, and the flow rates of FID gases of air and hydrogen were 400 and 40 mL min^{-1} , respectively. Helium was set as the carrier gas (1.0 mL min^{-1}). The oven program was 1 min at 180°C and 15°C min^{-1} to 280°C (25 min). The injector and detector temperatures were 270 and 300°C, respectively. Peak identification was by comparing the retention time of the samples peak with that of a β -sitosterol standard.

Statistical analysis

Initially, all data obtained from this study were tested for normality with the Shapiro-Wilk test and showed normal distribution of residuals. Levene's test was further conducted to check the homogeneity of variances. Finally, a two-way analysis of variance (ANOVA) was adopted to test the effect of the four locations, including Anyang, Fuping, Huangzhong, and Qingyang, and years (four years: 2015-2016, 2016-2017, 2017-2018, 2018-2019) of fall-seeded camelina (Tongliao excluded due to crop failure) on

seed yield, seed oil content, oil yield, seed protein content, saturated fatty acid, monounsaturated fatty acid, and polyunsaturated fatty acid contents, total flavonoid, and β -sitosterol contents. Additionally, the data obtained from the Fuping location were subjected to ANOVA to test i) the effect of different sowing seasons (fall- or spring-seeded camelina); and ii) different planting types (spring-seeded camelina or field camelina seedling transplanting) and years (4 years: 2015–2019) on parameters mentioned above. Mean values were presented for those reported parameters. When ANOVA revealed statistically different means, the Tukey *post-hoc* test was conducted to separate means ($P \leq 0.05$). All statistical analyses were conducted using R 3.2.4 (R Core Team, 2016).

Results and discussion

Climates and camelina development

Meteorological data during the study period (2015-2019), including mean bimonthly air temperature and accumulated bimonthly precipitation (Supplementary Table 1), were broadly comparable to the long-term average (1981-2010) at all experimental locations (Supplementary Table 2). Among all the locations, Huangzhong and Tongliao were the coldest, with the mean bimonthly air temperature ranging from -8.9 (January- February) to 16.9°C (July-August) and from -9.5 (January-February) to 25.2°C (July-August), respectively, whereas Anyang and Fuping were relatively warmer with a similar mean bimonthly air temperature range (Anyang: 0.4 - 28.3°C ; Fuping: 0.4 - 28.2°C) (Supplementary Table 1). At Qingyang, the minimum and maximum mean bimonthly air temperatures were -6.1 (January-February) and 23.2°C (July-August), respectively. Accumulated bimonthly precipitation amounts during the same period in each year at each location were generally different; however, precipitation was relatively evenly distributed throughout the growing season at all experimental locations (Supplementary Table 1). Throughout the entire experimental period, Huangzhong received relatively higher precipitation (2602 mm in total), followed by Anyang (2599 mm) and Qingyang (2182 mm), compared to Fuping (1975 mm) and Tongliao (2034 mm).

The GDD accumulated from sowing to harvest significantly varied with location and sowing season (fall or spring sowing) (Table 1). In the case of fall camelina sowing, among all the locations, the GDD was the most significant at Qingyang (1139-1248), which was comparable to the reported mean of 1250 GDD in northern Italy (fall sowing in October) (Matteo *et al.*, 2020) and 1186-1405 GDD at Fargo and Prosper, North Dakota (sowing in the 1st week of October) (Wittenberg *et al.*, 2020), but much lower than the value of fall-seeded camelina grown in Aliartos (Greece) (1758) where the mean monthly temperature during the whole growth cycle of camelina (from September to July) was 14.2°C (Walia *et al.*, 2021). The GDDs were similar at Anyang (725-847) and Fuping (792-909). By contrast, Huangzhong had the lowest GDD (355-451) with a mean monthly temperature of 1.3°C during the camelina growth cycle, which was comparable to the value calculated for camelina (247-549) at Storrs, Connecticut (planting between the 2nd and 3rd week of October) where the mean monthly temperature was 2.3°C during the crop growth cycle (Zhang *et al.*, 2019). At Tongliao, fall camelina sowing was only conducted for two years (2015-2016 and 2016-2017) due to crop failure caused by a heavy sandstorm and bird damage during the early stages of camelina development, indicating potential limitations when

camelina is seeded as a winter annual in this area. Spring camelina sowing in the present study was characterised by higher GDD, ranging from 1217–1443 at Fuping and 1444–1594 at Tongliao, respectively, compared to fall-seeded camelina. This is in contrast to studies that reported higher GDD for fall-seeded camelina (Walia *et al.*, 2018; Krzyżaniak *et al.*, 2019). Differences in GDD between these studies may be due to the different base temperatures (4 vs 5°C), genotype used, and/or growing temperature conditions. The GDD derived from spring camelina sowing in the present study were consistent with the reported values of 1101–1216 at Morris, Minnesota (Gesch, 2012), 1096–1411 across multi-locations in Europe and Canada (Zanetti *et al.*, 2017), 1019–1389 at Olsztyn, Poland (Krzyżaniak *et al.*, 2019), and mean ranges of 971–1037 at Sheridan, Wyoming (Sintim *et al.*, 2016).

Crop yield of fall- or spring-seeded camelina

Although camelina has recently gained considerable research interest due to its multiple applications (*i.e.*, biofuels, oleochemical compounds, food, and feed) in both Europe and North America (Gesch and Archer, 2013; Augustin *et al.*, 2015; Bansal and Durrett, 2016; Berti *et al.*, 2017a, 2017b), it is considered a relatively new oilseed *Brassicaceae* in Asian countries such as China. We previously evaluated the spring-seeded ‘Xiaoguo’ in northern China. Here, a further agronomic evaluation of this cultivar seeded in fall or spring was conducted at more wide-ranging locations in central and northern China.

Fall-seeded camelina

In the present study, the fall-seeded camelina ‘Xiaoguo’ grew in all test environments (except for Tongliao), surviving low winter temperatures, thus displaying its extensive environmental adaptability (Table 1). Seed and oil yield of fall-seeded camelina ‘Xiaoguo’ were significantly affected by location but not year (Table 2); both traits varied greatly, ranging from 1708–2497 and 599–1059 kg ha⁻¹ (Figure 2). Among the test locations, ‘Xiaoguo’ grown at Huangzhong had the highest seed (2497 kg ha⁻¹) and oil yield (1059 kg ha⁻¹) in comparison to the lowest seed (1708 kg ha⁻¹) and oil yield (667 kg ha⁻¹) at Qingyang. The relatively cooler temperature (mean monthly temperature: 5.1°C; range: -8.9–17.3°C) coupled with evenly distributed precipitation throughout the growing season (accumulated yearly precipitation: 1200 mm) at Huangzhong appears to have favored camelina seed and oil production (Supplementary Table 1). Data reported by others also indicated that relatively cool conditions are associated with higher camelina seed and oil yield (Gesch, 2014; Gugel and Falk, 2006). Concerning the other two locations, Anyang produced a relatively higher seed (2219 kg ha⁻¹) and oil yield (936 kg ha⁻¹) across experimental years compared to Fuping (seed yield: 2033; oil yield: 842 kg ha⁻¹) (Figure 2). When averaged across experimental locations and years, ‘Xiaoguo’ showed mean seed and oil yields of 2115 and

794 kg ha⁻¹, respectively, which fell within the range of seed yield for several winter camelina genotypes (mean: 1905 kg ha⁻¹; range: 840–2795 kg ha⁻¹) evaluated in western Poland (Kurasiak-Popowska *et al.*, 2018). The yield data in this study were significantly greater than the reported values for a fall-seeded camelina cultivar ‘Italia’ (seed yield: 820 kg ha⁻¹; oil yield: 320 kg ha⁻¹) evaluated in Bologna, Italy, under Mediterranean climate conditions (Matteo *et al.*, 2020).

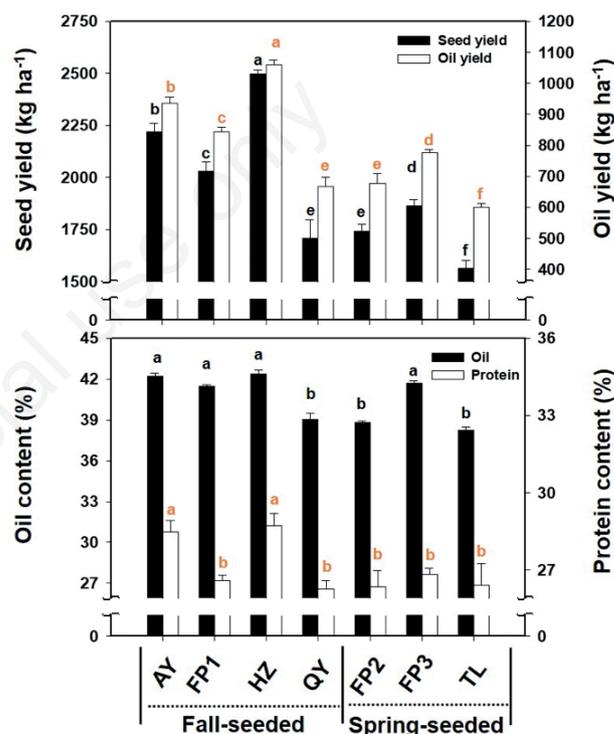


Figure 2. The mean seed yield, oil yield, oil content, and protein content of spring- and fall-seeded camelina cv ‘Xiaoguo’ across the experimental years (2015–2019) evaluated at Anyang (AY), Fuping (FP), Huangzhong (HZ), Qingyang (QY), and Tongliao (TL). FP1, FP2, and FP3 represent fall camelina seeding, spring camelina seeding, and spring camelina seedling transplanting at Fuping, respectively. Different letters represent the significant different values determined by Tukey *post-hoc* test ($P \leq 0.05$).

Table 2. The ANOVA for factors effect and interactions of fall-seeded camelina cv ‘Xiaoguo’ at Anyang, Fuping, Huangzhong, and Qingyang during 2015–2019 on seed yield (SY), oil content (OC), oil yield (OY), protein content (PC), saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA), total flavonoids (TF), and β -sitosterol.

Source of variation	DF	SY P	OC P	OY P	PC P	SFA P	MUFA P	PUFA P	TF P	β -sitosterol P
Location (L)	3	<0.001***	<0.001***	<0.001***	<0.001***	0.003**	0.03*	0.04*	0.02*	0.03*
Year (Y)	3	0.07ns	0.6ns	0.07ns	0.52ns	0.02*	0.04*	0.04*	0.68ns	0.15ns
L × Y	9	0.28ns	0.8ns	0.37ns	0.14ns	0.002**	0.54ns	0.35ns	0.36ns	0.7ns

*, **, and *** represent significant at 0.05, 0.01, and 0.001 probability level, respectively. ns, not significant.

Spring-seeded camelina

The seed and oil yield of spring-seeded camelina 'Xiaoguo' in northern China, including Anyang, Huangzhong, and Qingyang, has recently been published (mean seed yield: 1946 kg ha⁻¹; mean oil yield: 598 kg ha⁻¹) (Zhang *et al.*, 2021). In the present study, the spring seeding of this cultivar at Fuping and Tongliao produced a mean seed and oil yield of 1654 and 637 kg ha⁻¹, respectively. However, when averaged across experimental locations (5 different environments) and years (2010-2019) (data of 2010-2014 from Zhang *et al.*, 2021), the mean seed and oil yields of spring-seeded camelina 'Xiaoguo' were 1751 and 657 kg ha⁻¹, respectively, which were 364 and 147 kg ha⁻¹ lower than the mean yield of fall-seeded 'Xiaoguo' in the present study. Similarly, Kurasiak-Popowska *et al.* (2018) reported that the mean seed yield of eleven winter camelina genotypes was significantly higher than that of nine spring cultivars (1905 vs 1300 kg ha⁻¹) in western Poland. The significant effect of sowing season (fall or spring camelina seeding) on seed and oil yield was also demonstrated by ANOVA with the 4-year camelina seeding data at Fuping in the present study (Table 3).

Spring camelina seedling transplanting

To the best of the authors' knowledge, there is no study on the evaluation of the productive performance of field transplanting of camelina seedlings. To evaluate and compare the yield differences between spring-seeded camelina and spring seedling transplanted, spring camelina seedling transplanting ('Xiaoguo' at 2-3 leaf stage; planting density: 3~5×10⁶ plants ha⁻¹) was undertaken at Fuping during the 4-year study (Table 1). In comparison with spring-seeded camelina, the camelina field transplanting required less GDD to reach harvest maturity (mean: 1279 vs 1405) with a higher mean seed (1864 vs 1741 kg ha⁻¹) and oil yield (778 vs 677 kg ha⁻¹) (Figure 2; Table 1). The ANOVA revealed a significant effect of planting types on camelina crop yield (Table 3). Considering the labour cost for seedling transplanting, it would not be a good option for large-scale camelina cropping. However, the relatively higher crop yield from the seedling transplanting (compared to seed sowing) provides an alternative approach, especially in smallholder farming systems in Asia (*i.e.*, China, Korea), to increase the yield of the camelina crop.

Seed quality of fall- and spring-seeded camelina

The ANOVA showed that camelina oil content was significantly affected by location, sowing season (fall or spring camelina seeding), and planting type (seed sowing or seedling transplanting), but not year (Tables 2 and 3). The average oil content across

the 4-year study for oil content of fall-seeded camelina 'Xiaoguo' was higher at Anyang (mean: 42.2%; range: 40-44%) and Huangzhong (mean: 42.4%; range: 40-45%) compared to that of camelina seeded at Fuping (mean: 41.4%; range: 29-45%) and Qingyang (39.1%; range: 35-41%) (Figure 2). In addition to the favourable weather conditions (even and higher precipitation) during the crop growth and development at Anyang and Huangzhong, the relatively appropriate soil type and pH (Anyang: fluvo-aquic soil with a pH of 6.8-7.3; Huangzhong: chernozem with a pH of 6.9-7.2) compared to other locations (Fuping: yellow-cinnamon soil with a pH of 7.2-7.8; Qingyang: loessial soil with a pH of 7.3-7.9) might be the key factor that resulted in the higher oil content in camelina (Moore *et al.*, 2019).

To the best of the authors' knowledge, few reports apart from the current study evaluate the oil content of a camelina genotype seeded both in spring and fall. However, in the present study, when averaged across locations and years, the mean oil content of fall-seeded camelina 'Xiaoguo' was significantly higher ($P < 0.05$) (mean: 41.5%) than that of spring-seeded (mean: 38.4%). This may be explained by the relatively cooler temperature and the longer growth period during the fall growing season favouring seed development and accumulating more oil in camelina seed compared to the spring growing season (Berti *et al.*, 2011; Gesch, 2014). Other studies have also reported that winter camelina 'Joelle' showed a relatively higher mean oil content ranging from 35 to 42% when seeded in September or October in multi-environments across Europe and Canada (Gesch *et al.*, 2014; Walia *et al.*, 2018 and 2021). Instead, a lower oilseed content of 'Joelle' (25-30%) across six different seeding dates (30th June-23rd October) has been reported at Fargo and Prosper, North Dakota (Wittenberg *et al.*, 2020). All these data indicate the significant environmental effect such as cumulative precipitation, winter temperature, and snow cover on plant stand establishment, winter survival, and persistence to the following spring for fall-seeded camelina compared to spring-seeded. Those eventually resulted in a wide variation in crop yield and seed quality, including oil content.

The significant effect of planting type on the oil content of spring-seeded camelina 'Xiaoguo' was revealed by ANOVA (Table 3). As shown in Figure 2, at Fuping, while a relatively less GDD was required for spring seedling transplanted plants to reach crop maturity compared to direct seeding, the oil content for the former was significantly higher ($P < 0.05$) (mean: 41.7%) compared to that of for the latter (mean: 38.8%). As described above, while spring field seedling transplanting showed a higher seed and oil yield compared to the direct seeding, due to the expensive labour cost, it would not be appropriate for the large-scale cultivation of cameli-

Table 3. The ANOVA for factors effect and interactions of spring- or fall-seeded camelina cv 'Xiaoguo', or spring seedling transplanting at Fuping during 2015-2019 on seed yield (SY), oil content (OC), oil yield (OY), protein content (PC), saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA), total flavonoids (TF), and β -sitosterol.

Variables	Source	DF	SY P	OC P	OY P	PC P	SFA P	MUFA P	PUFA P	TF P	β -sitosterol P
Fall or spring camelina seed sowing season	Sowing (S)	1	0.01*	0.03*	0.02*	0.08ns	0.002**	0.19ns	0.26ns	0.72ns	0.78ns
	Year (Y)	3	0.07ns	0.9ns	0.65ns	0.14ns	0.007**	0.009**	0.92ns	0.22ns	0.31ns
	S × Y	3	0.96ns	0.85ns	0.96ns	0.06ns	0.005**	0.02*	0.39ns	0.73ns	0.72ns
	Residual	24	-	-	-	-	-	-	-	-	-
Spring seed sowing or seedling transplanting	Planting types (PT)	1	<0.001***	0.04*	<0.001***	0.6ns	0.06ns	0.35ns	0.92ns	0.05ns	0.2ns
	Year (Y)	3	0.13ns	0.67ns	0.2ns	0.07ns	0.87ns	0.23ns	0.33ns	0.69ns	0.2ns
	PT × Y	3	0.9ns	0.9ns	0.92ns	0.2ns	0.7ns	0.13ns	0.17ns	0.4ns	0.41ns
	Residual	24	-	-	-	-	-	-	-	-	-

*, **, and *** represent significant at 0.05, 0.01, and 0.001 probability level, respectively. ns, not significant.

na. Camelina crude protein content was affected by location but not sowing season, planting type, and year (Tables 2 and 3). Across the experimental years, the mean seed crude protein of fall-seeded camelina ‘Xiaoguo’ was 28.5% (range: 26.7-29.3%) and 28.7% (range: 26.5-29.8%) at Anyang and Huangzhong, respectively, which were significantly greater than those of 26.5% (range: 24.6-28.3%) and 26.2% (range: 24.3-28.8%) at Fuping and Qingyang (Figure 2). These values were consistent with previously reported values of 26.4-29.0% by Krzyżaniak *et al.* (2019), 27.0-28.3% by Neupane *et al.* (2020), 26.0-29.4% by Vollmann *et al.* (2007), and 26.8-29.9% by Wittenberg *et al.* (2020), and relatively higher than that of 24-27% by Zanetti *et al.* (2017). Except for the higher seed crude protein in fall-seeded camelina ‘Xiaoguo’ at Anyang and Huangzhong, there were no significant differences in seed crude protein in camelina regardless of location, planting type, or sowing season (spring- or fall-seeded camelina at Fuping) (Figure 2 and Table 3). Additionally, camelina ‘Xiaoguo’ seed with a higher oil content at Anyang and Huangzhong corresponded to higher protein content (Figure 2), which contradicts the previous report of Zanetti *et al.* (2017), indicating the suitable growing condition may also favour camelina seed protein production.

The ANOVA for factors effect and interactions on saturated, monounsaturated, polyunsaturated fatty acid, and individual major fatty acid content in camelina seed oil are presented in Tables 3 and 4. The ANOVA showed location and year, but not planting type, significantly affected saturated, monounsaturated, and polyunsaturated fatty acid in camelina seed oil, whereas sowing season only affected saturated fatty acid. The average content of saturated fatty acids of camelina ‘Xiaoguo’ across the experimental years ranged from 11.3% (spring seeding at Fuping) to 14.2% (fall seeding at Fuping) (mean: 12.3%), monounsaturated fatty acids from 31.4% (spring seeding at Tongliao) to 32.9% (spring seedling transplanting at Fuping) (mean: 32.0%), and polyunsaturated fatty acids from 53.3% (fall seeding at Fuping) to 56.9% (fall seeding at Qingyang) (mean: 55.6%) (Table 4). These values were consistent with the previously reported values for camelina (Vollmann *et al.*, 2007; Krzyżaniak *et al.*, 2019; Zhang *et al.*, 2021). Statistically similar contents of saturated fatty acids (11.3 vs 12.1%), monounsaturated fatty acids (32.8 vs 32.9%), and polyunsaturated fatty acids (55.7 vs 55.8%) in camelina seed oil for the two planting types at Fuping were revealed by Tukey post-hoc test (Table 4). Additionally, at Fuping, the planting season showed a significant effect on saturated fatty acid content but not on the other two fatty acid groups (Table 4). In the case of individual fatty acid content, oleic (C18:1) (mean: 14.2%; range: 12.9-15.2%), linoleic (C18:2) (mean: 17.1%; ranges: 15.3-19.5%), α -linolenic (C18:3) (mean:

33.1%; ranges: 30.2-35.7%), and eicosenoic acid (C20:1) (mean: 14.6%; ranges: 13.6-15.7%) were the most abundant fatty acids in camelina oil, accounting for almost 80% in total oil content, which is in line with the previous studies (Krzyżaniak *et al.*, 2019; Zhang *et al.*, 2021). The content of erucic acid (C22:1) was in the range of 2.4-2.9% (mean: 2.7%), which is in agreement with the previously reported values for camelina (Vollmann *et al.*, 2007; Walia *et al.*, 2018). In the present study, two unique unsaturated fatty acids of docosahexaenoic acid (DHA) (C22:6) and nervonic acid (C24:1) were also detected in camelina ‘Xiaoguo’ seeded at all test environments with a mean content of 0.14% (range: 0.06-0.23%) and 0.38% (range: 0.28-0.53%), respectively (Table 4).

Camelina seeds containing important bioactive compounds such as flavonoids and β -sitosterol have been demonstrated to possess many bioactive functions, including antioxidation, antibacterial, and anxiety reduction (Aziza *et al.*, 2010; Rahman *et al.*, 2018; Panayotis *et al.*, 2021). In the present study, the ANOVA showed that the content of these two compounds was only affected by location, but not year, sowing season, and planting type (Table 2 and 3). The mean total flavonoid content in camelina ‘Xiaoguo’ across experimental years was highest at Anyang and Huangzhong (3.8 mg g⁻¹), followed by Fuping and Qingyang (3.4 mg g⁻¹), and lowest at Tongliao (2.7 mg g⁻¹) (Table 4). The mean of flavonoid content across locations and years was 3.4 mg g⁻¹ (range: 2.7-3.8 mg g⁻¹), which is comparable to that reported by Russo *et al.* (2018) (mean: 3.6±0.1 mg g⁻¹), and lower than those of reported by Rahman *et al.* (2018) (mean: 6.8±0.7 mg g⁻¹) and Aziza *et al.* (2010) (mean: 18.5 mg g⁻¹), but higher in respect to Salminen *et al.* (2006) (mean: 1.3±0.2 mg g⁻¹). In the case of β -sitosterol, the mean content of this compound across experimental years showed a similar location-related pattern as total flavonoid content in camelina, with the highest β -sitosterol content obtained at Anyang and Huangzhong (1775 μ g g⁻¹), followed by Fuping and Qingyang (range: 1741-1757 μ g g⁻¹), and lowest at Tongliao (1680 μ g g⁻¹). The mean β -sitosterol content across locations and years was 1723 μ g g⁻¹ (range: 1680-1778 μ g g⁻¹), which fell within the range (1479-1772 μ g g⁻¹) of β -sitosterol content previously reported for camelina (Piravi-vanak *et al.*, 2021), and lower than the mean value (1884±144 μ g g⁻¹) reported by Shukla *et al.* (2002).

Potential of camelina in China

There is great potential for the large-scale cultivation of camelina in central and northern China on marginal land that may be suitable for growing this crop due to its adaptability to drought and low temperature. In a continuation of our previous study (Zhang *et al.*, 2021), the present results further confirm that

Table 4. Major fatty acids and fatty acid groups (% of total fatty acids), total flavonoids (TF) (mg g⁻¹), and β -sitosterol (μ g g⁻¹) of camelina cv ‘Xiaoguo’ grown in five different growing environments from 2015 to 2019.

Location	Sowing season	C18:1	C18:2	C18:3	C20:1	C22:1	C22:6	C24:1	SFA	MUFA	PUFA	TF	β -sitosterol
Anyang	Fall	13.3 ^{ab}	16.6 ^{bc}	35.7 ^a	15.1 ^{ab}	2.8 ^{ab}	0.23 ^a	0.53 ^a	11.4 ^b	31.8 ^{ab}	56.9 ^a	3.8 ^a	1775 ^a
Fuping	Fall*	14.6 ^{ab}	15.3 ^c	33.1 ^b	14.7 ^{ab}	2.7 ^{ab}	0.18 ^{ab}	0.46 ^{ab}	14.2 ^a	32.8 ^a	55.6 ^{abc}	3.4 ^{ab}	1743 ^{ab}
	Spring ^o	14.3 ^{ab}	16.7 ^{bc}	34.6 ^{ab}	15.7 ^a	2.4 ^b	0.12 ^{bc}	0.36 ^{bcd}	11.3 ^b	32.8 ^a	55.7 ^{abc}	3.4 ^{ab}	1757 ^{ab}
	Spring [#]	15.1 ^a	19.5 ^a	30.2 ^c	13.6 ^b	2.8 ^a	0.06 ^c	0.28 ^d	12.1 ^b	32.9 ^a	55.8 ^{abc}	3.1 ^b	1690 ^b
Huangzhong	Fall	15.2 ^a	18.2 ^{ab}	30.6 ^c	15.4 ^{ab}	2.4 ^b	0.10 ^c	0.29 ^{cd}	13.3 ^{ab}	33.5 ^a	54.2 ^{bc}	3.7 ^a	1778 ^a
Qingyang	Fall	12.9 ^b	16.7 ^{bc}	33.2 ^b	13.9 ^{ab}	2.9 ^a	0.13 ^{bc}	0.34 ^{bcd}	12.6 ^b	32.2 ^{ab}	56.9 ^a	3.4 ^{ab}	1741 ^{ab}
Tongliao	Spring	14.0 ^{ab}	17.0 ^{bc}	34.5 ^{ab}	14.1 ^{ab}	2.7 ^{ab}	0.18 ^{ab}	0.42 ^{abc}	12.0 ^b	31.4 ^{ab}	56.7 ^{ab}	2.7 ^c	1680 ^b
Mean		14.2	17.1	33.1	14.6	2.7	0.14	0.38	12.3	32.0	55.6	3.4	1723

*Fall camelina seeding; ^ospring camelina seeding; and [#]spring camelina seedling transplanting at Fuping during the study period (2015-2019). C18:1 (oleic acid), C18:2 (linoleic acid), C18:3 (linolenic acid), C20:1 (eicosenoic acid), C22:1 (erucic acid), C22:6 (docosahexaenoic acid), C24:1 (nervonic acid), saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and polyunsaturated fatty acid (PUFA). ^{a-d}Different letters within each column: significant different values (P \leq 0.05, Tukey post-hoc test).

camelina 'Xiaoguo' can grow across very different growing environments, including central (Henan province), northwestern (Gansu and Qinghai province), and northeastern (Inner Mongolia) China. When averaged across the 9 years (2010-2019) and 5 different locations, camelina 'Xiaoguo' showed a satisfactory mean seed (1946 kg ha⁻¹) and oil yield (696 kg ha⁻¹). Camelina has the best potential in Anyang (Henan province) and Huangzhong (Qinghai province) compared to other test locations. Based on the results, camelina looks promising as an alternative oilseed crop when seeded as a winter or spring annual crop with minimal agricultural inputs (*i.e.*, lower fertiliser requirements and pesticide application) compared to other oilseed crops (*i.e.*, oilseed rape, soybean). Currently, oil-crushing companies in China would not buy camelina because there is no market for it. Therefore, development in new markets is urgently needed for camelina to become an option for growers. Hence, the most pressing challenge for camelina development in China is to register this crop as a food raw material (Zhang *et al.*, 2012; Mao, 2020).

Moreover, greater effort is needed to introduce and promote camelina oil to the public. Research is necessary to minimise erucic acid content in camelina oil, making it more suitable for consumption. To the authors' knowledge, only two local camelina cultivars ('Xiaoguo' and 'Jilan') and several breeding lines are available in China (Huang *et al.*, 2005; Du *et al.*, 2018). Therefore, more research is required on the selection or breeding of spring and winter camelina cultivars with high-yielding and extensive environmental adaptability characteristics.

Conclusions

Coupled with our previous evaluation of the spring-seeded camelina trials, a Chinese camelina cv 'Xiaoguo' can grow across very different growing environments in central and northern China. It is potentially a good cultivar and could be seeded both in the spring and fall growing season with a satisfactory crop yield compared to the existing cultivars (*i.e.*, 'Calena,' 'Joelle,' 'Omega'). Based on the 9-year study, except for extreme weather conditions, sowing location and season, but not year, seem to be the key determinants for camelina seed yield. In the present study, field camelina seedling transplanting provides an alternative approach, especially for smallholder farming systems in Asian countries (*i.e.*, China and Korea) to increase crop yield; however, this approach is not suitable for large-scale cultivation of this crop in North America due to the expensive labour cost. While camelina showed wide environmental adaptability, caution should be taken for fall field seeding camelina in the northeastern area of China (*i.e.*, Tongliao) as fall and winter sandstorms often occur. Overall, camelina appears promising as an alternative edible oil source and also has great potential as an oilseed for biodiesel and as a source of bioactive compounds (*i.e.*, flavonoids, β -sitosterol) for the pharmaceutical industry in China.

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