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Safflower genotypes affected by nitrogen fertilization in subtropical conditions

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Abstract

Nitrogen fertilization is one of the main management systems that affects safflower (*Carthamus tinctorius*) production. However, the response of safflower to nitrogen fertilization may vary depending on the genotype and growing conditions. The objective of this study was to evaluate the influence of nitrogen fertilization on yield components, oil content, and protein content of safflower genotypes in subtropical conditions. Two experiments were performed to evaluate the effect of nitrogen fertilization (0 and 160 kg ha⁻¹) on six safflower genotypes. The experiment was designed in randomized blocks, in a 2 × 6 factorial design, with six repetitions. The height of the plants, yield, and oil and protein contents were determined 170 and 160 days after emergence in 2017 and 2018, respectively. The oil content and protein content of safflower genotypes were affected by nitrogen fertilization, but this effect varied depending on the genotype. Genotype 4 dominated with a grain yield of 1088 kg ha⁻¹ and an oil yield of 215 kg ha⁻¹. Nitrogen fertilization increased the grain yield, oil content, and protein content; thus, it is appropriate for the cultivation of safflower off-season in autumn-winter in subtropical conditions. Safflower genotype 4 could be a promising oilseed crop for southern Brazil when fertilized with 100 kg ha⁻¹ of N.

Keywords: Carthamus tinctorius L.; oilseeds; industrial crops.

Abbreviations: DAE_days after emergence; SOM_Soil organic matter; BS_ Base saturation.

Introduction

There is great need to adopt plants that have industrial potential and that are also tolerant to abiotic stresses. Characteristic of tropical climates, safflower (Carthamus tinctorius L.) is a candidate species that can be grown in autumn-winter in Brazil (Sarto et al., 2018). It is an oilseed crop with potential for cultivation in dry areas (Lovelli et al., 2007; Santos et al., 2017). Safflower seeds have a considerable high-quality oil content (35-45%), which can be used for human consumption and industrial use. Safflower oil has high levels of oleic acid (30%) and linoleic acid (70%), and can be used as raw material for biodiesel production (Ilkılıç et al., 2011). In Brazil, safflower can be used for the second harvest (autumn-winter harvest). This crop is still little known and, therefore, the performance of this species requires investigation. Due to the risk of irregular rainfall, farmers use little fertilizer and rarely perform nitrogen topdressing (Santos et al., 2019). However, nitrogen is fundamentally important for the growth and development of safflower, and is one of the most important safflower management techniques (Dordas and Sioulas, 2009). One of the most efficient ways for safflower to develop in tropical conditions is through the proper application of fertilizers. Nevertheless, it is necessary to elucidate the appropriate amount of fertilizer required.

Several studies have declared safflower to be responsive to nitrogen topdressing (Dordas and Sioulas, 2008; Dordas and Sioulas, 2009; Elfadl et al., 2009; Soleimani, 2010; Shahrokh; niaa and Sepaskhah, 2016; Shahrokhniaa and Sepaskhah, 2017; Santos et al., 2018). However, studies of the nitrogen fertilization of safflower have presented conflicting results. This discrepancy may be related to residual N in the soil, different safflower genotypes, and variable climatic conditions (Dordas and Sioulas, 2009). Yau and Ryan (2010) revealed that safflower did not respond to the application of N, which may have been due to its deep root system capable of absorbing N. Different genotypes exhibited differences in the accumulation and partitioning of N into different plant organs (Papakosta and Gagianas, 1991). Thus, the accumulation of dry matter can be affected by genotype (Koutroubas et al., 2004). The impact of N fertilization on safflower has not been extensively studied in Brazil. In this sense, the introduction of N-efficient genotypes may be important for safflower harvesting in subtropical conditions. Currently, there is insufficient information on the interaction between genotype and nitrogen with oil content and protein content. The response of safflower to nitrogen fertilization may vary according to genotype. The objective of this study was to evaluate the influence of nitrogen fertilization on yield components, oil content, and protein content of different North American safflower genotypes in subtropical conditions.

Results

Plant height

The plant height was significantly affected (P < 0.01) by the interaction of the genotype and nitrogen factors in 2017

(Table 1). The application of nitrogen increased the plant height of genotype 4 with values of 114.4 cm. The interaction of the factors demonstrated that the height of genotype 5 plants was reduced by nitrogen application (Table 2; Figure 1). In 2018 the application of nitrogen increased the plant height of all safflower genotype (Table 2). However, in 2018 the plant height of safflower genotypes 2 and 4 were similar (85.3 cm). The average plant height was 84 cm in 2017 and 76 cm in 2018.

Yield

Grain yield was not affected (P > 0.01) by the interaction of genotype and nitrogen in either year (Table 1 and 2). Thus, the effects of genotype and nitrogen are presented separately. The application of nitrogen led to significantly higher (P < 0.05) grain yields in both 2017 and 2018. In 2017, genotype 4, which had a yield of 1088 kg ha⁻¹, dominated over genotypes 1 and 6, which had yields of 701 and 573 kg ha⁻¹, respectively. In 2018, although genotype 4 appeared to have a higher grain yield (864 kg ha⁻¹), there was no significant variation between the genotypes (P > 0.05).

Oil content

The oil content was significantly affected (P < 0.01) by the interaction of genotype and nitrogen in both years (Table 2 and 3). The highest oil contents were observed for genotypes 2 and 6 in both years, with a maximum of 24% in 2017 (Figure 2A). Nitrogen fertilization increased the oil content of genotypes 5 and 6 in both years. However, the oil content of genotype 4 was reduced with nitrogen application. Meanwhile, the oil contents of genotypes 1 and 2 were not affected by nitrogen fertilization (Figure 2A and B).

Protein content

The protein content was significantly influenced (P < 0.01) by the interaction of genotype and nitrogen in both years (Table 2 and 3). Genotype 1 was notable for its protein content, benefiting from the application of nitrogen in both years (Figure 3A and B). The protein values for genotype 1 were 14 and 17% in the years 2017 and 2018, respectively (Figure 3A and B). The average protein content of all genotypes was 11.7% in 2017 and 8.4% in 2018 (Table 1 and 2).

Oil yield and protein yield

Oil yield was not affected (P > 0.05) by the interaction of genotype and nitrogen, so the effects of the factors are discussed separately (Table 1 and 2). In 2017, genotype 4 had the highest oil yield of 215 kg ha⁻¹, compared to genotypes 1, 5, and 6, which had values of 89, 123, and 126 kg ha⁻¹, respectively (Table 1 and 2).

As was the case with the grain yield, the oil yield was not significantly affected (P > 0.05) by different genotypes in 2018. The oil yield ranged from 58 to 122 kg ha⁻¹ in 2018. However, the application of nitrogen significantly increased (P < 0.05) the oil yield of genotypes (Table 2).

The protein yield was significantly affected (P < 0.05) by the interaction of genotype and nitrogen only in 2017 (Table 1). Nitrogen increased the protein yield in genotype 1 from 42 to 152 kg ha⁻¹ (Figure 4). In 2018, the application of nitrogen

increased the overall protein yield from 37 to 78 kg ha^{-1} (Table 2).

Discussion

Nitrogen fertilization increased the plant height of safflower (Table 1; Figure 1), which corroborates previous findings (Abbadi et al., 2008). Nitrogen is one of the most important nutrients for agricultural production, as it affects the production of dry matter, influencing the development and maintenance of the leaf area, as well as the photosynthetic efficiency (Shahrokhniaa and Sepaskhah, 2017). Under N deficiency, growth is inhibited, and the root to shoot ratio is decreased (Steer and Harrigan, 1986). The application of 46 kg ha⁻¹ of nitrogen has been shown to increase safflower height by 18% compared to unfertilized plants (Shahrokhniaa and Sepaskhah, 2017).

The grain yield benefited from the application of nitrogen, with an increase of 34% in 2017 and 43% in 2018 (Table 1 and 2). Dordas and Sioulas (2008) reported that nitrogen fertilization at a rate of 150 kg of N ha⁻¹ increased the production of safflower grains by 19%. Safflower grain yield can be expressed using several components. The components of the direct yield are the plant population, the number of heads per plant, the number of seeds per head, and the weight of seeds (Gilbert and Tucker, 1967). The relative importance of each component is affected by many factors, including genetic factors. High variation in grain yield was detected for several genotypes (923–3391 kg ha⁻¹) (Koutroubas et al., 2008). The maximum yield was 1088 kg ha⁻¹, which was observed in genotype 4. This yield was lower than the yield of Brazilian genotypes (3820–4532 kg ha⁻¹) of safflower grown in Southern Brazil (Sampaio et al., 2016; Zanão Júnior et al., 2017). The oil yield of the genotypes varied significantly only in the first year, with values from 89 to 215 kg ha⁻¹. Thus, the grain yields of the North American genotypes tested in the South Brazil were lower than previously reported. Referring to the work of Mazieiro et al. (2019), it can be suggested that this may have been due to the lack of adaptation of the genotypes to the subtropical conditions.

The oil content can be adjusted by applying nitrogen and by choosing the genotype (Figure 2A and B). The content of safflower oil is not generally affected by the application of N (Elfadl et al., 2009). The results of the present study revealed that some genotypes were affected by nitrogen application, but this effect was not consistent. The oil content ranged from 8 to 24%. Mazieiro et al. (2019) noted a variation of 12 to 23% in North American safflower genotypes. Zanão Júnior et al. (2017) observed a variation of 23 to 29%. Santos et al. (2018) reported a variation of 24 to 30% for a Brazilian genotype. The oil content is strongly dependent on the genotype (Hang and Evans, 1985). It is evident that the nitrogen application had a greater effect on the yield of safflower grains than on the oil content. A higher oil yield was observed due to the application of nitrogen, with an increase of 34 and 48% in the years 2017 and 2018, respectively. These results corroborate the findings of Dordas and Sioulas (2008), who observed no effect on oil content. However, the effect on grain yield compensated for the reduction in oil content.

The protein content was affected by the interaction of the genotype and nitrogen (Figure 3A and B). The protein concentration is an important property of safflower seeds due to the use of the seeds in animal feed (Shahrokhniaa

Fable 1. Plant height, grain yiel	l, oil content, and protein o	of safflower genotypes with	n nitrogen fertilization in 2017.
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Treatments		Grain Yield $(1 - 1)^{-1}$	Oil content	Protein	Oil yield (kg h_{2}^{-1})	Protein yield (kg
	Plant height (cm)	(kg na)	(%)	(%)	na)	na)
Genotype						
1	85.2 bc	701 b	12.8 e	12.6 ab	89 c	97 ab
2	83.1 bc	793 ab	23.6 a	13.0 a	186 ab	104 ab
3	86.9 bc	882 ab	18.9 c	12.0 abc	168 ab	107 ab
4	107.7 a	1088 a	19.9 bc	11.5 c	215 a	126 a
5	76.5 c	807 ab	16.1 d	9.4 d	133 bc	74 b
6	89.1 b	573 b	21.9 ab	11.7 bc	126 bc	67 b
Nitrogen (kg ha ⁻¹)						
0	87.1	690 b	18.6	10.9 b	130 b	76 b
100	89.0	925 a	19.1	12.4 a	175 a	114 a
ANOVA (P values)						
Genotype (G)	<0.000	0.005	<0.000	<0.000	<0.000	0.0039
Nitrogen (N)	0.399	0.002	0.2432	<0.000	0.0042	<0.000
G × N	0.008	0.854	<0.000	<0.000	0.9343	0.0126



Fig 1. Plant height in 2017. Values followed by a different lowercase letter are significant difference between genotypes same nitrogen fertilization. Values followed by a different capital letter are significant difference among nitrogen fertilization under same genotype (Tukey test, P <0.05).

Table 2. Plant height, grain yield, oil content, and prote	n of safflower genotypes with nitrogen fertilization in 2018.
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Treatments	Plant (cm)	height	Grain yield (kg ha ^{−1})	Oil content (%)	Protein (%)	Oil yield (kg ha ⁻¹)	Protein yield (kg ha ⁻¹)
Genotype							
1	70.8 bc		622	9.0 d	9.7 a	58	67
2	85.3 a		698	17.1 a	9.2 ab	120	67
3	76.3 b		607	13.6 b	8.8 bc	84	55
4	85.3 a		864	14.1 b	8.2 c	122	73
5	72.6 bc		654	11.7 c	6.5 d	80	42
6	65.8 c		472	15.9 a	8.5 bc	78	43
Nitrogen (kg ha ⁻¹)							
0	72.9 b		536 b	11.9 b	6.9 b	64 b	37 b
100	79.2 a		770 a	15.3 a	10.0 a	117 a	78 a
ANOVA (P values)							
Genotype (G)	<0.000		0.2564	<0.000	<0.000	0.0548	0.2093
Nitrogen (N)	0.003		0.0144	<0.000	<0.000	0.0005	<0.000
G×N	0.090		0.9944	0.0002	<0.000	0.9631	0.3387

Table 3. Soil chemical characteristics in the experimental areas before initiating the experiment.

Year	pH (CaCl ₂)	SOM†	P (resin)	H + Al	Exchangeable	Exchangeable	Exchangeable	BS‡
					К	Ca	Mg	
		g dm ⁻³	mg dm ⁻³		————cmol _c dm ^{−3} ——			%
2017	4.4	50	2.8	9.7	0.37	4.1	1.5	40
2018	5.0	32	9.4	6.2	0.33	5.2	2.9	58

⁺ Soil organic matter.[‡] Base saturation.



Fig 2. Oil content in 2017 (A) and 2018 (B). Values followed by a different lowercase letter are significant difference between genotypes same nitrogen fertilization. Values followed by a different capital letter are significant difference among nitrogen fertilization under same genotype (Tukey test, P <0.05).



Fig 3. Protein in 2017 (A) and 2018 (B). Values followed by a different lowercase letter are significant difference between genotypes same nitrogen fertilization. Values followed by a different capital letter are significant difference among nitrogen fertilization under same genotype (Tukey test, P <0.05).



Fig 4. Protein yield in 2017. Values followed by a different lowercase letter are significant difference between genotypes same nitrogen fertilization. Values followed by a different capital letter are significant difference among nitrogen fertilization under same genotype (Tukey test, P <0.05).



Fig 5. Behavior of meteorological variables of precipitation and mean temperature during safflower cultivation Cascavel, PR, Brazil, in 2017 and 2018.

and Sepaskhah, 2017). Nitrogen fertilization in mulch was effective in increasing the protein content of safflower, which is widely accepted in the literature and is in line with the findings of Dordas and Sioulas (2008) and Shahrokhniaa and Sepaskhah (2017). Higher protein values have been obtained with nitrogen fertilization due to the higher nitrogen accumulation and grain yield. The protein content has been shown to increase by 40% compared to control (Dordas and Sioula, 2008). The variation in protein content was 5 to 17% in the present study. Mazieiro et al. (2019) observed a protein content variation of 8 to 17% for North American safflower genotypes. Due to the strong effect of nitrogen application on protein content, it is evident that

protein yield was substantially increased by nitrogen application, at an average of 66% in 2017 and 47% in 2018.

Materials and methods

Plant materials

The safflower genotypes used were originally from the North American company.

Location and climatic conditions

Two experiments were performed in Cascavel, Paraná, Brazil. In 2017, the experiment was conducted at Fundetec

(Foundation for Scientific and Technological Development; 25°00'39" S, 53°17'22" W, altitude of 785 meters). In 2018, the experiment was conducted at UNIOESTE (Western Parana State University; 24°59'21.2" S 53°26'59.6" W, altitude of 781 m). According to the Köppen classification, the climate of the sites is subtropical Cfa type, without a defined dry season. The average temperature of the hottest month is higher than 22°C and the average temperature in the coldest month is lower than 18°C, with hot summers and infrequent frosts in winter. The behavior of the meteorological variables during the experiment is shown in Figure 5.

The soil in the experimental area was classified as Rhodic Acrudox (Soil Survey Staff, 2014). Before starting the experiment, the chemical characteristics of the soil were determined (0–20 cm; Table 3) according to the methods of Embrapa et al. (2009).

Experimental setup

Before implantation, glyphosate [isopropylamine salt of N-(phosphonomethyl) glycine] was applied (1.44 g acidequivalent ha^{-1}). Ten days after sowing, the pre-emergence herbicide, s-metolachlor, was applied (1 kg ha^{-1}).

The genotypes were sown on May 12, 2017 and May 2, 2018. The sowing was performed mechanically, using a seeder fertilizer machine, regulated to 32 seeds per meter. At the time of sowing, base fertilization was performed with 300 kg ha⁻¹ of fertilizer 08-20-20 (N-P2O5-K2O). For seed treatment, Imidacloprid was applied at 90 g a.i. per 100 kg of seed and Thiodicarb was applied at 30 g a.i. per 100 kg of seed.

Treatments and experimental design

The experimental design was in randomized blocks, in a 2 × 6 factorial design, with six repetitions. Six genotypes of safflower (1, 2, 3, 4, 5, and 6) and two rates of nitrogen application (0 and 160 kg ha⁻¹) were used. Nitrogen topdressing was performed 50 days after emergence (DAE), using encapsulated urea (46% N). Each plot consisted of five lines measuring 4 m in length, with 0.45 m spacing between lines.

Traits evaluated

The harvest occurred 170 and 160 days after the emergence of the plants in the 2017 and 2018 seasons, respectively. The height of the plants was determined by measuring the distance between the ground level and the apex of the plant with a graduated tape. Six random plants were measured within each plot. The plants were collected from 1 m² of each plot, before threshing and manual cleaning of the seeds were performed. The values were expressed in kg ha⁻¹. The moisture content was determined using the gravimetric method by drying a sub-sample for 24 h at 105°C and correcting for 12%. For the evaluation of the oil content, the extraction was performed using a Soxhlet extractor (IAL, 2008) under laboratory conditions using petroleum ether solvent. The extraction was performed with 2 g of ground seeds. The protein content was determined using the Bradford method (1976). The oil and protein yields were determined by multiplying the oil and protein content by the grain yield.

Statistical analysis

The statistical analysis was based on the analysis of variance and comparison of averages using the Tukey test with a significance threshold of 5% (P < 0.05). Statistical analyses were performed using the SISVAR 5.6 statistical software.

Conclusions

The oil content and protein content of safflower genotypes were affected by nitrogen fertilization, but this effect varied depending on the genotype. Nitrogen fertilization increased the grain yield, oil content, and protein content, and it is thus recommended for growing safflower off-season in autumn-winter. Nitrogen fertilization is important for safflower due to the severe rates of decomposition and nitrogen dynamics in the soil under subtropical conditions.

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