Dry mass, nutrient concentration and accumulation in safflower (*Carthamus tinctorius* L.) influenced by nitrogen and potassium fertilizations

Ellen Cristina Alves de Anicélio, Edna Maria Bonfim-Silva*, Tonny José Araújo da Silva and Márcio Koetz

Institute of Agricultural Sciences and Technology, Federal University of Mato Grosso, Department of Agricultural and Environmental Engineering, Rondonopolis, Mato Grosso, Brazil

*Corresponding author: embonfim@hotmail.com

**Abstract**

Safflower (*Carthamus tinctorius* L.) is a promising crop to disseminate in Brazil for biodiesel production, and nitrogen and potassium fertilization and the proper ratio between these nutrients in the soil are important factors in plant development. The objective was to evaluate the effect of combinations of nitrogen (N) and potassium (K) rates on dry mass, nutrient concentration and accumulation in safflower. Experiment was conducted in greenhouse, with oxisol being collected from 0.00-0.20 m under Cerrado vegetation. The experimental design was randomized blocks, and the treatments were arranged in 5x5 factorial scheme, corresponding to five nitrogen rates (0, 60, 120, 180 and 240 mg dm$^{-2}$) and five potassium rates (0, 50, 100, 150 and 200 mg dm$^{-2}$), with four replications. The following productive characteristics were evaluated: shoot dry mass and head dry mass; the following nutritional characteristics were evaluated: nitrogen and potassium concentration and nitrogen and potassium accumulation in the shoot and in the heads. The results were submitted to a variance analysis (F-test) and regression study with 5% probability (p<0.05). There was no significant interaction between nitrogen and potassium, with an isolated effect of the factors for all of the variables. Nitrogen fertilization increased all of the variables and adjusted the quadratic regression model, except for the nitrogen concentration in the shoot and in the heads as described by a linear regression model. Potassium fertilization increased all of the variables, adjusting the linear regression model. Nitrogen and potassium fertilization in isolation positively influenced the production and nutrition of safflower plants.

**Keywords:** Alternative for biodiesel production; Phytomass; Nutritional balance; Oilseed; Soil fertility.

**Abbreviations:** N_nitrogen; K_potassium; AMA_Mato-Grossense Cotton Institute; pH_hydrogen potential; P_phosphorus; Ca_calcium; Mg_magnesium; H_Hydrogen; Al_aluminum; CEC_cation exchange capacity; V_base saturation; OM_organic matter; PA_pure analytical-grade; B_Boron; Cu_copper; Mn_manganese; Zn_zinc; Mo_molybdenum; DAE_days after emergence; SAS_statistical analysis system; F_test Statistical test; RSREG_regression procedures; GLM_command general linear model procedure; $R^2$_coefficient of determination.

**Introduction**

The search for alternative energy sources is a requirement of sustainable production systems. Thus, the importance of oilseed crops, such as safflower, has increased, especially with the interest in biofuel production (Dordas and Sioulias, 2008). In addition to this purpose, safflower has been widely used in the food because the seeds of this plant species have a high oil content (35-40%) of excellent quality, as well as industrial use in the manufacture of paints and varnishes (Giayetto et al., 1999). Currently, the highest safflower producers are Kazakhstan, India, the United States and Mexico (Food and Agriculture Organization of the United Nations-Faostat, 2013). In Brazil, information for the cultivation of this crop is scarce. However, research is being conducted for the implementation and good production of this crop (Silva, 2013; Bellé et al., 2012). The safflower crop has important agronomic traits, such as high tolerance to drought, high temperatures, strong and hot winds, low relative humidity and saline soils (Kizil et al., 2008). Therefore, safflower adapts to adverse conditions. Considering that the Brazilian Cerrado soils are predominantly deep and well drained and that there are irregular rainfall patterns, safflower is a good crop for off-season crop production in this region, diversifying production, facilitating the management of soil, pests and diseases, increasing the income of the farmer. Due to the economic and environmental importance of safflower and studies with this culture being recent in the country, the need for basic research that investigates the management of soil fertility with the objective of meeting the nutrient requirements of this crop becomes evident. Among the essential nutrients to plants, nitrogen is the most extracted by plants and is considered one of the most important for the growth and development of crops. This nutrient is an essential component of amino acids and proteins, nucleic acids, hormones, and chlorophyll from organic compounds that are fundamental to the survival of plants (Cantarella, 2007). After nitrogen, potassium is the most required nutrient by plants in quantitative terms. This nutrient has many functions in the plant, in particular the activation of several enzyme systems, many of them participants in the processes of photosynthesis and respiration, and promotes the maximization of nitrogen effects on the plant (Marschner, 1995). Therefore, the nitrogen and potassium supply for plants in proper proportions results in a synergistic effect

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between these nutrients, increasing crop production. In this context, the objective of this study was to investigate the effect of combinations of nitrogen and potassium rates on dry mass, nutrient concentration and accumulation in safflower grown in oxisol.

Results and Discussion

Plant dry mass

The shoot dry mass was significantly influenced by the nitrogen and potassium rates in isolation (Fig. 1 and 2). Nitrogen was adjusted to a quadratic regression model with a nitrogen rate of 95 mg dm$^{-3}$, which promoted the highest dry mass production (33.22 g pot$^{-1}$) of safflower plants (Fig. 1). To increase the shoot dry mass of safflower, it is necessary the nitrogen adequate supply with the objective of maximizing the photosynthetic active area, which is responsible for intercepting solar radiation, promoting the growth of the crop (Megda and Monteiro, 2010).

The results of this study agree with Dordas and Sioulas (2009), who found that the dry mass of safflower varied according to the nitrogen rates that were applied to the soil; the largest amount of dry mass was found under higher nitrogen rates (200 kg ha$^{-1}$). An increase in the shoot dry mass production of safflower with nitrogen fertilization was also reported by Golzafar et al. (2011), who obtained the maximum production at a nitrogen rate of 150 kg ha$^{-1}$. The shoot dry mass production was also increased by the supply of potassium rates according to the linear regression model, varying from 23.9 to 37.97 g pot$^{-1}$, showing an increase of 37% compared to the higher potassium rate (200 mg dm$^{-3}$) with the absence of this nutrient in the fertilizer (Fig. 2). An increase in the shoot dry mass as promoted by rates lower than those that were found in this study was reported by Abbadi et al. (2008) who, when evaluating safflower growth, obtained the maximum estimated value of shoot dry mass production with a potassium supply of 90 mg dm$^{-3}$. The increased dry mass of safflower plants, according to the linear increase in the potassium rates, was also verified by Abbasieh et al. (2013), and 150 kg ha$^{-1}$ potassium promoted the maximum production of dry mass. Studies with other cultures also demonstrate the importance of potassium when increasing the dry mass, such as for sunflower, where potassium application increased the plant dry mass up to an estimated rate of 181.25 mg L$^{-1}$ (Jesus et al., 2013). For the head dry mass, there was significance in the isolation of nitrogen and potassium rates, with the results described by quadratic and linear regression models, respectively (Fig. 3 and 4). For nitrogen fertilization, the highest head dry mass (19.2 g pot$^{-1}$) was observed at the nitrogen rate of 124 mg dm$^{-3}$ (Fig. 3). Dordas and Sioulas (2009) also observed that nitrogen contributed to the increased head dry mass of safflower; however, these authors observed the maximum head production at the highest tested nitrogen rate (200 kg ha$^{-1}$). The results indicate that nitrogen affects head production and therefore the yield of the safflower crop. This result occurs because the production of plant dry mass is directly related to the nitrogen supply; when the supply of this nutrient is low, there is less production of dry mass, especially in the leaves, which affects the production of assimilates and the distribution of assimilates to the reproductive organs, reflecting the head dry mass (Dordas et al., 2008). However, quadratic results indicated that the excess nitrogen in the fertilizer resulted in decreased head production. The head dry mass of safflower plants increased linearly with the potassium supply, showing an increase of

![Fig 1. Shoot dry mass of safflower plants at 74 DAE, according to the nitrogen rates in the oxisol. SDM - shoot dry mass; N - nitrogen. *Significant at 5%.](image1)

![Fig 2. Shoot dry mass of safflower plants at 74 DAE according to the potassium rates in the oxisol. SDM - shoot dry mass; K - Potassium. *** Significant at 0.1%](image2)

![Fig 3. Head dry mass of safflower plants at 74 DAE, according to nitrogen rates in the oxisol. HDM - Head dry mass; N - Nitrogen. *Significant at 5%](image3)
Fig 4. Head dry mass of safflower plants at 74 DAE according to the potassium rates in the oxisol. HDM – Head dry mass; K - Potassium. *** Significant at 0.1%.

33% compared to a higher dose of potassium with the non-application of the nutrient to the soil (Fig. 4). In a study from Abbadi et al. (2008), the increase in potassium fertilizer also promoted the highest head dry mass of the safflower plants, in which the potassium rate of 90 mg dm$^{-3}$ promoted a 61% increase in the head production compared to that of the control. Fanaei et al. (2009) reported that the use of potassium fertilizer significantly affected the production components of oilseeds.

Potassium deficiency causes the irregular functioning of stomata, which may reduce CO$_2$ assimilation and the photosynthetic rate (Cecilio and Grangeiro, 2004), negatively affecting production, as observed in the treatment without potassium application.

**Concentration and nitrogen accumulation by plants**

The concentration and nitrogen accumulation in the shoot were significant for the nitrogen and potassium rates in isolation (Fig. 5 and 6). The nitrogen concentration in the shoots increased linearly with increasing nitrogen rates, ranging from 9.55 to 14.08 g kg$^{-1}$ (Fig. 5A). However, the resulting nitrogen accumulation in the shoot was described by a quadratic regression model with the greatest accumulation (391 mg pot$^{-1}$) found at the nitrogen rate of 169 mg dm$^{-3}$ (Fig. 5B). The nitrogen concentration in the shoots of safflower plants according to nitrogen application was studied by Dordas and Sioulas (2009), who observed variation from 9.43 to 11.41 kg ton$^{-1}$, values that were similar to those that were found in this study, at the concentration that was observed in the conditions of non-application of the nutrient and at a nitrogen rate of 200 kg ha$^{-1}$, the highest rate that was studied by these authors, which obtained the maximum concentration.

Primì et al. (2010) evaluated safflower plants with nitrogen fertilization and found that the concentration of crude protein increased from 11 to 17% dry mass, corresponding, respectively, to a nitrogen concentration of 1.76 and 2.72% of the dry mass comparing treatment with the absence of application with the higher nitrogen rate of the experimental interval, 105 kg ha$^{-1}$. Rastgou et al. (2013) also concluded that nitrogen fertilization significantly affects nitrogen uptake by safflower plants. For other crops, Mattos and Monteiro (2003) observed a linear increase in the nitrogen concentration in the newly expanded leaf of Signal grass to the nitrogen rate of 200 mg dm$^{-3}$ (maximum rate of the experimental interval). The quadratic response of nitrogen accumulation in shoots according to the nitrogen rate was due to the lower production of dry mass that was found at higher nitrogen rates of the experimental interval, which decreased nitrogen accumulation in safflower plants. The variation in the nitrogen concentration in the shoot dry mass according to potassium rates adjusted the quadratic regression model (Fig. 6A). The nitrogen concentration varied from 13.69 to 10.81 g kg$^{-1}$ with increasing potassium rates. The nitrogen concentration decreased with the increasing potassium supply. The maximum nitrogen concentration in the shoot dry mass (13.69 g kg$^{-1}$) was obtained when potassium was not applied, while the lowest concentration (10.81 g kg$^{-1}$) was obtained at a potassium rate of 130 mg dm$^{-3}$. However, the nitrogen accumulation in the shoot increased linearly, ranging from 304.1 to 406.3 mg pot$^{-1}$, with the increases potassium supply to the soil (Fig. 6B). The highest nitrogen concentration in the shoots of the safflower plants in the absence of potassium application to the soil was due to the effect of the concentration, resulting from the lower growth of plants caused by a lack of potassium. This response was also reported by Lavres Junior and Monteiro (2002) in Mombaça grass and Viana (2007) in wheat, in which the maximum nitrogen concentration occurred in the absence of potassium supply. However, there was an increase in the nitrogen accumulation in the shoot with the increased potassium rates due to the importance of this nutrient in nitrogen use by plants because potassium is essential for metabolism and for the nitrogen incorporation in the leaves (Xu et al., 2002). Keeney et al. (1967) found that potassium increased nitrogen uptake by corn, promoting an increase in the protein concentration in the grain due to a higher nitrogen supply.

There was significant effect of the nitrogen rates on the concentration and accumulation of this nutrient in the heads of safflower plants (Fig. 7). Note that the nitrogen concentration in the heads increases linearly with the supply of this nutrient, ranging from 27.4 to 33.4 g kg$^{-1}$ (Fig. 7A). However, the nitrogen accumulation in the heads adjusted the quadratic regression model, and the nitrogen rate of 154 mg dm$^{-3}$ resulted in the greater accumulation (596 mg pot$^{-1}$) of this nutrient (Fig. 7B).

Similar results were reported by Rastgou et al. (2013), who verified that the application of nitrogen fertilizer increases the nitrogen concentration in the reproductive structure of safflower plants. These authors found nitrogen concentrations from 3.08 to 4.03% of the dry mass of seeds, values that were obtained, respectively, from the non-application and nitrogen rate of 200 kg ha$^{-1}$ (higher dose of the experimental interval). This study demonstrated that the heads accumulated 60% of the total nitrogen that was absorbed by the shoot dry mass + heads, indicating a high translocation of this nutrient to the shoots. Gachon (1972) studied the absorption of nutrients and observed an intense translocation of nitrogen from the leaves and stems for the heads and especially for the achenes of sunflower plants after flowering stage.

The nitrogen concentration in heads of safflower plants varied with the potassium rates, as in the linear regression model (Fig. 8A), decreasing with the potassium supply from 32.8 to 27.9 g kg$^{-1}$. However, the nitrogen accumulation in the heads increased (from 469.2 to 607.6 mg pot$^{-1}$) as the potassium rates increased (Fig. 8B).

The increased potassium rates reduced the nitrogen concentration due to the linear increase in the dry mass of the heads as promoted by K fertilization. Thus, as the reproductive mass increased, the nitrogen concentration...
Fig 5. Concentration (A) and nitrogen accumulation (B) in the shoot of safflower plants at 74 DAE according to the nitrogen rates in the oxisol. NCS - Nitrogen concentration in the shoot; NAS – nitrogen accumulation in the shoot; N - Nitrogen. *** , ** and * Significant at 0.1, 1 and 5%, respectively.

Fig 6. Concentration (A) and nitrogen accumulation (B) in the shoots of safflower plants at 74 DAE according to potassium rates in the oxisol. NCS - Nitrogen concentration in the shoot; NAS – nitrogen accumulation in the shoot; K - Potassium. *** , ** and * Significant at 0.1, 1 and 5%, respectively.

Fig 7. Concentration (A) and nitrogen accumulation (B) in the heads of safflower plants at 74 DAE according to the nitrogen rates in the oxisol. NCH - Nitrogen concentration in heads; NAH – nitrogen accumulation in heads; N - Nitrogen. *** and ** Significant at 0.1 and 1%, respectively.
Fig 8. Concentration (A) and nitrogen accumulation (B) in the heads of safflower plants at 74 DAE according to the potassium rates in the oxisol. NCH - Nitrogen concentration in heads; NAH – nitrogen accumulation in heads; K - Potassium. *** Significant at 0.1%.

Fig 9. Potassium accumulation in the shoots of safflower plants at 74 DAE according to the nitrogen rates in the oxisol. PAS - Potassium accumulation in the shoot; N - Nitrogen; *Significant at 5%.

Fig 10. Concentration (A) and potassium accumulation (B) in the shoots of safflower plants at 74 DAE according to the potassium rates in the oxisol. PCS - Potassium concentration in the shoot; PAS - Potassium accumulation in the shoot; K - Potassium; *** Significant at 0.1%.
decreased in this plant compartment due to the dilution effect of this nutrient in safflower heads. The highest nitrogen concentration that was found in the absence of potassium application did not result in the increased production of heads because the dry mass of the heads responded with increasing the linear with potassium rates. Thus, there was not a positive correlation between the nitrogen concentration and the production of the heads. However, the increased nitrogen accumulation with increasing potassium fertilization demonstrates the importance of potassium in the incorporation of nitrogen in safflower plants.

Concentration and potassium accumulation by plants

Nitrogen fertilization did not affect the potassium concentration in the shoots of safflower plants. However, there was a significant nitrogen rate for potassium accumulation in the shoot, adjusting the quadratic regression model; the maximum accumulation was 763.9 mg pot⁻¹, as obtained at a nitrogen rate of 90 mg dm⁻³ (Fig. 9). The highest potassium accumulation with nitrogen fertilization occurs due to increased shoot dry mass as promoted by nitrogen, and the nitrogen rates of 90 and 95 mg dm⁻³ are the maximum values of potassium accumulation and shoot dry mass, respectively. Thus, nitrogen favored potassium accumulation in the shoots of safflower plants, corroborating Singh et al. (2002), who reported that nitrogen application increased the potassium uptake by crops of rice and wheat. The concentration and potassium accumulation in the shoots of safflower plants adjusted the linear regression model according to potassium rates (Fig. 10A and B). Thus, the maximum potassium accumulation with nitrogen fertilization were obtained when the highest rate of the nutrient was applied. The observed differences in the concentration and accumulation between the condition in which there is no applied potassium and a higher rate of nutrient application were between 5.18 and 17.6 g kg⁻¹ and between 431 and 932 mg pot⁻¹, respectively. The maximum potassium concentration at the higher rate of the experimental interval is according to Mattos and Monteiro (1998), who conducted an experiment in a greenhouse with nutrient solution, evaluating the nutritional diagnosis of Marandu grass. The author found that increased potassium rates in the nutrient solution increased the potassium concentration in the shoot components, similar to the results of this study. Nitrogen fertilization did not influence the potassium concentration in the heads of safflower plants. However, there was a significant effect of the nitrogen rate on potassium accumulation in the heads, adjusting the quadratic regression model, the maximum accumulation was 322.5 mg pot⁻¹, obtained at the nitrogen rate of 108 mg dm⁻³ (Fig. 11). It is possible to perceive that the potassium accumulation in the heads was positively influenced by the nitrogen rates, indicating the relationship between these two nutrients, in which the absorption of a nutrient increases the demand for the other. In other words, the nitrogen supply can influence the absorption and potassium distribution in plant tissue (Cantarrela, 2007). In addition, the increased dry mass of the heads as promoted by nitrogen fertilization contributed to the maximum potassium accumulation by the experimental unit. For K fertilization, there were significant answers regarding the concentration and potassium accumulation in the heads of safflower plants according to the potassium rates, adjusted to the linear regression model (Fig. 12A and B). In conditions of non-application of potassium the concentration and accumulation of this nutrient in the heads were 10.6 g kg⁻¹ and 145.5 mg pot⁻¹, respectively, while the higher concentration and accumulation were 21.1 g kg⁻¹ and 437.2 mg pot⁻¹, respectively, observed at the highest ratio the experimental interval. The potassium concentrations in this study are close to those found by Gerendas et al. (2008) studying safflower that were subjected to different rates of potassium. These authors also found that the optimum supply of potassium fertilizer was equivalent to a potassium rate of 268 mg dm⁻³ and was responsible for the highest potassium concentration (20.6 mg g⁻¹) in safflower. Grove and Sumner (1982) also observed for the sunflower that an increased potassium supply increased the concentration of that nutrient.

Materials and Methods

Localization, treatments and plant material

The experiment was conducted in a greenhouse in Rondonopolo, Mato Grosso State (16°27'49”S and 50º34'47”W), at 284 m of altitude, from July to September 2014. The experimental design was a randomized complete block with 25 treatments and four replications, arranged in a 5×5 factorial scheme with five nitrogen rates (0, 60, 120, 180 and 240 mg dm⁻³) and five potassium rates (0, 50, 100, 150 and 200 mg dm⁻³) totaling 100 experimental units. The safflower cultivar IMA 0213 was used as the plant species.

Soil characteristics

The soil that was used in the experiment was classified as an oxisol (Embrapa, 2013), collected from a layer of 0-0.20 m, with the following chemical and textural characteristics (Embrapa, 1997): pH 4.1 (CaCl₂); 2.4 mg dm⁻³ P; 28 mg dm⁻³ K; 0.3 cmolc dm⁻³ Ca; 0.2 cmolc dm⁻³ Mg; 4.2 cmolc dm⁻³ H; 1.1 cmolc dm⁻³ Al; 5.9 cmolc dm⁻³ CEC; base saturation of 9.8 (V%); 22.7 g dm⁻³ OM; 549 g kg⁻¹ sand; 84 g kg⁻¹ silt; and 367 g kg⁻¹ clay.

Soil fertilizations and sowing

The soil was sieved through 4-mm mesh for experimental implementation in the greenhouse, and soil samples of 8 dm³ were placed in plastic bags. The soil acidity was corrected with the incorporation of dolomitic limestone (Total neutralization power = 80.3%), increasing the base saturation to 60%. After liming, the soil samples were moistened at 60% water retention capacity, the bag was sealed to prevent water loss, and the soil was incubated for 30 days. After incubation with limestone for soil acidity correction, the soil samples were moved to the pots, and fertilization was implemented, consisting of the application of phosphorus, potassium and micronutrients. The phosphorus recommendation (P₂O₅) was 150 mg dm⁻³, and the potassium was used at rates corresponding to treatment; the fertilizer sources were superphosphate and pure analytical-grade potassium chloride (PA), respectively. Micronutrient fertilizer was performed using a solution containing 1 mg dm⁻³ B and Cu, 3 mg dm⁻³ Mn and Zn and 0.2 mg dm⁻³ Mo; the fertilizer sources were H₂BO₃, CuCl₂·2H₂O, MnCl₂·4H₂O, ZnSO₄·7H₂O and NaMoO₄·2H₂O, respectively. Phosphorus was applied in solid granular form at the layer corresponding to the third (1/3) of the upper pot to increase the efficiency of nutrient absorption by the roots. Samples with 2.5 dm³ of soil were removed from the pot, incorporated the fertilizer and returned to the pot. The potassium application via solution
was split into two applications, the first at sowing and the second 10 days after plant emergence.

Sowing included 20 seeds per pot, leaving four plants in each pot after thinning. Thus, each experimental plot consisted of a plastic pot with a capacity of 8 dm$^3$ containing four safflower plants. At 10 days after plant emergence, the first application of nitrogen was performed, corresponding to treatments using urea as a source. Nitrogen fertilizer was applied via solution and subdivided into three applications (10, 20 and 30 days after emergence).

Irrigation management

The soil moisture was maintained uniform in all of the experimental units by controlling the water tension in the soil, performed by a subsurface self-irrigated system that permitted continuous water replacement to the soil according to the evapotranspiration of the soil-plant system. This irrigation system was composed of porous cup (5 cm in diameter and 9 cm high), inserted into the soil, connected to a water reservoir (Mariotte tube) through a flexible microtube (Fig. 13) according to Bonfim-Silva et al. (2007). The water in this reservoir was replaced as necessary. The water potential in the soil was established by the height of water column between the pot and the reservoir (height = 30 cm), which corresponds to a controlled tension of 3 KPa.

Traits measured

At 74 days after emergence (DAE), the following productive characteristics were determined: shoot dry mass and head dry mass; the following nutritional characteristics were determined: nitrogen and potassium concentration and nitrogen and potassium accumulation in the shoot and in the heads.

The shoot was then cut close to the soil surface, and the leaf mass was separated from the heads. The collected material was placed in paper bags, dried in an air-forced circulation heater at 65 ºC for 72 hours, and then weighed. The nitrogen and potassium concentration were determined according to the methods that were proposed by Malavolta et al. (1997). Was calculated the accumulated amounts of nutrients in the plant by multiplying the nutrient concentrations in every part of the plant by the amount of dry mass that is produced per pot.
Fig 13. Allocation of the porous ceramic capsule center and the surface layer of the pot (A and B). The pot was filled with fertilized soil to cover the capsule (C); the capsule was connected to the water reservoir through a flexible microtube (D).

Statistical analysis

The data were evaluated statistically using the “Statistical Analysis System” (Sas, 2002). First, an analysis of variance was performed for the nitrogen and potassium rate combinations. If the result was significant for the F test for the interaction between nitrogen rates and potassium rates, the response surface was studied through the RSREG procedure. In cases in which the interaction was not significant, a regression study of the components of the first and second degree was performed using the GLM command. The maximum level of significance was 5% for all of the statistical tests.

Conclusions

The application of nitrogen and potassium fertilization influenced the isolated mode in the productive and nutritional characteristics of safflower plants. The nitrogen and potassium concentrations in the shoots and heads peaked when higher rates of these nutrients were applied to the soil. The nitrogen and potassium accumulation in the shoots and heads of safflower responds similarly to the dry mass regarding the application of these nutrients to the soil. Potassium promotes an increase in the nitrogen accumulation in plant tissue, demonstrating the need for balance between these two nutrients in the fertilization of safflower crops.

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