

## The effect of nitrogen fertilization on root characteristics of *Camelina sativa* L. in greenhouse pots

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**Abstract.** Climate change has made mandatory the introduction of new crops in Greece, such as the cultivation of camelina [*Camelina sativa* (L.) Crantz]. Nitrogen (N) and the development of root system are two important factors affecting crop growth and yield. Camelina has been studied mainly for its composition and oil. In the present study, root development of camelina crop was thoroughly investigated; mainly in terms of Nitrogen fertilization. Therefore, a camelina greenhouse experiment was established in Western Greece, in the region of Agrinio, in March 2019 in completely randomized design with four treatments, (control 0 ppm N, 30 ppm N, 60, ppm N and 90 ppm N). The N rates had statistically significant affected root density and root surface from 40 to 120 days after treatment (DAT) with highest values at 100 DAT and 90 ppm N, 52.54 cm of root 100 cm<sup>-3</sup> and 27.59 cm<sup>2</sup> of root 100 cm<sup>-3</sup>, respectively. The root volume was significantly affected by N fertilizer from 40 to 100 DAT and highest value was 13.18 cm<sup>3</sup> of root 100 cm<sup>-3</sup> soil in the 90 ppm at 120 DAT. The plant leaf area was significantly affected by the highest rate of N. Yield per plant had not statistically significant difference with the 60 and with the 90 and highest weight per plant 292.25 g plant<sup>-1</sup> in 90 ppm. In conclusion, N fertilization significantly affected growth or camelina's root system after 40 DAT. Plant growth was significantly affected by fertilization and the highest yield and 1,000 seed weight were recorded with the highest amount of N.

**Key words:** camelina, N rates fertilizer, root system, thousand-seed weight, yield.

Abbreviations: N Nitrogen; DAT Days After Transplanting.

### INTRODUCTION

Camelina [*Camelina sativa* (L.) Crantz] belongs to the family Brassicaceae and has many benefits, environmental, industrial, as well as nutritional, since seed oil has a unique profile of fatty acid (Angelopoulou et al., 2019). A direct result of climate change is the change of temperatures that offer alternative cultivation zones and allow in Greece the introduction of new species (Bilalis et al., 2017). *Camelina sativa* is well-adapted to semi-arid region and grown for its seed and oil (Obour et al., 2015). Camelina oil is rich in fatty acid with high levels of alpha-linolenic acid and linoleic acid (Toncea et al.,

2013). Also, in many areas it is used as a raw material for biofuels due to the great potential for plant mass growth (Solis et al., 2013). It is an alternative plant for biofuel production with significant economic and environmental impact, mainly in areas where corn and soybeans are grown. In these areas, camelina is a great option to increase biodiversity, while at the same time, prevents weed expansion and limits pathogenic weed cycles (Johnson & Gesch, 2013). The environmental impact of camelina cultivation is also characterized by the fact that camellia oil used as jet fuel has low emissions (Shonnard et al., 2010). In the past, it was an important oil crop, while today has additional characteristics such as low weed management need and low soil tillage requirements (Bilalis et al., 2017).

Although camelina is a non-legume plant and has high need of N, while compared with other oil plants, camelina is a crop that does not need high inputs (Dobre & Jurcoane, 2011; Gao et al., 2018). According Francis & Warwick (2009) important factors that influence the yield of camelina are sowing density and available nutrients. The application of N fertilizer significantly increases the yield (Solis et al., 2013; Wysocki et al., 2013) and plant growth (Dobre et al., 2014). The different fertilization in camelina result in different quality of feed (Henriksen et al., 2009). Additionally, the oil content decreased with the increase of the N rate (Solis et al., 2013). However, there is a great variability in the effect of N fertilization on yields and oil quality depending on climatic and soil conditions. (Malhi et al., 2014). The N rate should not be excessive because it can have negative effects. A negative effect of N fertilization is the reduction of crop productivity (Johnson & Gesch, 2013). Johnson & Gesch (2013) regarding its use as biodiesel. In this aspect fertilization should not exceed 800 kg ha<sup>-1</sup>. In organic farming, the yield of camelina cultivation was higher with compost addition, followed by inorganic fertilization (Bilalis et al., 2017). In another oil crop such as linseed cultivation, there was a high correlation between oil content and seed yield (Bilalis et al., 2010).

The plant's ability to absorb nutrients is directly related to the development of the root system especially in dry thermal conditions (Sidiras et al., 2001; Anderson et al. 2012). In safflower crop the linoleic acid and fatty acids contents were reduced to dry conditions (Ashrafi & Razmjoo, 2010). Therefore, in order to ensure the quality of the oil, the root system plays a primary role, and with the appropriate agricultural practices it has the highest density and absorbs more nutrients. In corn cultivation the N fertilization increased the length of the root while the weight of the root decreased with the increase of N rate (Durieux et al., 1994). The applied N has positive significant affected the ratio shoot/ root of camelina, even in dry conditions camelina has the root mechanisms to use N (Gao et al., 2018).

While camellia is considered a low-input plant, the exact amount of fertilizer that gives the best yield has not been determined. Also, since the literature shows great variability in yields depending on the climatic conditions of each region, there is a knowledge gap for implementing its cultivation in Greece. The objective of this study was to identify the effect of different nitrogen amounts on the development of the root system, growth of the aboveground part of the plant and seed yield. Our hypothesis is that different levels of nitrogen fertilization, influence the growth of the root system of camelina plants.

## MATERIALS AND METHODS

### Experimental Design

A camelina greenhouse experiment (*Camelina sativa* L.) was set up in Western Greece, in the region of Agrinio, in March 2019. The cultivated variety was Calena, produced by Saatbau Linz in Austria

The experiment followed a completely randomized design (CRD), with four treatments, different amounts of added nitrogen (control 0 ppm N, 30 ppm N, 60 ppm N and 90 ppm N). The fertilizer applied was potassium nitrate, KNO<sub>3</sub>, 13% N and 46% K. In the control treatment 0 g KNO<sub>3</sub> (without fertilizer) were added, in the 30 ppm N treatment, 3.655 g KNO<sub>3</sub> were added, in 60 ppm N, 7.311 g KNO<sub>3</sub> and in 90 ppm N, 10.966 g KNO<sub>3</sub>. The quantity of K that was changing was not taken into account why K fertilization does not have a direct effect on a small biological life plant cycle and the release of potassium ions often takes place over a long period of time as opposed to nitrates (Thorne, 1954).

A total of 320 pots, 20 pots per treatment and 80 pots per replicate were used. The capacity of the pots was 12 L. They were filled with 8 L of soil and 4 L of compost (natural product of aerobic degradation of Sea weed *Posidonia oceanica*). Nitrogen content of compost 2%. The soil was clay loam (CL) with pH 6.72, organic matter 2.17%, total nitrogen 0.1125%, N-NO<sub>3</sub> 10 ppm and 12 ppm N-NH<sub>4</sub>, 18% CaCO<sub>3</sub>, and a good supply of available phosphorus (P-Olsen 48 ppm) and potassium (335 ppm) The greenhouse conditions remained the same for all pots (temperatures, sunshine and water). Irrigation rate was 8 times of 0.5 L during the camelina life cycle.

So that all plants have the same biological life cycle and the same time point of emergence seedlings were prepared. The growth of seedlings was done in a seedbed with the method of the floating system, where the germination of the seeds as well as the growth of the plants took place. Large water tanks were created, in which the discs were placed. The discs contained plant substrate. The plants remained there until they reached a height of 5 cm. The plants were then transplanted into pots., one plant per pot.

### Measurements

The measurements were performed in different days after transplanting (DAT). Root characteristics (root density, root surface and root volume) and plants agronomic characteristics (height, seed yield, leaf area and 1,000 seed weight) were measured.

Regarding the root measurements, the samples were collected in 6 different DAT (20, 40, 60, 80, 100 and 120 DAT) 2 samples per treatment. There were washed over a 5 mm mesh sieve. Also there was used a formalin/acetic acid/alcohol (FAA) staining solution. Root density (cm of root 100 cm<sup>-3</sup> soil), root surface (cm<sup>2</sup> of root 100 cm<sup>-3</sup> soil), as well as root volume (cm<sup>3</sup> of root 100 cm<sup>-3</sup> soil) were determined in millimeters using a high resolution scanner, using DT-software (Delta-T Scan version 2.04; Delta Devices Ltd, Burwell, Cambridge, UK) (Bilalis et al., 2012).

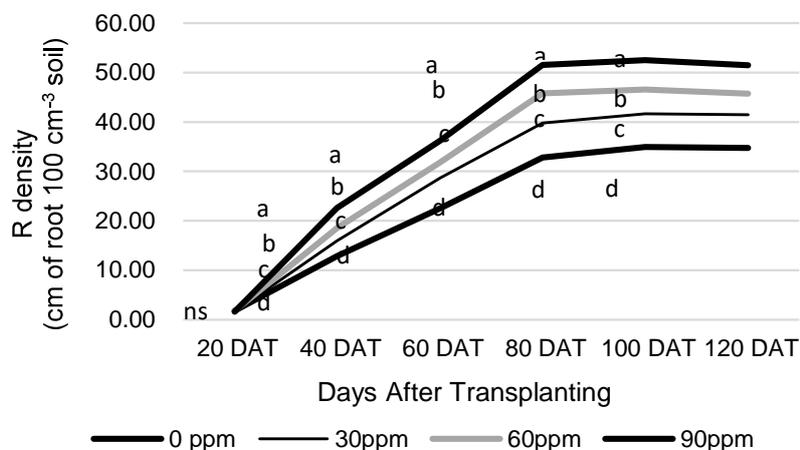
In terms of plant characteristics, plant height (cm), was measured in 100 DAT. At the end of the experiment, 120 DAT, there were measured the plantseed yield (g), the leaf area (cm<sup>2</sup> plant<sup>-1</sup>) and the 1,000 seed weight (g). The leaf area was determined in 100 DAT, by the use of an automatic leaf area meter (Delta-T Devices Ltd., Burrwell, Cambridge, UK).

### Statistical Analysis

The experimental data analysis was conducted using the software Statistica (StatSoft, 1996), according to the completely randomized design. Differences among the means were compared using the Least Significant Difference (LSD) test, at the 5% level of significance ( $P \leq 0.05$ ). The tests of correlation coefficients and linear regression by Statistica software were set at two levels with significance ( $\alpha = 0.05$ ) and remarkable significance ( $\alpha = 0.01$ ).

## RESULTS

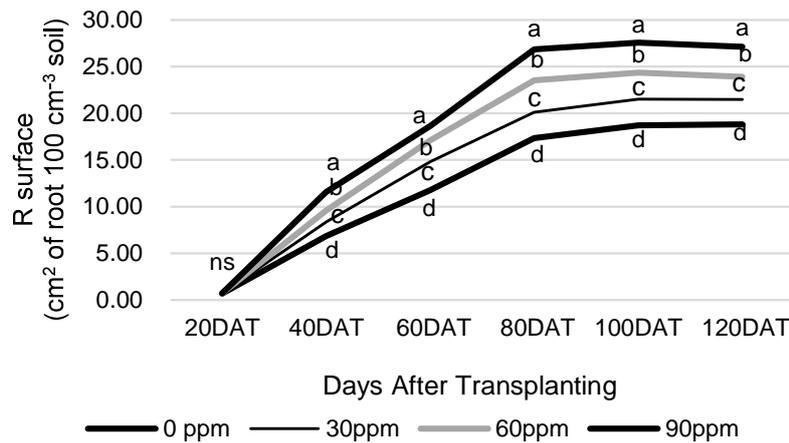
In Fig. 1, the changes in the root density are presented. The nitrogen treatments had statistically significant difference between them, from 40 to 120 DAT. At 20 DAT no treatment was statistically significant. The 0 ppm treatment had the lowest value of root density, in all measurements from the 20 DAT to 120 DAT and the 90 ppm had the highest value from the first measurement to the last (Fig. 1). At 80 DAT, at the 0 ppm treatment, the root density was 32.82 cm of root 100 cm<sup>-3</sup>, in the 30 ppm was 39.77 cm of root 100 cm<sup>-3</sup>, in the 60 ppm was 45.82 cm of root 100 cm<sup>-3</sup> and in the 90 ppm was 51.58 cm of root 100 cm<sup>-3</sup>. At 100 DAT the highest value was recorded, which was 52.54 cm of root 100 cm<sup>-3</sup> soil in the 90 ppm and the lowest was 12.85 cm of root 100 cm<sup>-3</sup> in the 0 ppm treatment at 40 DAT. Up to 100 DAT there was an increase in root density, but at 120 DAT there was a small decrease in all treatments (Fig. 1).



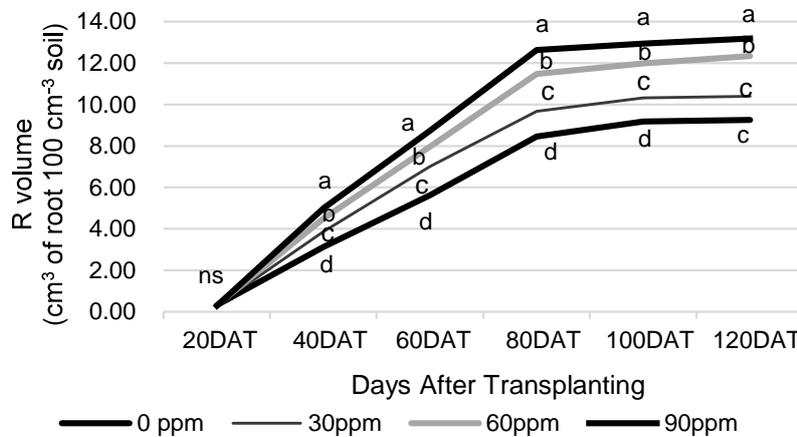
**Figure 1.** Changes in root density (affected by different nitrogen levels) in different Days After Transplanting (DAT) ('ns': not statistically significant at  $P = 0.05$ ).

Moreover, in Fig. 2, at 20 DAT none treatment were statistically significant, concerning the root surface. From 40 DAT to 120 DAT, all treatment had statistically significant difference between them. At 120 DAT there was a declining trend at all levels of nitrogen. The 0 ppm had the lowest values in all Days After Transplanting. On the other hand, the 90 ppm had the highest values in all Days After Transplanting.

The values ranged from 6.85 to 27.59 cm<sup>2</sup> of root 100 cm<sup>-3</sup> soil. The highest value was 27.59 cm<sup>2</sup> of root 100 cm<sup>-3</sup> soil at 100 DAT, in the 90 ppm and the lowest was 6.85 cm<sup>2</sup> of root 100 cm<sup>-3</sup> soil at 40 DAT, in the 0ppm (Fig. 2). Also, in the root surface and in the root density the same path is presented in the different DAT, increasing trend from 20 DAT to 100 DAT and in 120 DAT there is a decrease in all treatments.



**Figure 2.** Changes in root surface (affected by different nitrogen levels) in different Days After Transplanting (DAT) ('ns': not statistically significant at  $P = 0.05$ ).



**Figure 3.** Changes in root volume (affected by different nitrogen levels) in different Days After Transplanting (DAT) ('ns': not statistically significant at  $P = 0.05$ ).

Furthermore, in root volume changes, which are shown in the Fig. 3, at 20 DAT no treatment was statistically significant. From the 40 DAT to 100 DAT all nitrogen levels had statistically significant difference between them. At 120 DAT the 60 ppm had not statistically significant differences with the 90 ppm. The lowest value was 3.12 cm<sup>3</sup> of root 100 cm<sup>-3</sup> soil in the 0 ppm and the highest was 13.18 cm<sup>3</sup> of root 100 cm<sup>-3</sup> soil in the 90 ppm at 120 DAT. Compared to the other measurements of the root system, the

growth trend continues up to 120 DAT. The largest increase was observed from 40 DAT to 80 DAT (Fig. 3). At 40 DAT the values were, in 0 ppm 3.12 m<sup>3</sup> of root 100 cm<sup>-3</sup> soil, in 30 ppm 3.86 m<sup>3</sup> of root 100 cm<sup>-3</sup> soil, in 60 ppm 4.50 m<sup>3</sup> of root 100 cm<sup>-3</sup> soil and in 90 ppm 4.99 m<sup>3</sup> of root 100 cm<sup>-3</sup> soil. At 80 DAT the values were, in 0 ppm 8.45 m<sup>3</sup> of root 100 cm<sup>-3</sup> soil, in 30 ppm 9.67 m<sup>3</sup> of root 100 cm<sup>-3</sup> soil, in 60 ppm 11.47 m<sup>3</sup> of root 100 cm<sup>-3</sup> soil and in the 90 ppm 12.63 m<sup>3</sup> of root 100 cm<sup>-3</sup> soil (Fig. 3).

Also, in the plant height the values ranged from 57 to 72.75 cm. All treatments had statistically significant differences between them. The highest value was 72.75 cm in the 90 ppm and the lowest was 57 in the 0 ppm (Table 1). In the leaf area, the 30 ppm had not statistically significant differences with the 0 ppm and with the 60 ppm. The lowest value was 1.41 cm<sup>2</sup> plant<sup>-1</sup> in the 0 ppm and the highest was 2.26 cm<sup>2</sup> plant<sup>-1</sup> in the 90 ppm. Moreover, the yield per plant of the 30 ppm had not statistically significant differences to the 60 ppm and to the 90 ppm. The highest value was 292.25 g per plant in the 90 ppm and the lowest was 169.50 g per plant in the 0 ppm (Table 1). In the 1,000 seed weight measurement, all treatments had statistically significant differences between them. The values ranged from 1.29 to 1.66 g. The lowest value was 1.29 g in the 0 ppm and the highest was 1.66 g in the 90 ppm.

**Table 1.** The agronomic characteristics as affected by different nitrogen levels

	Plant height (cm)	Leaf area (cm <sup>2</sup> plant <sup>-1</sup> )	Yield (g plant <sup>-1</sup> )	1,000 seed weight (g)
0 ppm	57.00 <sup>a</sup>	1.41 <sup>a</sup>	169.50 <sup>a</sup>	1.29 <sup>a</sup>
30 ppm	63.00 <sup>b</sup>	1.92 <sup>ab</sup>	208.75 <sup>b</sup>	1.36 <sup>b</sup>
60 ppm	66.50 <sup>c</sup>	2.12 <sup>b</sup>	231.00 <sup>bc</sup>	1.48 <sup>c</sup>
90 ppm	72.75 <sup>d</sup>	2.26 <sup>c</sup>	292.25 <sup>c</sup>	1.66 <sup>d</sup>
F <sub>ppm</sub>	74.16 <sup>***</sup>	25.25 <sup>***</sup>	66.57 <sup>***</sup>	141.75 <sup>***</sup>

*F*-test ratios are from ANOVA. Different letters within a column indicate significant differences according to LSD ( $P = 0.05$ ). Significance levels: \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ ; ns, not significant ( $p > 0.05$ ).

## DISCUSSION

It is known that the development of the root system is affected by the availability of nutrients (Drew, 1975). According to Pavlista et al. (2012), who studied camelina and two other crops, the fertilization as well as irrigation are critical factors for the first six weeks of root growth. This is also shown in the figures above, as there was an almost linear increase in all root characteristics during the first 40 DAT (Fig. 1, Fig. 2, and Fig. 3). In addition, there was positive correlation between the roots with the agronomic characteristics (Table 2). More specifically, the root density was positively correlated with the plant height, the yield, the leaf area and with the 1,000 seed weight ( $r = 0.94$ ,  $P < 0.001$ ;  $r = 0.90$ ,  $P < 0.001$ ;  $r = 0.91$ ,  $P < 0.001$  and  $r = 0.92$ ,  $P < 0.001$  respectively), (Table 2). In several studies was reported that higher plant growth and yield were related with root system characteristics (Vamerali et al., 2000; Den Herder et al., 2010).

Moreover, there was positive correlation between the root characteristic. Thus root density was positively correlated with root surface ( $r = 0.98$ ,  $P < 0.001$ ) and with root volume ( $r = 0.94$ ,  $P < 0.001$ ), while root volume was positively correlated with the root surface ( $r = 0.94$ ,  $P < 0.001$ ) (Table 2). The different amounts of N application significantly affected the development of roots, in accordance with several studies who

observed that the variation in nitrogen supply leads to differences in root development (Tausz et al., 2017).

**Table 2.** Correlation matrix between root and agronomic characteristics

	Root density 120 DAT	Root surface 120 DAT	Root volume 120 DAT	Plant Height	Yield	Leaf area per plant	1,000 seed weigh
Root density 120 DAT	1	0.98***	0.94***	0.95***	0.90***	0.91***	0.92***
Root density 120 DAT	0.98***	1	0.94***	0.93***	0.87***	0.94***	0.95***
Root density 120 DAT	0.94***	0.94***	1	0.89***	0.86***	0.85***	0.88***
Plant height	0.95***	0.93***	0.89***	1	0.89***	0.80***	0.90***
Yield	0.90***	0.87***	0.86***	0.89***	1	0.77***	0.85**
Leaf area per plant	0.91***	0.94***	0.85***	0.80***	0.77***	1	0.92***
1,000 seed weight	0.92***	0.95***	0.88***	0.90***	0.85***	0.92***	1

Significance levels: \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ ; ns, not significant ( $P > 0.05$ ).

Urbaniak et al. (2007), reported that the plant height of the camelina increased as the applied dose of nitrogen fertilizer increased. Similar results were obtained in our study where the highest plant height was recorded in the higher amounts of nitrogen. Furthermore, Johnson & Gesch (2013) presented in their study that different nitrogen levels had no effect on the photosynthetic capacity of the leaves. On the other hand, Field & Mooney (1986); Pan et al. (2011) emphasized that as nitrogen levels increased, resulted in greater leaf area, leaf N, as well as increased leaves photosynthetic capacity. Also, Karydogianni et al. (2020) reported that the effect of nitrogen on canopy photosynthesis was due to the effect of nitrogen on the production of the leaf area as well as on light interception. In our study, the leaf area increased as the applied dose of nitrogen increased.

In addition, the yield per plant increased with increased rate of nitrogen fertilizer. Czarnik et al. (2017), reported that increasing nitrogen fertilization significantly increases the number of silicles per plant, as well as the number of seeds per silicles. Henriksen et al. (2009), said that the camelina showed an increase in yield at 40 kg N ha<sup>-1</sup> compared to the control. Czarnik et al. (2017), demonstrated that the 1,000 seeds weight increased significantly as the nitrogen applied to the fertilizer increased. Similar results were presented in our study, where at 90 ppm the largest weight of 1,000 seeds was recorded. The yield per plant had a positive correlation with the plant height ( $r = 0.89$ ,  $P < 0.001$ ) as well as with the weight of 1,000 seeds ( $r = 0.85$ ,  $P < 0.001$ ), (Table 2).

## CONCLUSION

In conclusion, the different levels of nitrogen fertilization, influenced the growth of the root system, as well as the agronomic characteristics of the camelina plant. More specifically, at the level of 90 ppm nitrogen, the highest growth rates were recorded for

the characteristics of the root such as root density, as well as in the agronomic characteristics such as the yield per plant. Also in the measurements of the root system at 100 DAT the maximum growth was presented.

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