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Nitrogen and Phosphorus Addition to Soil Improves Seed Yield, Foliar Stomatal Conductance, and the Photosynthetic Response of Rapeseed (*Brassica napus* L.)

Esmaeil Zangani [1,*], Kamran Afsahi [1], Farid Shekari [1], Eileen Mac Sweeney [2] and Andrea Mastinu [2,*]

[1] Department of Plant Production and Genetics, Faculty of Agriculture, University of Zanjan, Zanjan 45371-38791, Iran; afsahi@znu.ac.ir (K.A.); shekari@znu.ac.ir (F.S.)
[2] Department of Molecular and Translational Medicine, Division of Pharmacology, University of Brescia, 25123 Brescia, Italy; e.macsweeney@studenti.unibs.it
* Correspondence: zangani@znu.ac.ir (E.Z.); andrea.mastinu@unibs.it (A.M.)

Abstract: The effects of nitrogen and phosphorus levels on the physiological traits, yield, and seed yield of rapeseed (*Brassica napus* L.), were studied in a farm research project of Zanjan University. Three levels of nitrogen (0, 100, and 200 kg/ha) and three levels of phosphorus (0, 75, and 150 kg/ha) were considered. The results showed that an increase in nitrogen level caused an increase in the leaf chlorophyll content so that the application of 200 kg/ha of nitrogen increased the chlorophyll content of the leaves until the mid-grain filling stage. Nitrogen application lowered leaf stomatal conductance in the early flowering stage whereas the stomatal conductance was increased during the late flowering stage. Nitrogen application (100 and 200 kg/ha) also increased the quantum yield of photosystem II. On the other hand, with the application of 150 kg/ha and 75 kg/ha of phosphorus, the leaf stomatal conductance and the quantum yield of photosystem II in the early flowering stage increased respectively. The results showed that the application of 200 kg/ha of nitrogen and 75 kg/ha of phosphorus significantly increased seed and oil yield compared to the control. In addition, the number of siliques per plant and the weight of 1000 seeds showed an increasing trend that was affected by nitrogen and phosphorus levels. This study demonstrated that nitrogen enhanced the chlorophyll content, leaf area, and consequently, the quantum yield of photosystem II. Nitrogen also augmented the seed filling duration, seed yield, and oil yield by increasing gas exchange. As a result, the application of 100 kg/ha of nitrogen together with 75 kg/ha phosphorus showed the greatest effect on the qualitative and quantitative yield of rapeseed. However, the application of 200 kg/ha of nitrogen alone or in combination with different levels of phosphorus did not significantly increase many of the studied traits.

Keywords: canola; chlorophyll; gas exchange; oil yield; quantum yield

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1. Introduction

Nitrogen (N) is the most limiting nutrient in soils used for agronomy in many regions of Iran [1]. Nitrogen is an essential nutrient for plant growth and is a constituent of secondary metabolites and amino acids, which play an important role in all plant metabolic processes [2–5]. Phosphorus (P) is required for energy metabolism and as part of plant structural components [6–8], and is therefore added to the soil as a fertilizer to increase seed production in many crops. The enrichment of the soil with nitrogen and phosphorus can contribute to increasing the nutritional value of plants for human and animal use [9–11]. Moreover, response to fertilizer application can depend on soil type, nutrients present, and weather conditions [12]. Tabak and colleagues, investigating the effects of nitrogen on the agronomic and physiological characteristics of wheat, reported on the agronomic efficiency for doses of 200 and 250 kg N ha^{-1} and on the physiological efficiency for a dose of 200 kg N ha^{-1} [13].

Rapeseed (*Brassica napus* L.) is an oil seed and belongs to the *Crucifereae* family that is cultivated in the many climates of Iran. In addition, it is a crop grown in rotation with wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), and rice (*Oriza sativa* L.). Adequate application of mineral fertilizers increased plant growth, seed filling time, and oil yield in canola. Rapeseed requires higher amounts of N and P compared to small seed cereal crops [14–16]. In autumn, additional nitrogen is needed for the overwinter survival in early-sown rapeseed [17]. In Iran, rapeseed production increases when N fertilizer is used while the use of P fertilizer has little effect, however, some soils are poor in P and rapeseed production can be increased by adding both P and N fertilizers. P deficiency problems limit crop production in many soils due to high pH and the presence of P in its insoluble form [12]. Previous research has shown that P deficiency can reduce oil concentration in rapeseed under different conditions [18]. Furthermore, N deficiency accelerates maturation, decreases leaf chlorophyll content, phosphoenolpyruvate carboxylase (PEPCase) activity, and affects the quantum yield of PSII photochemistry (Fv/Fm). As a result, it reduces leaf area and biomass yield [19,20]. Hossain et al. (2010) reported that leaf chlorophyll content is significantly influenced by the levels of N and P, as the chlorophyll content of the leaf is negatively correlated with the age of the plant during the treatment period with N and P [21]. Some studies report that N fertilizer increases the seed yield of rapeseed [22–25]. Some experiments show that nitrogen is the primary factor for lower rapeseed yields in Iran.

Therefore, since N and P affect the growth and physiology of plants, it is essential to establish the ideal concentrations of N and P required to increase the quality and yield of plants for agronomic purposes. For this reason, given the agronomic importance of rapeseed in the world, this plant species was chosen to evaluate the effects of supplementing the soil nitrogen and phosphorus. Much research has studied the effects of nitrogen and phosphorus supplementation [16,26–28], but few studies have focused on geographic areas with semi-arid soils. Thus, the objectives of this study were to identify the ideal concentrations of nitrogen and phosphorus in order to improve certain physiological traits as well as the quantitative and qualitative yield of rapeseed. In particular, given the criticalities in the growth of plants in arid soils, this work will define the synergistic effects of N and P in the improvement of photosynthetic processes. Indeed, this integration could improve the response of photosynthetic systems in *Brassica napus*. At the same time, integration with N and P could increase the reserve lipid content in the seeds. The reserve lipid content in the seeds can be considered a fundamental investment that the mother plant makes towards its progeny.

2. Materials and Methods

2.1. Growth Conditions

The study was conducted in the University of Zanjan, Faculty of Agriculture Research Farm, Zanjan province, Iran (latitude: 36°41′ N, longitude: 48°24′ E and altitude 1620 m above sea level). The field soil texture was sandy loam. Based on the USDA soil taxonomy, the study area presented an *Aridisol* type soil. Some of the soil characteristics are shown in Table 1. The area is semiarid with a 10-yr average air temperature of 10.6 °C and an annual precipitation of 285 mm. The effects of nitrogen and phosphorus levels on the physiological traits and yield parameters of rapeseed were evaluated. Three levels of nitrogen (0, 100, and 200 kg/ha) and three levels of phosphorus (0, 75, and 150 kg/ha) were considered. The research was carried out in a randomized complete block design with factorial restriction, replicated three times. The plots for the entire experiment were 27. The seeds were sterilized with 0.2% carboxinthiram fungicide prior to sowing. The seeds were sown on 10 September 2017 in Okapi cultivar, the area with the highest level of cultivation in cold temperature regions. The surface of the plots were comprised of five 4 m rows spaced 40 cm apart. Fifty percent of the N fertilizer was applied before sowing, while the remainder was applied before the stem was elongated. Phosphorus was added to the soil before cultivation. Ammonium nitrate and ammonium phosphate with the addition of

50% P_2O_5 were the fertilizers used for the experiments. At the beginning of the experiment, full irrigation (supplied by irrigation tape) was applied to all experimental plots until the plants were fully established.

Table 1. Some physical and chemical characteristics of the field soil.

Sand	Silt	Clay	pH	EC	O.C	K	P	N
(%)	(%)	(%)		ds/m	(%)	ppm	ppm	(%)
14	51	35	7.8	0.59	0.84	152	8.5	0.12

2.2. Physiological and Biochemical Parameters

The chlorophyll content, leaf stomatal conductance, and quantum yield were measured after two stages (the early and the end flowering stages, with a 14-day interval) by randomly selecting five plants. Leaf chlorophyll content was measured using a chlorophyll meter device (CCM–200-Opti-science, Hoddesdon, UK) [21]. A porometer (AP4-Delta-T, Cambridge, UK) was used to assess the leaf stomatal conductance in order to evaluate the gas exchange in the leaf [21]. In order to measure quantum yield of photosystem II, the chlorophyll fluorescence of the leaves was measured and registered in a darkened environment using a fluorimeter device (OSI 30, Hoddesdon, UK). For this purpose, leaves were covered with a specific clip for 20 min and then the quantum yield of photosystem II (Fv/Fm) was obtained [21].

2.3. Seed Yield Measurements

Two m^2 per plot were harvested manually after the plants reached physiological maturity. The organic material yield and seed yield were determined. The components of the seed yield including the number of siliques per plant, number of seeds per silique, and the 1000-seed weight were calculated by sampling 10 plants per plot randomly [21]. The seed oil content was measured using a seed analyzer device (NearInfra Red system). The oil yield was calculated by the multiplication of seed yield by oil content [21]. Seed and oil yield were reported in kg/ha.

2.4. Statistical Analysis

All data are expressed as mean ± standard deviation. Data was analyzed using SAS statistical software (ANOVA; SAS, Version 9.1, Cary, NC, USA) and mean scores were compared using Duncan's multiple range test at $p = 0.05$ level.

3. Results

3.1. Chlorophyll

The variance analysis results for the leaf chlorophyll showed that in the early flowering stage, different levels of nitrogen cause significant differences in the leaf chlorophyll content. On the contrary, the application of phosphorus had no significant effect on this trait (Table 2). In addition, nitrogen and phosphorus interaction in the late flowering stage was significant (Table 2).

The results demonstrated that an increment in nitrogen level significantly increased leaf chlorophyll content (Figure 1). The interaction between nitrogen and phosphorus revealed that applying 200 kg/ha of nitrogen significantly increased the leaf chlorophyll content, although there was no significant difference compared to the application of 100 kg/ha N and 150 kg/ha P. Therefore, it can be concluded that the application of nitrogen increased the leaf greenness index until the middle of the seed filling stage.

Table 2. Variance analysis of some physiological characteristics of rapeseed under N and P levels.

Variables	df	Mean Square					
		Chl. Content (First Stage) mg g^{-1}	Chl. Content (Second Stage) mg g^{-1}	Stomatal Conductance (First Stage) $mmol^{-2}$ s^{-1}	Stomatal Conductance (Second Stage) $mmol^{-2}$ s^{-1}	Quantum Yield PSII (First Stage) Fv/Fm	Quantum Yield PSII (Second Stage) Fv/Fm
Repeat	2	94.18	0.048	9326.8	1286.8	0.044	0.022
Nitrogen (N)	2	664.6 **	36.11 ^{ns}	7851.9 **	5037.9 *	0.032 ^{ns}	0.037 *
Phosphorus (P)	2	15.81 ^{ns}	94.75 ^{ns}	2457.8 **	267.8 ^{ns}	0.059 *	0.010 ^{ns}
N*P	4	46.2 ^{ns}	198.03 **	1270.6 ^{ns}	1327.6 ^{ns}	0.005 ^{ns}	0.010 ^{ns}
Subplot error	16	52.3	32.42	477.5	1245.5	0.014	0.008
CV		17.2	15.3	26.5	18.9	15.2	10.6

ns: not significant, * and ** respectively represent significance at probability level of 5% and 1%.

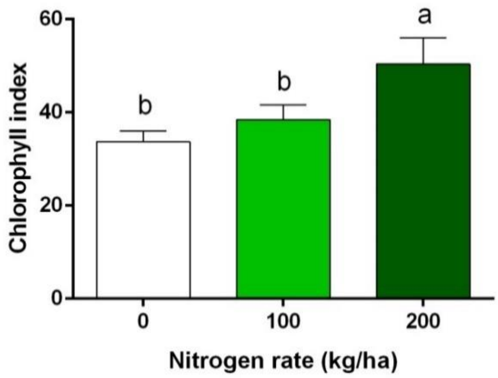

Figure 1. Effects of N application on chlorophyll content index in early flowering. Different letters on the top of the bars indicate significant difference at $p < 0.05$ by Duncan.

3.2. Stomatal Conductance

A significant difference for nitrogen and phosphorus levels at 1% probability level was observed for leaf stomatal conductance in the early flowering stage. This difference for the simple effect of nitrogen was significant in the late flowering stage (Table 2). Results showed that an increase in the nitrogen level caused a decrease in leaf stomatal conductance in the early flowering stage. The application of 100 kg/ha N significantly increased leaf stomatal conductance in the late flowering stage compared to the non-application of N (Figure 2B). In addition, results revealed that unlike N, increases in P levels significantly augmented leaf stomatal conductance in the early flowering stage compared to the control (Figure 2C).

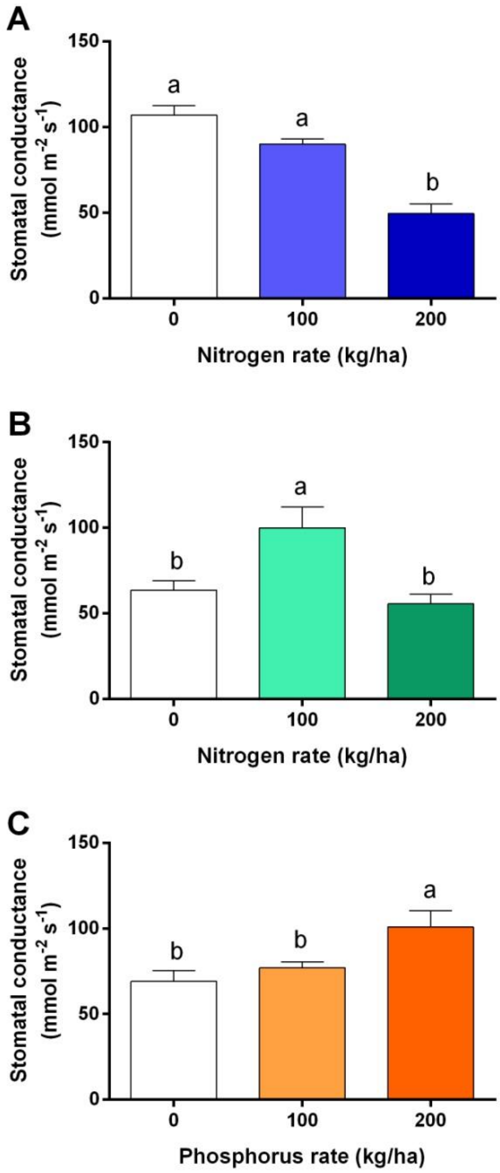

Figure 2. Effects of N application in early flowering (**A**) and late flowering (**B**) on foliar stomatal conductance and effect of P application on foliar stomatal conductance in early flowering (**C**). Different letters on the top of the bars indicate significant difference at $p < 0.05$ by Duncan.

3.3. Quantum Yield of Photosystem II

Results of variance analysis for the quantum yield of photosystem II showed that there was no significant difference between N levels in the early flowering stage while P application revealed a significant effect on this trait (Table 2). The application of N in the late flowering stage significantly increased the quantum yield of PS II compared to the non-application of this fertilizer. Different levels of P did not cause a significant increase in the quantum yield of PS II (Table 2). The results demonstrated that the application of 75 kg/ha P in the early flowering stage increased the quantum yield of PS II compared to the higher level of P but this increment was not significant compared to the control (Figure 3A). In addition, the application of N significantly enhanced the quantum yield of PSII in the late flowering stage compared to the control (Figure 3B).

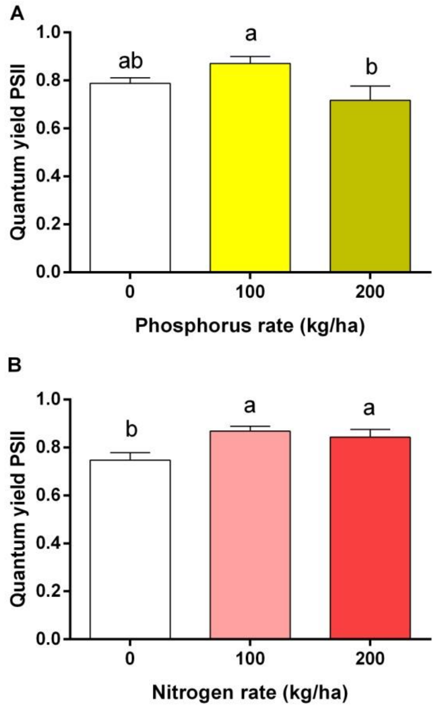

Figure 3. Effect of P application in early flowering (**A**) and effect of N application in late flowering (**B**) on quantum yield PSII. Different letters on the top of the bars indicate significant difference at $p < 0.05$ by Duncan.

3.4. Yield and Seed Yield Components

Results of variance analysis for the seed yield components revealed that the effect of N on the number of siliques per plant and the effect of N×P on the 1000-seed weight were significant. However, the effects of N, P, and their interaction did not show a significant difference on the number of seeds per siliques (Table 3). Mean comparisons of the number of siliques per plant revealed that application of 200 kg/ha of nitrogen increased the siliques per plant significantly compared to the control and the 100 kg/ha N level (Figure 4). The highest weight of 1000 seeds was obtained with 100 kg/ha N and 75 kg/ha P but there was no significant difference with some of the treatments (Table 4).

Table 3. Variance analysis of yield and yield components of rapeseed under N and P levels.

Variables	df	Mean Square						
		Seed Yield (kg/ha)	Oil Yield (kg/ha)	Oil Content (%)	Seed per Silique	Siliques per Plant	1000 Seed Weight (g)	Harvest Index (%)
Repeat	2	2,107,874	421,173	0.712	5.53	607.7	0.147	86.5
Nitrogen (N)	2	3,939,121 **	732,087 **	2.89 *	2.61 ^{ns}	377.4 *	0.218 ^{ns}	45.9 ^{ns}
Phosphorus (P)	2	1,106,963 ^{ns}	210,125 ^{ns}	0.161 ^{ns}	13.46 ^{ns}	295.5 ^{ns}	0.713 ^{ns}	16.3 ^{ns}
N*P	4	1,903,944 *	405,293 *	1.21 ^{ns}	11.08 ^{ns}	109.8 ^{ns}	1.055 *	74.4 *
Subplot error	16	570,051	102,704	0.65	10.38	103	0.339	18.3
CV		28.6	27.4	2.3	12.4	19.6	15.4	18.3

ns: not significant, * and ** respectively represent significance at probability level of 5% and 1%.

Variance analysis showed that the seed yield increased significantly with the application of N (1% level) and N × P interaction (Table 2). The results showed that the application of N (both levels) as well as 75 kg/ha P increased the seed yield significantly compared to the control; for example, the application of 200 kg/ha N increased the seed yield by 164% compared to the control (Table 4). Variance analysis of the harvest index revealed a significant effect of N×P on this trait (Table 3). The highest harvest index was obtained using 200 kg/ha N treatment although there was no significant difference compared to 100 kg/ha N and 150 kg/ha P treatments (Table 3).

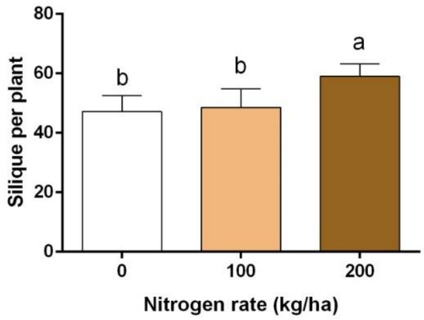

Figure 4. Effects of N application on siliques per plant. Different letters on the top of the bars indicate significant difference at $p < 0.05$ by Duncan.

Table 4. Effects of N and P application on seed yield and yield components of rapeseed.

Nitrogen (kg/ha)	Phosphorus (kg/ha)	Seed Yield (kg/ha)	Oil Yield (kg/ha)	1000 Seed Weight (g)	Harvest Index (%)	Chl. Content (Second Stage)
	0	1606.7 ^{c}	722.6 ^{d}	3.4 ^{abc}	19.4 ^{d}	35.5 ^{bc}
0	75	2427.1 ^{bc}	1077.5 ^{bcd}	4.27 ^{ab}	29.7 ^{ab}	37.2 ^{bc}
	150	1781.8 ^{c}	795.2 ^{cd}	3.53 ^{abc}	19.9 ^{cd}	32.4 ^{c}
	0	1974.1 ^{c}	860.6 ^{cd}	3.3 ^{bc}	25.5 ^{abcd}	36.4 ^{bc}
100	75	3284.3 ^{ab}	1466.0 ^{ab}	4.3 ^{a}	25.9 ^{abcd}	34.6 ^{c}
	150	2888.5 ^{bc}	1304.1 ^{bc}	4.1 ^{ab}	27.2 ^{abc}	45.5 ^{ab}
	0	4234.8 ^{a}	1880.7 ^{a}	4.3 ^{ab}	32.6 ^{a}	50.8 ^{a}
200	75	3292.8 ^{ab}	1440.9 ^{ab}	3.6 ^{abc}	24.5 ^{bcd}	31.8 ^{c}
	150	2235.9 ^{bc}	971.3 ^{bcd}	3 ^{c}	25.0 ^{bcd}	31.3 ^{c}

Means sharing the same letters in a column in per trait do not differ significantly at $p \leq 0.05$ according to Duncan's multiple range tests.

3.5. Oil Content and Oil Yield

Results of variance analysis for the oil content and oil yield showed that N application was significant (Table 3). However, N×P interaction was significant only for oil yield (Table 2). Mean comparisons showed that an increase in the amount of nitrogen caused a decrease in the seed oil content although the reduction was not significant compared to the 100 kg/ha N level (Figure 5). The highest oil yield was obtained by the application of 200 kg/ha N, which showed a 160% increase compared to the control plants (Figure 5). Phosphorus also had a positive effect on the seed oil content while N×P interaction (100 or 200 kg/ha nitrogen and 75 kg/ha phosphorus) increased the oil yield compared to the non-application of these fertilizers (Table 4).

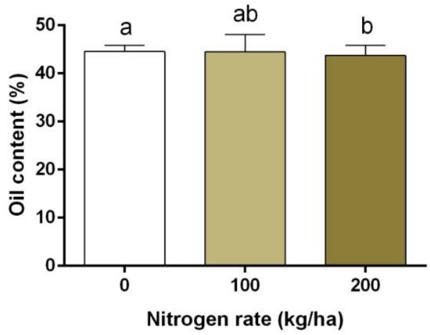

Figure 5. Effects of N application on seed oil content of rapeseed. Different letters on the top of the bars indicate significant difference at $p < 0.05$ by Duncan.

4. Discussion

Rapeseed is a rotation crop for semiarid terrain and diversifies production to add a high oil and protein content crop to cereal rotations [29,30]. In this experiment, the effect of nitrogen and phosphorus on some traits of rapeseed was significant.

The leaf chlorophyll content was significantly influenced by N and P levels during plant growth [21]. Nitrogen is a component of chlorophyll that is responsible for photosynthesis. The researchers have demonstrated that up to 75% of leaf nitrogen is found in the chloroplasts and most of it is found in ribulose bisphosphate carboxylase [31]. Therefore, nitrogen deficiency often results in a reduction in the chlorophyll index and rubisco activity and hence a reduction in photosynthesis. As a result, yield and growth are reduced [32].

In this study, nitrogen deficiency caused a reduction in chloroplast photochemical reactions and the quantum yield of photosystem II (Fv/Fm) [19]. It seems that the activity of the xanthophyll cycle increases in response to N deficiency, to distribute excess energy under high light conditions [33]. Moreover, this could be associated to a loss in chlorophyll content and to imbalances in the allocation of assimilates due to decreased plant growth due to N decline [34]. Some researchers found that a reduction in N has no effect on the Fv/Fm, but some reports have determined that N limitation reduces the efficiency of PSII photochemistry and the quantum yield of PSII in spinach [33,35,36]. However, some evidence confirms that Fv/Fm is associated with a loss in chlorophyll and perhaps related to the stability of oxidized PSII caused by N deficiency [33,36]. Wang et al. (2001) also found the quantum yield of PSII (Fv/Fm) to be sensitive to phosphorus stress [37].

Our results demonstrated that plants grown in high N conditions during the early flowering stage were not associated with adjustments of the density or apertures of stomas [19]. It seems that the decreased gas exchange at low N and P levels in the end and early flowering stages might be due to both the smaller stomatal conductance and lower biochemical performance of chloroplasts [21]. Broadley et al. (2001) suggested that N limits gas conductance across stomata or limits leaf morphology by reducing cell division and cell expansion in epidermal cells [38]. This data is consistent with data reported for sunflowers (*Helianthus annus*) [39], lettuce (*Lactuca sativa*) [38], and castor beans (*Ricinus communis* L.) [40].

The effect of N and P levels on the yield components of rapeseed showed that their application improved the qualitative and quantitative traits compared to the non-application of these elements. Other researchers also found an increase in yield due to the application of nitrogen fertilizer [41–44] whereas nitrogen application in excess can induce plant lodging, reduce oil content, and increase chlorophyll content of the seed [45]. On the other hand, the P necessary for high yields usually results from P addition or from being combined with different levels of nitrogen [45,46]. Furthermore, P application can accelerate growth and decrease the time until harvest. It seems that the application of different levels of N for rapeseed changes the seed yield response to P fertilizer. Reduction in the seed oil content with increases in nitrogen could be due to increases in seed protein [47]. Consequently, application of high levels of N decreased the seed filling period and as a consequence reduced grain oil content as reported by several authors [17,48,49].

5. Conclusions

Based on the results of this investigation, N deficiency can affect the physiological characteristics of plants and thus it reduces the quantitative and qualitative yield of rapeseed while the addition of P fertilizer is only favorable if soil testing indicates P deficiency. Although nitrogen and phosphorus supplementation improved the physiology of rapeseed, some questions remain unanswered. In particular, in order to better understand how the plant interacts with the soil, the nitrogen and phosphorus soil levels will have to be quantified in subsequent studies. In addition, it will be important to measure the nitrogen and phosphorus content inside the plant. This information completes the results for the optimization of rapeseed physiology and could better clarify the pathways involved.

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