The Effect of Nitrogen and Sulphur Fertilizers and Growth Regulator on the Seed and Oil Yield of Canola as Second Crop in Rice Fields

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ABSTRACT

The effect of chemical nitrogen (N) and sulphur (S) fertilizers, as well as a growth regulator, was studied on quantitative and qualitative traits of canola cv. 'Hyola 401' as a second crop in paddy fields in Baz Kia Gurab located in Lahijan, Guilan province, Iran in 2021-2022 in a split-plot experiment based on a randomized complete block design with three replications. The experimental factors included a growth regulator (Shootex) at two rates of 0 or 200 mg/L as the main plot and pure N fertilizer at four levels of 0, 90, 180, or 270 kg/L (from a urea source containing 46% N) and S fertilizer at three levels of 0, 35, or 70 kg/ha (from an ammonium sulphate source containing 24% S and 12% N) as the subplot. The results showed that the highest seed and oil yields (4081 and 1135 kg/ha, respectively) were obtained from the treatment of the growth regulator, 270 kg/ha N, and 70 kg/ha S, and the lowest (1086 and 368 kg/ha, respectively) from the treatment of the growth regulator, no N fertilizer, and 70 kg/ha S. To achieve the best results, the use of vegetative growth regulator, nitrogen and sulphur fertilizers, 270 and 70 kg/ha each, is recommended for rapeseed in the growing conditions of the region.

Keywords: canola, nutrition, paddy, yield.

INTRODUCTION

Canola (*Brassica napus* L.), an important oilseed in the world, is the third leading source of oil production after soybean and oil palm. Canola seeds contain more than 40% oil (Ghasem Beiki et al., 2020). Canola oil is the only edible oil whose fatty acids contain sulphur. The fatty acids of canola oil are mostly unsaturated (Ohara et al., 2009). This plant, which has high oil content, can be cultivated in Iran, so it can compensate for the failure to supply the oil demand of this country. Like sunflower, soybean, and corn, canola oil is nutritionally valuable since it contains unsaturated fatty acids and no cholesterol.

In addition to having suitable nutritional properties, it can be cultivated in many parts of the world from higher to lower latitudes since it has autumn and spring cultivars (Naderifar et al., 2019). Global population growth and the improvement of living standards have increased canola production. Its cultivation has also increased due to the development of specific cultivars and markets (Safikhani et al., 2015). China, Canada, India, and Germany are the leading canola-producing countries, accounting for over 32% of the world's canola (Ghasem Beiki et al., 2020). Canola oilseed has been imported to Iran in recent years and has provoked interest owing to the improvement in the quality of its oil and meal (Rabiee et al., 2012). The total cultivation area of canola was 183,431 ha, with an annual production rate of 295,123 t in 2018-2019 (Anonymous, 2020).

Soil fertility and the supply of nutrients required by canola play an essential role in its seed production and quantitative and qualitative yield. Using chemical fertilizers can effectively increase fertilizer use efficiency and contribute to achieving the maximum yield of canola (Mostafavirad et al., 2018). The application of inorganic chemical fertilizers is the fastest way to supply the nutrients that plants need (Abdolahi et al.,

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2020). The rate and type of fertilizers applied to canola influence its oil quality (Asare and Scarisbrick, 1995). Nitrogen (N) is one of the most important nutrients and a key factor in achieving optimal crop yields. Currently, the most important method of nitrogen supply is using nitrogen fertilizers. Nitrogen management is crucial to economically producing crops and meeting society's food needs (Tedone et al., 2018). Canola has a high nitrogen requirement. Accordingly, its optimal production in Iran requires the application of nitrogenous fertilizers because most farms in this country suffer from the scarcity of absorbable nitrogen and organic matter. Canola response to fertilization depends on the environmental conditions, including regional climate, soil type, soil moisture, and genotype (Rabiee et al., 2012; Gholamhoseini et al., 2020). Canola takes up about twice as much nitrogen from the soil as wheat. In irrigated farming, high nitrogen application may be necessary and economical for the optimal growth of canola, but when it comes to rainfed farming, in which it has low potential production, less fertilizer is required, and the plant will show lower response to nitrogen fertilization (Rabiee and Tousi Kehal, 2010).

Sulphur is the fourth major nutrient required by most crops after nitrogen, phosphorus, and potassium. Sulphur shortage not only reduces the yield due to improper nutrition but also impairs crop quality, such as protein and oil content (Malhi and Gil, 2006; Ghaderi et al., 2017). Today, due to the high price of chemical fertilizers and the destructive effect of excessive fertilization on the environment on the one hand and the significance of crop quantity and quality on the other, sulphur has caught much attention as a nutrient required for crop production (Ghaderi et al., 2017).

It is a component of various compounds, including amino acids such as cysteine and methionine. It is also a part of proteins, thereby playing an essential role in making vitamins in plant cells (Grant et al., 2012). Sulphur is involved in the formation of chlorophyll, enzyme-activating compounds, biotin, vitamin B, and photosynthesis. It is also present in the reduced organic form in glutathione and ascorbate, so it is involved in reducing the toxicity of oxygen radicals and hydrogen peroxide (Ghasem Beiki et al., 2020).

The biological oxidation of sulphur in the soil is mainly carried out by thiobacillus bacteria. These bacteria have little population and activity in most of Iran's farmlands due to their unfavorable conditions, including low organic matter and the lack of the previous use of sulphur and their inoculant. These bacteria are chemolithotrophs, gram-negative, and rod-shaped, and obtain their energy requirement from the oxidation of sulphur-reducing compounds (Besharati and Motalebifard, 2016). It should be noted that the chemical oxidation of elemental sulphur is very slow in the soil, and its biological oxidation, on the other hand, depends on the thiobacillus population, soil moisture, temperature, ventilation, soil acidity, and soil organic matter. Sulphur deficiency affects both crop yields and quality. Its deficiency reduces plant tolerance by decreasing the amount of sulphur-containing defense compounds. Environmental stress induces the synthesis of reactive oxygen species (ROS) or free radicals in living organisms, which can disrupt the normal life of living organisms and even lead to some pathological cases in plants (Besharati and Motalebifard, 2016). Sulphur plays a significant role in the production of oilseeds, including canola (Jamal et al., 2010). It takes part in the structure of coenzyme A as a thiol group. This coenzyme is of special importance in the formation of acetyl coenzyme A as the initiator of the fatty acid biosynthesis pathway (Salwa et al., 2010). Sulphur increases reproductive growth, and its deficiency may stop the growth of reproductive organs or lead to siliqua sterility.

Canola plants grown in sulphur-deficient soil may suffer from silique shedding due to sulphur shortage. For this reason, canola's need for sulphur is about three times as much as grains. In sulphur-deficient soils, sulphur fertilization at a proper rate can increase canola yield by nearly four times. The symptoms of sulphur deficiency appear more in soils with low organic matter and heavy texture, in dry years, and in areas where nitrogen is applied at high rates (Mostafavirad et al., 2018). The combined application of sulphur and nitrogen significantly increased the lateral branches, siliques per plant, seeds per siliques, and oil yield of canola compared to the control (Habibi et al., 2014). In general, nutrient availability and fertilization rate come into interaction with soil texture in the cultivation of any crop. Soil texture influences fertilization management directly by affecting nutrient retention and absorption, cation exchange capacity, and soil moisture (Sparks, 2003). It can also affect plant growth and seed yield by influencing plant nutrition (Naderifar et al., 2019).

Plant hormones, often called phytohormones, are non-food organic compounds synthesized in low concentrations in meristem or young tissues and regulate important physiological activities in plants in the target tissue (Ljung, 2013). Growth regulators are compounds that change physiological processes. They adjust plant growth and development by imitating hormone effects, reducing hormone production, and eliminating, transferring, or changing the place of the effect of a hormone. It can be said that all hormones are growth regulators, but all growth regulators are not necessarily hormones (Moradi, 2015). The market value of plant growth regulators is about 4.2 billion USD, and their annual sales are approximately 1.2 billion USD (Piraste Anoushe and Imam, 2019).

The present research aimed to explore the effect of chemical nitrogen and sulphur fertilizers, along with the application of growth regulators, on the quantitative and qualitative traits of canola as a second crop in paddy fields.

MATERIAL AND METHODS

The research was carried out in a field located in Baz Kia Gurab in Lahijan, Iran (Lat. 37°17' N, Long. 49°55' E) in 2021-2022 as a split plot experiment based on a randomized complete block design with three replications. The study site has a temperate and humid climate with an average 10-year annual rainfall of 1330 mm (Guilan Meteorological Quarterly, 2019). Table 1 presents the climatic data of the region during the research. Before the experiment, the physical and chemical characteristics of the soil were measured (Table 2).

The experimental factors were applied in two crop years of 2021-2022 on the farm located in Baz Kia Gurab. The experimental factors included the application of the growth regulator [Shootex is a type of auxin (IBA)] at two rates of 0 and 20 mg/L as the main plot and pure N fertilizer at four rates of 0, 90, 180, and 270 kg/ha (from a urea source with 46% N) and S fertilizer at three rates of 0, 35, and 70 kg/ha (from an ammonium sulfate source with 24% S and 12% N) as the subplot. Nitrogen fertilizer was applied in three steps: one-third during canola sowing, one-third during stem elongation, and one-third before flowering.

Table 1. Climatic data for the study site for 2021-2022

Item	Year	Oct 23-Nov 21	Nov 22-Dec 21	Dec 22-Jan 20	Jan 21-Feb 19	Feb 20-Mar 12	Mar 13-Apr 20	Apr 21-May 20
T_{mean} (°C)	2021	13.4	13.7	8.7	9.3	10.6	13.7	17.4
	2022	15.1	12.1	8.7	7.6	12.3	14.2	18.1
Rainfall (mm)	2021	327.5	53.4	197.5	86.6	128.7	31.2	40.4
	2022	123	158.8	58.5	77.8	59.2	89.7	106.8
Humidity (%)	2021	82	77	80	75	84	75	83
	2022	84	86	84	86	85	87	88

Table 2. The physical and chemical properties of the soil in the study site

Year	Total N (%)	K (mg/kg)	P (mg/kg)	pH	Organic matter (%)	Electrical conductivity (dS/m)	Texture
2021	0.18	217	15.1	7.4	1.7	1.17	Clay-silty
2022	0.54	213	14.5	7.4	1.7	1.02	

Ammonium sulfate fertilizer was applied to supply sulphur at the stem elongation stage (Mostafavirad et al., 2018). The soil of the study site was silt-clayey, with an acidity of 7.4 and an organic carbon content of 1.9%. Each experimental plot was composed of 8 three-meter-long sowing lines spaced by 25 cm. After harvesting rice in late September, the farm was plowed twice with a rotavator set at 10-15 cm depth. The weeds were controlled by Treflan® applied at a rate of 3 L/ha. Based on the soil analysis, the farm was fertilized with 150 kg/ha ammonium phosphate and 150 kg/ha potassium sulfate. To drain the water and prevent the field from being flooded, drains with a depth of 30-40 cm and a width of 35-40 cm were built around the farm. The treatments were spaced by 1 m, and the replications by 2 m. The seeds were sown by hand in late October.

The seeding rate was 10 g/plot calculated based on 10 kg/ha. According to the experimental factor, the growth regulator was sprayed on the canola plants in the rosette stage. The necessary agricultural operations, such as weeding and controlling the pests, were carried out during the growing season. Herbicide Gallant was applied at a rate of 3 L/ha at the six-leaf stage to control narrow-leaf weeds. Snails were controlled by using metaldehyde at two stages - first during canola emergence and second at the 3-4-leaf stage.

To calculate agronomic traits, including plant height, number of siliques per plant, and number of seeds per silique, 20 plants were randomly selected from each plot to measure these traits. The averages were recorded for statistical calculations. Thousand-seed weight was determined on 1,000 seeds randomly selected from each plot with a seed counter. The seed oil was extracted using acetone solvent and a Soxhlet extractor, and its amount was calculated as a percentage. The oil yield was calculated as the product of oil percentage and seed yield (Kocheki and Sarmadanya, 1999). Finally, an area of 2 m^2 was harvested from each plot with a sickle, and the grain yield was determined (Solimanzadeh et al., 2008). Data were analyzed by SAS (Ver. 9.1) software. The means were compared by Tukey's test at the $P<0.05$ level.

RESULTS AND DISCUSSION

Plant height

Plant height reflects growth and use of environmental resources and is influenced by the number of nodes per stem and internode length. Based on the analysis of variance (ANOVA), this trait was significantly affected by growth regulator and N and S fertilization treatments (Table 3). The tallest plants were, on average, 171.3 cm related to the treatment of the growth regulator, 270 kg/ha N, and 70 kg/ha S. The same statistical group included the plants treated with the growth regulator, 270 kg/ha N, and 35 kg/ha S (and 180 kg/ha N, and 70 kg/ha S with 170.2 cm), whose average height reached 171.2 cm. The lowest plant height was 137.3 cm related to the treatment of no growth regulator, no N fertilizer, and 35 kg/ha S (Table 4).

Plant height is a genetic trait, but it is influenced by the environment, too. In this regard, agronomic practices, e.g., nutrient application to the soil, are essential factors influencing it. Plant height increases with N fertilization, which is associated with the increase in foliage and internode length. Plant height is a parameter that influences seed yield because stems store a great part of photosynthates, which may be consumed by reproductive organs in various ways, during their growth and immediately after elongation. This, in turn, acts as a source of carbohydrates and nitrogen, which are mobilized during the seed-filling period. Flower initiation in canola is accompanied by a fast increase in plant height through the increase in internode length and flowering stem growth (Safari et al., 2021). Rabiee et al. (2012) reported that the combined application of Azotobacter and Azospirillum was related to the highest plant height of, on average, 121.3 cm, and the treatment of no biofertilizers was related to the lowest one of, on average, 108.4 cm. Higher plant height is associated with more capsules per plant and, therefore, more seeds per plant.

Table 3. Variance analysis of parameters measured in nutritional conditions and growth regulator of Canola in two Years

SOV	df	Plant height	Siliques per plant	Seeds per silique	Thousand-seed weight	Seed yield	Oil yield	Oil percentage
		MS						
Year	1	0.56	5.8	1.17	0.0067	389168*	14.06	88446.7
Rep	2	311.3	530.5	5.6	0.26	329257	49	15929
Growth Regulator	1	416.8*	2	39.06*	0.27*	5178658**	11.67	411073**
N	3	5854**	52200.3**	473.5**	5.17**	43515149**	576**	2373923**
S	2	93.3	1170.5**	36.5*	0.123	625158**	5.75	81675.2**
Year (Rep)	6	59.8	10	8.07	0.062	50984	1.23	11877.8
Year×GR	1	7.56	0.17	0.56	0.000011	18814	1.56	69
GR×N	3	92.6*	36.1	7.28	0.01	419916**	0.87	34440.8*
GR×S	2	18.8	14.2	0.562	0.142	9745	21*	5374.2
Year×N	3	4.6	3.6	1.13	0.0065	109380*	2.89	16758*
N×S	6	165.9**	141.3*	40.4**	0.171*	228760**	3.35	34296**
Year×S	2	0.14	5.4	0.09	0.026	6251	1.27	1143.8
GR×N×S	6	11.8*	93.2*	7.31*	0.037*	47760**	4.62*	4774.5*
Year×GR×N×S	17	1.15	1.2	0.38	0.0077	18282	0.51	1405.4
Error $_{total}$	88	30.53	57.9	5.14	0.061	33639	4.04	5702.9
CV (%)		3.57	6.06	9.7	7.17	7.8	6.29	10.5

Number of siliques per plant

The number of siliques per plant, including those on the auxiliary branches and those on the main branches, is an important seed yield component because they contain seeds and supply photosynthates required by the seeds and, finally, seed weight (Omidi et al., 2005). ANOVA revealed that the treatments of growth regulator and N and S fertilization influenced this trait significantly (Table 3).

The number of siliques is a vital canola trait that the seed yield strongly depends on because siliques play a key role in plant photosynthesis after flowering, during which plant leaf area decreases. The highest number of siliques per plant was 182 obtained from the plants treated with the growth regulator, 270 kg/ha N, and 70 kg/ha S, and the lowest number was 82 related to the plants treated with the growth regulator but no N or S fertilizers (Table 4). Nutrient availability in meristems increases branching, the number of siliques per plant, seed yield, and oil percentage owing to its efficiency in producing auxin (Payandeh et al., 2018).

Table 4. Mean comparison of interaction effects for investigated traits under nutritional conditions and growth regulator of Canol

Growth regulator	N	S	Plant height	Siliques per plant	Seeds per silique	Thousand-seed weight	Seed yield	Oil yield	Oil percentage
Absence	0	0	138.5fg	86.2lm	18.8de	3.1cd	1168.8jk	34.5bc	403.9hi
	0	35	137.3g	83.0mn	17.3e	2.9de	1129.7jkl	34.8bc	385.4ij
	0	70	137.5g	83.3mn	18.5de	2.9de	1098.7kl	35.7bc	398.1hi
	90	0	148.8d	96.7l	20.8d	3.4bc	1470.2hi	34.3bc	498.8gh
	90	35	149.0d	110.3ij	23.8bc	3.2c	1617.8gh	37.8a	612.3fg
	90	70	144.7e	113.2i	22.2c	3.4bc	1622.2gh	36.7ab	587.4fg
	180	0	168.0ab	135.2fgh	25.5b	3.6bc	2390.0ef	29.5de	702.8ef
	180	35	156.5cd	137.5fg	22.7c	3.2c	2533.2de	31.0cd	785.3de
	180	70	165.0b	149.7d	25.5bc	3.2c	2525.2de	30.8d	778.5de
	270	0	161.8c	164.2c	23.0bc	3.9a	3156.8c	26.3h	831.4d
	270	35	164.0bc	173.7b	28.5ab	3.9a	3450.7bc	26.7gh	921.4bcd
	270	70	161.7c	174.5b	28.0ab	3.8ab	3734.2b	28.8ef	1078.8ab
Presnce	0	0	144.3e	82.3n	21.0cd	3.1cd	1320.8hij	34.2bc	452.1ghi
	0	35	141.5ef	82.5n	19.7d	3.1cd	1247.2ij	34.7bc	433.4ghi
	0	70	140.3efg	85.2lm	18.3de	3.0cde	1085.8l	34.0bc	368.1j
	90	0	149.2d	104.2jkl	22.0c	3.2c	1720.0gh	36.2ab	622.9efg
	90	35	147.7de	107.8jk	23.0bc	3.5bc	2070.0fg	34.5bc	711.7e
	90	70	146.3de	113.3i	23.2bc	3.5bc	2115.7fg	35.5bc	748.5de
	180	0	167.7ab	131.2gh	23.5bc	3.7ab	2851.2cd	31.2cd	890.3c
	180	35	156.5cd	140.0ef	24.3bc	3.3bc	3157.5c	29.3de	925.1bc
	180	70	170.2a	142.8e	26.3b	3.5bc	3254.7bc	29.3de	957.5bc
	270	0	167.5ab	165.0c	24.8bc	3.9a	3682.3b	27.0fg	995.6b
	270	35	171.2a	168.3bc	30.0a	4.0a	3862.3ab	26.5gh	1026.1ab
	270	70	171.3a	181.8a	31.0a	4.0a	4081.2a	27.8fg	1135.1a

Number of seeds per silique

As the number of seeds per silique (plant) increases, more sink is provided for storing photosynthates produced by the plants, eventually increasing the yield. ANOVA showed that the effect of growth regulator and N and S fertilizers was significant on the number of seeds per silique (Table 3). The plants treated with the growth regulator, 270 kg/ha N, and 70 kg/ha S had the highest number of seeds per silique (31 seeds), and those treated with no growth regulator, no N fertilizer and 35 kg/ha S had the lowest number (17 seeds). This finding reflects the positive effect of N on reducing flower abortion due to more optimal growth and nutrition. The treatment of 150 kg/ha N had the highest number of seeds per main silique (on average, 20.83 seeds) (Table 4).

The effect of micronutrients on increasing the number of seeds per silique may be ascribed to higher photosynthesis, the facilitation of root growth, or the inoculation of florets (Shabanzadeh and Galavi, 2011). On the other hand, the higher number of seeds in plants sprayed with micronutrients can be associated with the lack of source limitation when applying fertilizers containing trace elements (Payandeh et al., 2018). A study on the effect of nutrients on canola indicated that the foliar application significantly increased the number of seeds per silique (Ahmadi, 2010).

Thousand-seed weight

Thousand-seed yield is a seed yield component that plays an essential role in expressing production potential and is influenced by environmental and genetic factors. As ANOVA showed, this trait was significantly affected by the growth regulator and N and S fertilization (Table 3). The highest 1000-seed weight was 4 g related to the application of the growth regulator, 270 kg/ha N, and 70 kg/ha S, and the lowest was 2.9 g related to the application of no growth regulator and no N or S fertilizer (Table 4). The combined application of biofertilizers, including Azotobacter and Azosperilium, resulted in the highest 1000-seed weight of canola (on average, 2.12 g).

Thousand-seed weight has a lower variance range than other traits because it is influenced by genetic factors, and environmental factors, including light, moisture, and temperature, are less influential (Rao and Mendham, 1991). The application of nitroxin increased the 1000-seed weight of canola (Safari et al., 2021). Since 1000-seed weight is genetic, proper fertilization seems to contribute to producing heavier seeds due to the increase in photosynthesis and photosynthate storage in the seeds (Rabiee et al., 2012). The availability of nutrients in adequate amounts and their translocation to seeds increase seed weight. Research shows that nutrient application improves green area longevity and prevents the loss of 1000-seed weight (Homayouni et al., 2013).

Seed yield

The results of ANOVA revealed that the growth regulator and N and S fertilizers significantly ($P<0.01$) affected seed yield (Table 3). The comparison of means showed that the highest seed yield (4081 kg/ha) was obtained from the treatment of the growth regulator, 270 kg/ha N, and 70 kg/ha S, and the lowest (1086 kg/ha) from the treatment of the growth regulator, no N fertilizer, and 70 kg/ha S (Table 4).

Yield per unit area is a vital trait assessed in all research. It represents production per unit area as per the use of environmental factors per unit area. The increase in canola's seed yield is attributed to the increase in dry matter production. Higher yields are often accompanied by the production of more siliques per unit area (Pasbaneslam, 2016). The application of nitroxin increased photosynthesis and dry matter by improving soil microbial activity and availability of hormones, growth stimulators, and nutrients, finally leading to higher seed yield (Mostafavirad et al., 2018). The higher seed yield of the canola under higher N rates is mainly related to the higher number of siliques and higher seed weight, whereas the number of seeds per pod is not influenced (Safari et al., 2021).

Seed oil percentage and yield

The effect of the N and S fertilizers was significant ($P<0.01$) on seed oil yield and percentage (Table 3). The treatment of the growth regulator, 90 kg/ha N, and 35 kg/ha S produced the highest oil percentage (37.8%), and the treatment of no growth regulator, 270 kg/ha N, and no S fertilizer produced the lowest one (26.3%) (Table 4). Research shows that N application usually reduces seed oil because N consumption directly correlates with protein increase. When N increases, nitrogenous protein precursors increase, so more photosynthates are consumed for protein formation, resulting in a decrease in photosynthates available for synthesizing fatty acids. As a result of the allocation of more photosynthates to protein formation, the potential of hydrocarbon mobilization decreases. This factor reduces the seed oil percentage of canola at higher N levels (Fathi et al., 2001; Rabiee and Tousi Kehal, 2010).

Oil yield is a function of oil content and seed yield (Safari et al., 2021). The highest oil yield of 1215.7 kg/ha was obtained from the treatment of the growth regulator, 270 kg/ha N, and 70 kg/ha S, and the lowest (357.6 kg/ha) from the treatment of the growth regulators, no N fertilizer, and 70 kg/ha S. A regional study by Ahmadi (2010) showed that in addition to genetic factors, environmental factors influenced oil percentage, too. If nutrients are supplied in adequate amounts, stimulating microorganisms can become more efficient, although these bacteria can still increase plant growth and yield in undesirable nutritional conditions (Heshmati et al., 2017).

CONCLUSIONS

Proper nutrition and growth in the canola plant are of particular importance and in case of lack of proper nutrition, especially nitrogen fertilizer, the yield will be severely reduced. On the other hand, due to the importance of oil in rapeseed cultivation, excessive consumption reduces the quality of the oil and its percentage, and the use of sulphur fertilizer along with nitrogen fertilizer, in addition to increasing the amount of oil, also increases its quality. The use of vegetative growth regulators in rapeseed will increase the height, the number of branches in the plant, and as a result, the number of silique per branch and silique, which contributes to a higher and more reliable performance of the rapeseed oil plant. According to the climatic conditions of the growth regulator treatment area, the use of 270 kg/ha of N fertilizer and 70 kg/ha of S fertilizer is recommended for farmers to produce rapeseed after rice harvest.

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