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Growth, Nitrogen Fixation and Nutrient Uptake by Chickpea (*Cicer arietinum*) in Response to Phosphorus and Sulfur Application under Rainfed Conditions in Pakistan

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ABSTRACT

A field experiment was conducted at Barani Agriculture Research Institute Chakwal Punjab, Pakistan to assess the seed yield, nitrogen fixation and nutrient uptake by chickpea (*Cicer arietinum* L.) in response to application of different levels of phosphorus (P) and sulfur (S). The treatments comprised three levels (0, 40 & 80 kg P_2O_5 ha^{-1}) of P and three levels (0, 15 & 30 kg S ha^{-1}) of S from two sulfur S sources (gypsum & ammonium sulfate) in different combinations. The trial was laid out according to randomized complete block design with split split plot arrangement. Application of P and S resulted in significant increase in seed yield by 21 and 12% over control, respectively. Effect of combined application of P and S was synergistic at nutrient application rate of $P_{40}S_{15}$, while antagonistic at $P_{80}S_{30}$. Sulfur application had significant effect on percent nitrogen derived from atmosphere (% N_{dfa}), while effect of P was non-significant. There was significant increase in protein content of chickpea seed due to application of S. Application of both P and S resulted in increase in nitrogen (N) fixation by 16%. Value cost ratio was less than 2 for higher level of P either sole or in combination with S. A fertilizer combination of $P_{40}S_{30}$ is more economical and cost effective for chickpea production under rainfed conditions.

Key Words: Natural abundance technique; Percent nitrogen derived from atmosphere; Soil N balance; Value cost ratio

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is an important pulse crop of rainfed tract in Pakistan. Average chickpea yield in Pakistan is much lower than developed countries of the world such as China (2.4 Mg ha^{-1}), Canada (1.9 Mg ha^{-1}) and USA (1.7 Mg ha^{-1}) (FAO, 2009). A number of factors including genetic and environmental are responsible for this low yield and imbalanced fertilization is the key among them.

Phosphorus is one of the major essential nutrient element required for optimum growth of grain legumes. In many soil types, P is the most limiting nutrient for the production of crops (Jiang *et al*., 2006). It plays primary role in many of the physiological processes such as the utilization of sugar and starch, photosynthesis, energy storage and transfer. Legumes generally have higher P requirement because the process of symbiotic nitrogen fixation consumes a lot of energy (Schulze *et al.*, 2006).

Sulfur is the 4th major essential plant nutrient after N, P and K. Its amount required by the plant is even higher than phosphorus and comes after N and K. Sulfur is becoming deficient in soil due to use of high grade S free fertilizers, cultivation of high yielding varieties and lack of industrial activity/deposition (Scherer, 2009). Soils of rainfed area in Pakistan are particularly deficient in S (Khalid *et al.*, 2009). Sulfur is also a vital part of the ferredoxin, an iron-sulfur protein occurring in the chloroplasts. Ferredoxin has a significant role in nitrogen dioxide and sulfate reduction and assimilation of N by root nodule and free living N-fixing soil bacteria (Scherer, 2008; Scherer *et al.,* 2008).

In Pakistan, work done regarding crop response to S application is limited to oilseeds and their oil contents only (Islam *et al.,* 2009). Research work regarding interaction of P and S and their role in legume's growth and nitrogen fixation is very rare. Further more, phosphate fertilizers have become very costly and their efficiency is very low in rainfed agriculture. Economic and judicious use of this precious input has become very important. Although, S is

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deficient in the soils of rainfed region yet neither its recommendation for inclusion in nutrient management programme for chickpea exists nor it is used by farmers for enhancing seed yield of pulses. In the scenario of ever increasing prices of food items, threats to food security and huge burden for import of pulses on economy (34 billion rupees according to estimates of government of Pakistan during fiscal year 2010-2011), it has become very important to efficiently utilize our existing resources. Therefore, present study was conducted to assess the interactive effect of S and P application on seed yield, N fixation and nutrient uptake of chickpea under rainfed conditions of northern Punjab, Pakistan.

MATERIALS AND METHODS

A field experiment was conducted using chickpea cultivar Balkassar 2000 at Barani Agriculture Research Institute (BARI), Chakwal during crop growing season 2007-2008. Physical and chemical properties of the experimental site are shown in Table I. The trial was laid out in randomized complete block design with split split plot arrangement (plot size of 1.5 ×3.5 m) keeping P in main plots, S sources in sub plots and S levels in sub subplots. There were eighteen treatments having different combinations of P (0, 40, 80 kg ha^{-1}) and S rates (0, 15, 30 kg ha^{-1}) from two S sources (gypsum and ammonium sulfate). Starter dose (26 kg ha^{-1}) of N was applied in the form of urea. However, in S treatments, urea dose was adjusted accordingly after taking in to consideration the addition of N from ammonium sulfate (AS). Phosphorus was applied in the form of triple super phosphate (TSP). All the treatments were replicated three times. Chickpea crop was sown maintaining row to row distance of 30 cm. All the fertilizers were applied as basal dose. Crop was grown under rainfed conditions and no supplemental irrigation was applied. Sample of plant dry matter tissue of legume and non-legume reference plant were taken for $\delta^{15}N$ determination (Unkovich *et al.* 2008).

Percent nitrogen derived from air (% N_{dfa}) =100 × ($\delta^{15}N$ (soil N)-$\delta^{15}N$ legume N)/(δ ^{15}N (soil N)-B).

Where $\delta^{15}N$ (soil N) is commonly obtained from a non N fixing reference plant grown in the same soil as the legume; B is the $\delta^{15}N$ of the same N fixing plant when grown with N as the sole source of N and its value is -2.0 (Kyei-Boahen *et al.,* 2002).

Legume N uptake (kg ha^{-1}) = legume dry matter yield (kg ha^{-1}) × N in plant tissue (%).

Amount of N fixed (kg ha^{-1}) = legume N uptake (kg ha^{-1}) × % N_{dfa}.

Total rainfall during cropping season (October to March) was 90 mm. At physiological maturity, crop from an area of one meter square in the middle of each plot was harvested separately. The plant samples were dried and data were recorded for seed, straw and dry matter yield. Representative samples of 100 g from both seed and straw were collected from bulk sample, oven dried and ground and analyzed for N and P (Ryan *et al.* 2001) and S (Verma, 1977). Nutrient uptake was determined by multiplying the respective nutrient concentration with dry matter yield. Protein content in seed were determined by multiplying N content (%) with factor of 6.25 (Nagur *et al.,* 1992). Soil N balance after chickpea production was obtained by subtracting N output from N input as follows (Amanuel *et al.,* 2000).

$$B = (N_f + N_{dfa}) - N_g$$

Where B is soil N balance, N_f is the applied N, N_{dfa} is the total fixed N and N_g is the N removed by chickpea.

Nutrient interactions (synergistic or antagonistic) were calculated by comparing the increase in yield or nutrient uptake (in terms of kg ha^{-1} over control) due to combined P and S application with that of individual/separate applications (Fageria, 2001). The economics of applied fertilizer was measured by value cost ratio (VCR). The VCR value was calculated by following formulas (Ahmad & Rashid, 2003).

$$VCR = \frac{\text{Value of increased yield obtained}}{\text{Total cost of fertilizer}}$$

Prices of input and output prevailing in the market during fiscal year 2007-2008 were taken into account for economic analysis (NFDC, 2009). Data on all observations were subjected to analysis of variance (ANOVA) by using software MSTATC. Treatment means were compared by least significant difference (LSD) test. Correlation analysis was also done to study the relationship among different parameters.

RESULTS

Seed and straw yield: There was significant increase in seed and straw yield of chickpea with P application (Table II). Seed and straw yield increased from 0.70 to 0.85 Mg ha^{-1} and from 1.42 to 1.58 Mg ha^{-1}, respectively, as P rate was increased from 0 to 80 kg P_2O_5 ha^{-1}. Difference between lower (40 kg P_2O_5 ha^{-1}) and higher level (80 kg P_2O_5 ha^{-1}) of P was significant. A similar trend was also observed for S regarding both seed and straw yield. Two S sources i.e., gypsum and AS were statistically similar to each other. The P by S interaction was not significant for seed yield but significant for straw yield. The highest straw yield was recorded in $P_{80}S_{30}$, which was followed by $P_{80}S_{15}$ and $P_{40}S_{30}$ and the lowest in control (Table IV). Harvest index did not show any variation among different treatments (Table II).

Phosphorus and sulfur uptake: There was significant increase in P uptake due to P and S application (Table II). Phosphorus uptake increased from 3.77 to 4.68 kg ha^{-1} and from 3.89 to 4.51 kg ha^{-1} as P rate was increased from 0 to 80 kg P_2O_5 ha^{-1} and S from 0 to 30 kg ha^{-1}, respectively.

Table I: Location, rainfall and physical and chemical properties of soils of the experimental sites

Parameter	Unit	Value
Latitude	N	32.5°
Longitude	E	72.4°
Mean annual rainfall (1979-2009)	mm	630
Cropping season (October to March) rainfall during 2007-2008	mm	90
Sand	%	69
Silt	%	21
Clay	%	10
Texture	-	Sandy loam
pH	-	7.6
ECe	$dS\ m^{-1}$	0.32
Total organic carbon	$mg\ g^{-1}$	3.7
$CaCO_3$	%	5.2
Total N	%	0.02
NO_3-N (AB-DTPA extractable)	$\mu g\ g^{-1}$	11.2
Phosphorus (AB-DTPA extractable)	$\mu g\ g^{-1}$	3.0
Sulfate- Sulfur ($CaCl_2$ extractable)	$\mu g\ g^{-1}$	6.4
Zinc (AB-DTPA extractable)	$\mu g\ g^{-1}$	0.75
Copper (AB-DTPA extractable)	$\mu g\ g^{-1}$	1.21
Iron (AB-DTPA extractable)	$\mu g\ g^{-1}$	7.82
Manganese (AB-DTPA extractable)	$\mu g\ g^{-1}$	2.98

Lower (40 kg P_2O_5 ha^{-1}) and higher level (80 kg P_2O_5 ha^{-1}) of P were different from each other. A similar trend was also recorded for S uptake with the exception that lower (15 kg S ha^{-1}) and higher level (30 kg S ha^{-1}) of S were similar to each other. Gypsum and AS were similar to each other regarding P uptake but were different in respect of S uptake. The P×S interaction was not significant for P uptake but significant for S uptake. The highest S uptake was recorded in $P_{80}S_{30}$, which was followed by $P_{80}S_{15}$ and $P_{40}S_{30}$ and the lowest in control (Table IV).

The S source by S level interaction regarding S uptake was significant (Table II). It was observed that application of 30 kg S ha^{-1} in form of AS was superior to same level of S application in form of gypsum in respect of S uptake (Table V). It was also observed that application of 30 kg S ha^{-1} in the form of gypsum was statistically similar to application of 15 kg S ha^{-1} in the form of AS.

Nitrogen fixation and uptake: Effect of P application on $\%N_{dfa}$ was not significant, while that of S was significant (Table III). Percent nitrogen derived from atmosphere increased from 52 to 55% as S application rate was increased from 0 to 30 kg S ha^{-1}.

There was significant increase in N uptake with both P and S application. Nitrogen uptake increased from 45 to 54 kg ha^{-1} and from 47 to 53 kg ha^{-1} as P application rate was increased from 0 to 80 kg P_2O_5 ha^{-1} and S application rate from 0 to 30 kg ha^{-1}, respectively. There was significant difference between lower and higher rates of both P and S application. An exactly same trend was recorded for amount of N fixed. Two S sources were alike in respect of $\%N_{dfa}$, N uptake and amount of N fixed.

Amount of N taken from soil and soil N balance: Phosphorus application resulted in significantly higher amount of N taken from soil (Table III). Amount of N derived from soil increased from 20 to 25 kg ha^{-1} due to application of 80 kg P_2O_5 ha^{-1}. Concomitantly, soil N balance declined with increase in N removal from soil from 6 to 1 kg ha^{-1}. Contrary to P, sulfur application had not any appreciable effect on both N derived from soil and soil N balance.

Protein composition of seed: Sulfur application resulted in significant increase in protein content of chickpea seed from 23.8 to 24.7% as S application rate was increased from 0 to 30 kg S ha^{-1} (Table II). Effect of P application was not significant on protein content of seed. Two S sources were statistically similar to each other in respect of protein composition of seed.

Interaction between phosphorus and sulfur: Combined application of P and S resulted in higher response in terms of seed yield at lower level of nutrient application ($P_{40}S_{15}$) as compared to sole application (Table VI). However, at higher level of nutrient application ($P_{80}S_{30}$), increase in seed yield due to combined application was less as compared to individual one. Therefore, interaction between nutrients was synergistic at lower level and antagonistic at higher level in respect of seed yield. Similar trend was also observed regarding P uptake. However, nutrient interaction was synergistic at both lower and higher level of nutrient application regarding S uptake.

Economic analysis: Value cost ratio is the rate of return on money spent on fertilizers. If VCR is greater than one, the fertilizer will be profitable. A VCR of 2 represents a 100 percent return on money invested on fertilizer. For high technology, recommended VCR is 2 as it ensures a good net return. At VCR lower than two, farmer's margin of return becomes low and there is risk of losing his money if there is poor management or bad weather. Due to risk factors, VCR of 2 is considered satisfactory. In our study, VCR value was higher for S as compared to P and among S sources; higher VCR value was recorded for gypsum as compared to AS (Table V). Among different P and S combinations, sole application of P as well as combination of higher rate of P with different S level resulted in VCR less than 2 (Table IV).

DISCUSSION

There was an increase of 21% in the seed yield of chickpea due to application of 80 kg P_2O_5 ha^{-1}. Hayat and Ali (2010) reported almost similar magnitude of response to application of 80 kg P_2O_5 ha^{-1} using mung bean and mash bean as test crops under similar climatic conditions. However, in irrigated conditions, much higher response (75%) of chickpea to application of 90 kg P_2O_5 ha^{-1} has been reported (Khan, 2002). Lower response in our study might be due to rainfed conditions where crop is under drought stress and fertilizer use efficiency is generally low (Ahmad & Rashid, 2003). There was 12% increase in seed yield of chickpea due to S application, which is in line with the findings of Hussain (2010) who reported 15% increase in seed yield of soybean due to application of 30 kg S ha^{-1}.

Table II: Seed and straw yield as function of P and S levels and S sources

Effect	Seed yield ($Mg\ ha^{-1}$)	Straw yield ($Mg\ ha^{-1}$)	Harvest Index (%)	P uptake ($kg\ ha^{-1}$)	S uptake ($kg\ ha^{-1}$)	Protein composition of seed (%)
P levels (kg P_2O_5 ha^{-1})						
0	0.70 c	1.42 c	33	3.77 c	4.56 b	23.9
40	0.81 b	1.52 b	35	4.25 b	5.55 a	24.2
80	0.85 a	1.58 a	35	4.68 a	5.85 a	24.4
LSD value	0.03**	0.02**	NS	0.4**	0.40**	NS
S sources						
Gypsum	0.78	1.50	34	4.20	5.23 b	24.3
Ammonium sulfate	0.80	1.52	34	4.27	5.41 a	24.0
LSD value	NS	NS	NS	NS	*	NS
S levels (kg S ha^{-1})						
0	0.74 c	1.46 c	34	3.89 c	4.85 b	23.8 b
15	0.80 b	1.51 b	35	4.29 b	5.38 a	24.0 b
30	0.83 a	1.56 a	35	4.51 a	5.75 a	24.7 a
LSD value	0.02**	0.01**	NS	0.12**	0.5**	0.5**
Interactions						
P × S sources	NS	NS	NS	0.18*	NS	NS
P × S levels	NS	0.03*	NS	NS	0.21*	NS
S sources × S levels	NS	NS	NS	NS	0.17*	NS
P × S sources × S levels	NS	NS	NS	NS	NS	NS

Table III: Nitrogen uptake and fixation as function of P and S levels and S sources

Effect	Percent N derived from atmosphere (% N_{dfa})	N uptake ($kg\ ha^{-1}$)	Amount of N fixed ($kg\ ha^{-1}$)	Amount of N taken from soil ($kg\ ha^{-1}$)	Soil N balance ($kg\ ha^{-1}$)
P levels (kg P_2O_5 ha^{-1})					
0	54	45 c	25 c	20 b	6
40	54	51 b	27 b	24 a	2
80	54	54 a	29 a	25 a	1
LSD value	NS	1.2**	1.5**	1.7*	-
S sources					
Gypsum	54	50	27	23	3
Ammonium sulfate	54	50	27	23	3
LSD value	NS	NS	NS	NS	-
S levels (kg S ha^{-1})					
0	52 b	47 c	25 c	22	4
15	54 a	50 b	27 b	23	3
30	55 a	53 a	29 a	24	2
LSD value	1.8**	1.4	1.1**	NS	-
Interactions					
P × S sources	NS	NS	NS	NS	-
P × S levels	NS	NS	NS	NS	-
S sources × S levels	NS	NS	NS	NS	-
P × S sources × S levels	NS	NS	NS	NS	-

Means with different letters differ significantly according to Least Significant Difference (LSD) test ($P<0.05$). NS stands for non significant difference, * and ** denote significance at $P < 0.05$ and $P < 0.01$ levels, respectively

Increase in seed yield of chickpea may be due to the fact that S availability to the plant enhances many physiological processes such as photosynthesis, assimilation of carbohydrates and protein formation (Brady & Weil, 2005).

Increase in amount of N fixed was mainly as a result of increase in plant growth and increased N uptake and partly due %N_{dfa} as indicated by the correlation analysis. Value of correlation coefficient showed very strong positive correlation of seed yield (R = 0.90, $P < 0.01$) and N uptake (R = 0.83, $P < 0.01$) with amount of N fixed, while a weak correlation with % N_{dfa} (R = 0.59, $P < 0.01$). Phosphorus uptake had strong correlation with amount of N fixed (R = 0.86, $P < 0.01$), while weak correlation with %N_{dfa} (R = 0.31, $P < 0.05$). This confirms earlier findings that P affects the process of N fixation by affecting the growth of host plant rather than its direct involvement in this process (Somado *et al.,* 2006).

There was increase in N taken from soil with P application and consequently soil N balance declined as P rate was increased. This is also confirmed by positive correlation of seed (R = 0.75, $P < 0.01$) and straw yield (R = 0.74, $P < 0.01$) with N taken from soil and negative correlation (R = -1.0, $P < 0.01$) between soil N balance and N taken from soil. In present study, although crop residues were removed from soil yet soil N balance was positive. Contrary to this, previous studies have shown that soil balance after legume harvest is positive in that case when

Table IV: Straw yield as a function of phosphorus with in each sulfur level

Treatments	Straw yield (kg ha^{-1})	S uptake (kg ha^{-1})	Value cost ratio (VCR)*
P_0S_0	1.39 f	4.24 g	-
P_0S_{15}	1.44 e	4.58 f	4.53
P_0S_{30}	1.47 d	4.86 e	3.43
$P_{40}S_0$	1.48 d	5.00 e	1.55
$P_{40}S_{15}$	1.52 c	5.58 c	2.26
$P_{40}S_{30}$	1.58 b	6.06 b	2.33
$P_{80}S_0$	1.52 c	5.31 d	1.24
$P_{80}S_{15}$	1.59 b	5.91 b	1.47
$P_{80}S_{30}$	1.64 a	6.33 a	1.54

Means with different letters differ significantly according to Least Significant Difference (LSD) test ($P<0.05$). * For economic analysis, price of urea, TSP, gypsum and ammonium sulfate was taken as Rs. 581, 1458, 120 and 867 per bag of 50 kg while that of gram as Rs. 1600 per 40 kg

Table V: Sulfur uptake (kg ha^{-1}) as function of sulfur levels from two sulfur sources

Treatments	Sulfur uptake (kg ha^{-1})	Value cost ratio (VCR)
Phosphorus levels (kg P_2O_5 ha^{-1})		
0	4.56 b	-
40	5.55 a	1.75
80	5.85 a	1.19
Gypsum (kg S ha^{-1})		
0	4.86 d	-
15	5.23 c	7.17
30	5.59 b	6.50
Ammonium sulfate (kg S ha^{-1})		
0	4.84 d	-
15	5.48 b	3.93
30	5.91 a	2.69

Means with different letters differ significantly according to Least Significant Difference (LSD) test ($P<0.05$).

Table VI: Interaction effect between phosphorus and sulfur application

Effect	Seed yield (kg ha^{-1})	Phosphorus uptake (kg ha^{-1})	Sulfur uptake (kg ha^{-1})
Increase due to sole P over control			
with 40 kg P_2O_5 ha^{-1}	98	0.51	0.76
with 80 kg P_2O_5 ha^{-1}	157	0.89	1.06
Increase due to sole S over control			
with 15 kg S ha^{-1}	56	0.32	0.34
with 30 kg S ha^{-1}	85	0.68	0.62
Increase due to combined P and S			
with 40 kg P_2O_5 and 15 kg S ha^{-1}	171	0.86	1.34
with 80 kg P_2O_5 and 30 kg S ha^{-1}	232	1.46	2.09
Type of interaction			
with 40 kg P_2O_5 and 15 kg S ha^{-1}	synergistic	synergistic	synergistic
with 80 kg P_2O_5 and 30 kg S ha^{-1}	antagonistic	antagonistic	synergistic

crop residues are returned to soil (Habtemichial *et al.,* 2007). One of the reasons for positive soil N balance might be low dry matter and seed yield due to drought stress as there was only 90 mm rainfall through out growing season, which resulted in lesser nutrient uptake and less soil nutrient depletion.

Sulfur and phosphorus interaction was positive at lower rate of nutrient application and negative at higher rate. Interaction between P and S occurs mainly due to two reasons. Firstly, P and S occur in soil in the form of phosphate (PO_4^{-3}) and sulfate (SO_4^{-2}) ions. These compete for adsorption on exchange sites in soil as both are anions. However, adsorption strength of PO_4^{-3} is more than that of SO_4^{-2} and presence of PO_4^{-3} results in reduction in SO_4^{-2} adsorption and accelerates downward movement (Abdin *et al.,* 2003). Secondly, application of S fertilizers to calcareous soils results in reduction in pH although temporarily and locally. This prevents the conversion of primary orthophosphate ($H_2PO_4^-$) and secondary orthophosphate (HPO_4^{-2}) into PO_4^{-3}. As phosphorus is mainly taken up by plant in the form of $H_2PO_4^-$ and $HPO4^{-2}$ hence, P availability for plant uptake is increased (Taalab *et al.,* 2008). Tiwari and Gupta (2006) observed that on a soil deficient in both and P and S, there was no interaction between P and S in terms of seed yield at $P_{40}S_{20}$, positive at $P_{60}S_{40}$ and negative at $P_{80}S_{60}$ when pegion pea was used as test crop. Contrary to these findings, Paliwal *et al.* (2009) observed that interaction of P and S exhibited strong synergistic relationship regarding soybean nutrition on Alfisol deficient in both P and S. Therefore, it can inferred from this and previous studies that type of interaction between P and S depends upon a number of factors such as initial soil fertility status, climatic conditions, level of nutrient applied and test crop used.

A comparison of two S sources indicated that both were statistically similar in respect of seed yield. However, when two S sources were compared in respect of S uptake, effect of application of 30 kg S ha^{-1} in the form of gypsum was statistically similar to 15 kg S ha^{-1} in the form of AS but significantly lower than 30 kg S ha^{-1} (Table V). Lower S uptake by plant in gypsum treated plot as compared to AS may be due to slow release of S from this source (Girma *et al.,* 2005). Ghosh *et al.* (2000) drawn conclusion after a series of trials that where immediate relief from S deficiency is necessary, readily soluble sources like AS out classed less soluble sources such as gypsum. They also observed that in calcareous soils, gypsum was less effective as compared to soluble sources like AS. Our study was conducted under rainfed conditions, where release of S from gypsum was enough to meet crop demand due to slow growth, therefore seed yield from gypsum plot was at par with that of AS.

Economic analysis showed that application of 80 kg P_2O_5 ha^{-1} whether alone or in combination with S was not profitable as VCR was less than 2. Therefore keeping in view the economic analysis, fertilizer combination of $P_{40}S_{30}$ is suitable for chickpea under rainfed conditions.

CONCLUSION

Application of P and S resulted in significant increase in seed and straw yield. Interaction between P and S was positive at lower rate and negative at higher rate of nutrient application. Effect of S on the process of N fixation was direct while that of P was indirect through its effect on overall plant growth. Sulfur application resulted in improvement in protein content of chickpea seed. Economic analysis showed that fertilizer combination of $P_{40}S_{30}$ is more profitable than $P_{80}S_{30}$. Sulfur should be included in nutrient management progrmme in order to get maximum yield of pulses. This will result in increased fertilizer use efficiency and saving of this precious and costly input.

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