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The Effects of Nitrogen Starter Fertilizer and Plant Density on Yield, Yield Components and Oil and Protein Content of Soybean (*Glycine max* L. Merr)

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Abstract: Effects of nitrogen starter fertilizer and plant density on yield and oil and protein content of soybean (*Glycine max* L. Merr) are not well understood, because nitrogen starter fertilizer and plant density has been tested separately. Two years field experiment was conducted to evaluate effects of these factors on yield, yield components, oil and protein content in 2006 and 2007 in Kermanshah, Iran. The experiment was conducted on soybean (var. Williams) as a split-plot based on randomized complete blocks design with three replications. Nitrogen starter fertilizer treatments were arranged in three rates (0, 40, 80 kg ha⁻¹) as main plots and plant density as sub plots arranged with three levels (15, 30, 45 plant m⁻²). Based on similarity treatments and experimental designs, the results of analysis of combined variance and mean comparisons showed significant (528.4 kg ha⁻¹) yield increase as density increased from 30 to 45 plant m⁻² and nitrogen starter fertilizer increased from 0 to 40 kg ha⁻¹ in two years. Analysis of correlation showed a positive significant correlation between yield and number of seed per plant ($r = 0.724$), number of pods and yield ($r = 0.463$), thousand seed weight and yield ($r = 0.437$). A linear regression was found between yield and number of seed per plant, number of pods and thousand seed weight (yield = $37.58 + 0.73x_1 - 0.14x_2 + 0.7x_3$; $r^2 = 0.56$; $p < 0.01$). Seed protein was unaffected by plant densities, but nitrogen application changed it. Dissimilarly, oil content has a diverse respond to treatments. This experiment showed density of 45 plant m⁻² and application of nitrogen starter fertilizer 40 kg h⁻¹ are optimum and increase grain yield under condition of our experiment. We suggest to conduct some experiments for understanding of linear relationship for number of pod for understanding of linear relationship for number of pod for levels of nitrogen starter and quadratic relationship for number of seed for levels of density.

Key words: Correlation, linear regression, split-plot, contrast, combined ANOVA

INTRODUCTION

Today, soybean is the world's leading source of oil as well as by-products and protein-rich seed meal. The highest domestic consumption is in Asia where soybean is a basic food since ancient times. Besides the oil content of about 20%, the high protein content (about 35%) is of special importance as a protein source in the world.

Although nitrogen fertilization of soybean is not a common practice there is speculation that the ability of soybean to fix atmospheric N is not always adequate for maximum yield. While researchers have investigated the effect of nitrogen fertilizer on soybean yield and quality. Because there are a number of factors influencing soybean nitrogen fixation and the response to applied nitrogen as soil temperature, moisture and pH that affect soybean response to applied nitrogen. On the other hand determined nitrogen fixation begin 14 days after planting only when plants were grown under optimum moisture and temperature conditions, thus a small amount of nitrogen at planting could be beneficial to early growth.

Then, concluded nitrogen applied before planting could be beneficial to soybean, given that nodules were not present until at least 9 days after soybean emergence (Osborne and Riedell, 2006).

Broadcast nitrogen (50 kg ha⁻¹) applied at planting increased of seed yield of determinate and indeterminate stem-termination type by at least 8% (Starling *et al.*, 1998). Greenhouse studies have also shown an increase in early soybean plant growth as a result of applied nitrogen (Eaglesham *et al.*, 1983). However, results from field studies on the effect of nitrogen fertilizer on soybean yield have been mixed. Many studies have shown an increase in yield and associated dry matter accumulation as a result of nitrogen application to soybean (Afza *et al.*, 1987; Michael *et al.*, 2001; Osborne and Riedell, 2006; Wood *et al.*, 1993).

As a result of widespread adoption of highly productive management practices such as solid seeding or narrow rows, soybean growers have become more aware of the importance of optimum plant populations and seeding rates in soybean production

systems. Besides following new management practices, growers also have been able to control plant populations with considerable precision because of the availability of high quality seed and improved planting equipment. New management practices and seed quality improvement indicate growers should reevaluate their current seeding practices.

Seiter *et al.* (2004) showed that high seed production in soybean, followed by low plant spacing. As well increasing of plant density associated by seed yield in soybean (Egli, 1988). In drought condition, there was an increasing of yield by rising plant population too (Houlshouser and Whittaker, 2002). Addition to Boquet (1990), demonstrated that plant height and seed yield were greater than before when plant population augmented.

Within row spacing affected yield of soybean in 1 of 4 years (Alessi and Power, 1982). Yield was most responsive to row spacing when the total July and August rainfall ranged from 100 to 270 mm (Bowers *et al.*, 2000). Seed rates did not affect the height of plant, number of seed per pod and 100 seeds weight but affected its branch height, the first pod height and side branches number, number of pod per plant and grain yield (Nuri, 2003). For the soybean treatments, there was a trend of increasing residual nitrogen at the 2 highest population densities (40-60 kg N ha⁻¹) (Blumenthal *et al.*, 1988). Branch seed yields in narrow rows, averaged ranged from 14 to 57% of total seed yield while 47 to 74% of total seed yield was produced on branches in wide rows (Norsworthy and Emerson, 2005).

The objectives of this study were to evaluate the impact of starter fertilizer nitrogen rates and determine the optimum plant population for production of soybean in Kermanshah-Iran region.

MATERIALS AND METHODS

Two years field experiment carried out during 2006 and 2007 growing season at the research station of Agriculture faculty, Razi University, in Kermanshah, Iran (Latitude: 34° 19' N, Longitude: 47° 3' E, Altitude: 1322 m, Köppen climate classification; CS₃). Mean annual temperature and precipitation in 2006 and 2007 ranged 15.0 to 15.5°C and 430.3 to 343.9 mm, respectively. The soil of experiment of field was clay loam with 7.1 pH.

The experiment was conducted on soybean (var. Williams) as a split-plot based on randomized complete blocks design with three replications. Nitrogen starter fertilizer treatments were arranged in three rates (0, 40, 80 kg ha⁻¹) as main plots and plant density as sub plots arranged with three levels (15, 30, 45 plant m⁻²). Each sub plot consists of six rows, with 5 m long. Inter-row and intra-row spacing were 50 and 5 cm, respectively. Conventional tillage was performed in the fall of each

year, with seedbed preparation in the spring using a furrower and hand cultivation after inoculation with *Rhizobium japonicum* in early May. Derived from soil analysis, phosphorus and potassium were applied at planting to each plot at 240 kg phosphate potassium ha⁻¹ and 130 kg K ha⁻¹ as phosphate potassium in 2006 and 2007, respectively.

Whole plant samples were collected by taking 1 m² of sub plots. At maturity, biologic yield (gr m⁻²) grain yield (gr m⁻²) harvest index, number of seed per area, number of pods per area, thousand seed weight, percent of seed oil and protein content were determined by harvesting 1 m² of the center two rows from each plot by hand and it was adjusted to 130 g kg⁻¹ moisture. Addition to, grain moisture and test weight were determined. Thousand seed weight determined from average of four 100 seed sample. Whole seed analysis for oil and protein content was determined by using near infrared reflectance spectroscopy (Inframatic 8620, Perten, Percon). Calibration equations used to determine oil concentration have a standard error of 0.05% (Cho *et al.*, 1998).

Data analysis variance, correlation and regression were performed by the PROC MIXED procedure of SAS (Littell *et al.*, 1996). Comparison of means accomplished by Duncan multiple range test ($\alpha < 0.05$).

RESULTS AND DISCUSSION

Combined analysis of variance: The results of combined analysis of variance for grain yield and main yield components showed significant differences for years for grain yield and main yield components ($p < 0.05$) (Table 1). Maximum grain yield was attributed to 2006 (3256.26 kg ha⁻¹) compared with the 2007 (2753.7 kg ha⁻¹) growing seasons (Table 1).

Mean comparison: Mean performance (Table 2, 3) of grain yield and main yield components and percent of content of protein and oil showed significant differences among densities for grain yield ($p < 0.05$) with the largest grain yield at 45 plants m⁻² (3871.1 kg ha⁻¹) compared to the 15 plants m⁻² (2513.6 kg ha⁻¹) and 30 (3342.7 kg ha⁻¹) densities. Egli (1988) have found similar results for soybean grain yield and plant density association. As a result of plant population rising, there is an increasing in number of pods and number of seed per pod comparatively that followed by yield increased significantly. Holshouser and Whittaker (2002) found similar results for impacts of density on soybean grain yield, they reported that population of 208000 plants ha⁻¹ was adequate for maximum yields at a site having only a brief period of stress; populations over 600000 plants ha⁻¹ were required to maximize yield where drought stress limited leaf area production. Kapustka *et al.* (1990)

Table 1: Combined analysis of variance for soybean characters

Scaled squares					
Source of variation	df	Grain yield (g m ⁻²)	No. of seed per unit area	Thousand seed weight	No. of pod per unit area
Year	1	12604.1**	3520.2**	1.57	12100.0**
Replication (Year)	4	826.8**	1113.1**	27.65**	19.5
Nitrogen	2	12890.0**	8656.9**	2930.35**	12544.0**
Year×Nitrogen	2	20548.6**	19715.2**	0.29	7533.3**
Replication×Nitrogen (year)	8	65.4	152.2	15.80	149.5
Density	2	45684.4**	13066.7**	9674.0**	10038.6**
Year×Density	2	17553.1**	20297.1**	1.79	325.6
Nitrogen×Density	4	5649.6**	13368.1**	2747.38**	6857.6**
Year×Density×Nitrogen	4	14804.8**	15738.6**	2.34	144.0
Error	24	3026.6	141.6	3.94	155.6

**Significant at the 0.01 probability level

Table 2: Mean comparisons of yield, oil and protein content

Treatments		Characters			
N fertilizer rate (kg ha ⁻¹)	Plant density (plant m ⁻²)	Biologic yield (g m ⁻²)	Grain yield (g m ⁻²)	Percent of protein	Percent of oil
0	15	924.3c	289.3d	35.28ab	21.92abc
0	30	956.2c	326.1bc	35.94a	21.53abc
0	45	1088.0bc	365.6b	35.84a	21.68abc
40	15	977.0c	197.4e	35.08ab	22.22a
40	30	1267.0b	353.5b	35.90a	21.81abc
40	45	1605.0a	433.4a	35.35ab	22.10ab
80	15	951.5c	267.4d	35.20ab	22.03ab
80	30	959.0c	323.2bc	34.78b	21.91abc
80	45	1200.0b	362.4b	34.80b	21.59abc

Mean values with the same letters are not statistically significant using Duncan's multiple range test

Table 3: Mean comparisons of main yield components and harvest index

Treatments		Characters			
N fertilizer rate (kg ha ⁻¹)	Plant density (plant m ⁻²)	Harvest index	No. of seed per unit area	Thousand seed weight	No. of pod per unit area
0	15	0.313ab	2153e	134.40b	142.3bc
0	30	0.341a	2431d	134.00b	137.2cd
0	45	0.336a	2738c	133.50b	145.5bc
40	15	0.202e	2762c	71.46e	77.5ef
40	30	0.279cd	2540c	139.00a	125.5d
40	45	0.270d	3406b	126.90c	163.2a
80	15	0.281cd	3725a	71.61e	67.8f
80	30	0.337a	2594cd	124.40d	156.2ab
80	45	0.302bc	2625c	138.10a	91.0e

Mean values with the same letters are not statistically significant using Duncan's multiple range test

reported increasing plant densities resulted in higher yields per unit land as we found this result.

Seed yield was significantly increased by 678.0 kg ha⁻¹ resulted in 40 kg N ha⁻¹ application compared to control with same density (45 plant m⁻²). The same result was demonstrated by Starling *et al.* (1998).

For maximum density (45 plant m⁻²), application of 80 kg nitrogen starter fertilizer ha⁻¹ decreased seed yield by 30 kg ha⁻¹ significantly (Table 2). There is may be shown by excessive nitrogen availability at soybean growth early in the season that resulted in nodule mass formation deficiently and consequently, there is inadequate plant nitrogen essential during growing season specially during seed filling period that followed by seed yield declining. Osborne and Riede (2003) have found that application of nitrogen as starter fertilizer may increase initial growth of soybean, but may also negatively impact nitrogen fixation when environmental conditions improve.

The largest significant No. of seed per plant was obtained at 45 plant m⁻² density (Table 3). Number of seed per plant maybe effect on seed yield directly because of a significant correlation between seed yield and number of seed per plant (0.724, p<0.01). Even though, there were positive significant correlations between seed yield with pod width and thousand seed weight.

Correlation analysis: Based on correlation analysis (Table 4), seed protein was unaffected by plant densities, but nitrogen application changed it. Dissimilarly, oil content has a diverse response to treatments due to an association of nitrogen in protein molecular structure that is fined in nitrogen fertilizer. Hence, appropriate application of nitrogen as starter fertilizer because of nitrogen supplying, can increase protein anabolism. It seems that, further application of nitrogen at early season growth due to excessive nitrogen availability, not only followed by deficiency in nodule formation but also there

Table 4: Correlation coefficients for yield and yield components and oil and protein content and biologic yield

Variables	No. of pod per area	Thousand seed weight	No. of seed per area	Harvest index	Percent of oil	Percent of protein	Grain yield	Biologic yield
No. of pod per area	1.00							
Thousand seed weight	0.654**	1.00						
No. of seed per area	0.542**	0.341*	1.00					
Harvest index	0.238	0.219	0.591**	1.00				
Percent of oil	0.053	-0.089	-0.209	-0.280*	1.00			
Percent of protein	0.222	0.113	0.049	-0.087	-0.319*	1.00		
Grain yield	0.463**	0.437**	0.724**	0.475**	0.019	0.111	1.00	
Biologic yield	0.218	0.198	-0.195	-0.687**	0.404**	0.439**	0.61*	1.00

**Significant correlation coefficients at the 0.01 probability level; *Significant correlation coefficients at the 0.05 probability level;

Table 5: Analysis ANOVA for testing contrasts for different levels of density and N. starter

Scaled squares					
Source of variation	df	Grain yield	No. of seed per unit area	Thousand seed weight	No. of pod per unit area
Linear starter	1	1600.0*	6373.3**	4610.8**	1223.1**
Quadratic starter	1	24180.1**	10940.4**	1249.8**	19.5
Linear density	1	87912.2**	23104.0**	14628.4**	12544.0**
Quadratic density	1	3456.6**	3029.4	4719.6**	7533.3**
Linear vs. Linear	1	2926.0**	2604.1	6800.3**	600.0
Linear vs. Quadratic	1	1485.1	36001.3**	756.2**	13944.5**
Quadratic vs. Linear	1	13530.1**	11100.5**	1024.3**	10512.5**
Quadratic vs. Quadratic	1	4657.4**	3766.6**	2408.7**	2373.40

**Significant at the 0.01 probability level; *Significant at the 0.05 probability level

Table 6: Analysis of variance of regression

Source of variation	df	MS
Regression	3	55739.37**
Residual	50	2548.284
Total	53	

**Significant at the 0.01 probability level

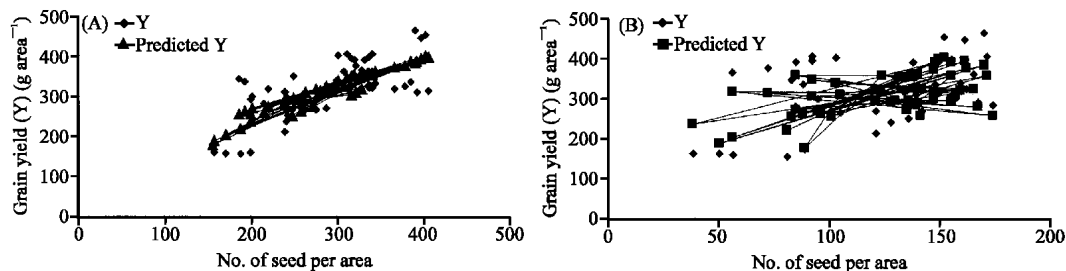


Fig. 1: Relationship between number of seed per area and grain yield (A), number of pod per area and grain yield (B)

is a lack of nitrogen during seed fill period possibly. La-Favre and Eaglesham (1987) reported inhibition of nodulation of soybean by high temperature is exacerbated by: the presence of available nitrogen and the complete absence of applied nitrogen. Because of negative genetical interaction between proteins and oil metabolism in soybean ($r = -0.319$), there is a persuasion into protein synthesis than of oil synthesis due to presence of nitrogen plant metabolism probably.

Analysis ANOVA for testing contrasts: Analysis ANOVA for testing contrasts (Table 5) showed high significant linear and quadratic relationship for grain yield and thousand seed weight, for all levels of treatments. But linear relationship for No. of pod for nitrogen starter and

quadratic relationship for No. of seed for density was not significant.

Analysis of regression: Analysis of multiple regression (Table 6) indicated that a best described response as a linear regression for yield and number of seed per area, yield and number of pod per area, yield and thousand seed weight ($\text{yield} = 37.58 + .73x_1 - 0.14x_2 + 0.7x_3$, $r^2 = 0.56$; $p < 0.01$) (Table 6, Fig. 1).

CONCLUSION

As Lee *et al.* (2008) found (economically optimum plant populations were 7 to 33% less than optimum plant populations), this experiment showed density of

45 plant m⁻² (450000 plant ha⁻¹) and application of nitrogen starter fertilizer (40 kg ha⁻¹) are optimum and increase Grain yield and protein content under condition of this experiment. Because linear relationship for No. of pod for levels of nitrogen starter and quadratic relationship for No. of seed for levels of density was not significant, we suggest to conduct some experiments for understanding of this relationships.

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