Effects of nitrogen application and planting density on growth and yield of *Sesbania* pea grown in saline soil

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Planting density and nitrogen (N) application rate are the two major factors affecting crop productivity. The present study was carried out to evaluate the effects of N application rate and planting density on the growth and yield characteristics of *Sesbania* pea in saline soil. Two planting densities (D1: 120,000 plants ha^{-1} and D2: 200,000 plants ha^{-1}) and three N application rates (N1: 0, N2: 180 and N3: 360 kg ha^{-1}) were applied in this study. The higher planting density decreased plant height, root length, pod number per plant and seed number per pod, but increased dry weight, N uptake and total seed yield. Increasing N application rate promoted plant height, SPAD reading, dry weight, pod number per plant, seed number per pod, seed yield and N uptake, whereas further increase in nitrogen rate played only a minor role in growth and yield (except plant height and SPAD reading) at D1. Moreover, planting density and N application rate had no significant effects on 1000-seed weight. According to the present study, D1 combined with N2 is an effective strategy to increase individual growth of *Sesbania* pea in saline soil, whereas D2 combined with N3 is the effective strategy for total seed yield.

Keywords: Nitrogen application, planting density, saline soil, *Sesbania* pea.

SESBANIA pea (*Sesbania cannabina* (Retz.) Poir.) is a rapidly growing annual herbal shrub that is widely adaptable to adverse climatic conditions such as high salinity, drought, waterlogging and so on[1]. It grows extensively in the tropical regions of Asia, Australia and Africa as a green manure crop to improve soil fertility and reclaim soils contaminated by heavy metals[2,3]. In China, *Sesbania* pea has been planted in the hills, ditches and wetlands of the Yangtze River region and introduced into the Yellow River Delta to increase soil fertility and reduce soil salinity[4]. *Sesbania* is also a good feed for poultry, livestock and fish. The gum produced from *Sesbania* seeds is highly water-soluble and widely used in oil production, mining and metallurgy, medicine and pesticide, and daily chemicals as well as textile and dyeing industry[5]. At present, *Sesbania* pea is mainly cultivated as a green manure crop and functional values of the seeds are far from being fully explored.

As mentioned in a previous study, crop yield typically showed a curvilinear response to plant densities or nitrogen fertilizer rates, and it reached a maximum level at the optimal plant density or nitrogen fertilizer application rate[6]. Normally, planting density is one of the most basic and effective agronomic factors affecting crop productivity and is highly dependent on soil fertility, cultivar, climatic conditions and farming system[7]. Nasto *et al.*[8] reported that increasing planting density to a reasonably high level led to higher yield (kg ha^{-1}) of bell pepper. Crops planted at suitable low planting densities can produce more branches and pods per plant. With the increase in planting density, the decrease in individual plant productivity is related to decrease in the number of branches and pods per plant[9–11]. In fact, changes in planting density can affect crop morphology, alter the ability of plants to capture resources (nutrients, sunlight, space, etc.) and induce different yield responses to nitrogen fertilizer[11–14].

Nitrogen is considered one of the essential macronutrients required by the plants for growth, development and production[15]. Optimizing nitrogen application is one of the main ways to increase crop yield and achieve food and environment security[16]. Almost all surveys showed that nitrogen fertilizer at suitable rates increased seed yield substantially, even under diverse and contradicting conditions[17]. Bani-Saeedi[18] reported that nitrogen produced higher seed yield per hectare by reducing flower shedding and sequentially affecting 1000-seed weight, increasing silique number per unit area and decreasing seed number per silique. Tumbare and Niikam[19] noticed that nitrogen fertilizer increased fruit weight, yield and fruit number of chilli peppers. As one of the important contributing factors to high seed yield, nitrogen fertilizer can effectively control the number of pods per plant[9–11].

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However, excessive use of nitrogen fertilizer can lead to higher production costs, increase the risk of nitrate leaching and water pollution, and reduce nitrogen use efficiency[20,21].

Planting density and nitrogen application rate can have an interactive effect on crop growth and productivity. Majnoun-Hosseini *et al.*[22] and Mobasser *et al.*[23] reported that plant height increased as planting space and use of nitrogen fertilizer decreased. Fathi *et al.*[24] suggested that increasing nitrogen fertilizer and planting density can boost seed yield in rapeseed. However, there is little knowledge available on the effects of planting density and nitrogen application rate on the growth and physiology, and yield of *Sesbania* grown in saline soil. This is of critical importance for developing a strategy to increase *Sesbania* production in such soils. Keeping this in mind, the objectives of the present study were to evaluate the effects of planting density and nitrogen application rate on growth, physiology and yield of *Sesbania* pea, and to explore the optimal combination of planting density and nitrogen application rate for *Sesbania* production in saline soil.

Materials and methods

Site description and treatment

A field study was conducted in the Coastal Forest Farm of Dafeng, Dafeng town (33°20′N, 120°47′E), Yancheng City, Jiangsu Province, China from April to October 2017, which provided *Sesbania* pea seeds. Before planting, soil samples were collected in the surface layer (0–20 cm) at the site to determine the physical and chemical soil properties. Soil total N was measured using the Kjeldahl method[25]. Soil Olsen-P was determined following the method of Page *et al.*[26]. Soil available K was measured according to McLean and Watson[27]. Soil organic matter was determined using the method of Tiessen and Moir[28]. Soil pH was determined following the method of Hendershot *et al.*[29]. Salt content of soil was measured according to Lu[30]. Experimental field had loamy soil, with a textural class of clay loam and the initial fertility status of N = 0.72 g kg^{-1}, P = 1.45 mg kg^{-1}, K = 279 mg kg^{-1}, organic matter of 19.75 g kg^{-1}, pH of 8.8 and salt content of 1.68 g kg^{-1}.

The experiment was a split-plot design using a randomized complete block arrangement with three replications. The total experimental area was 259.2 sq. m, and the size of each plot was 1.2 × 12 m (width × length). The treatments consisted of planting density at two levels (D1 = 120,000 plants ha^{-1} and D2 = 200,000 plants ha^{-1}) as the main plots and N application rate at three levels (N1 = 0, N2 = 180 and N3 = 360 kg ha^{-1}) as subplots. Urea containing 46% N was used as the nitrogen source in two split doses: 50% at sowing and 50% at 77 days after planting (DAP). In addition to the nitrogen, calcium superphosphate was applied at 120 kg ha^{-1} of P_2O_5 for all treatments in equal proportion at planting and 77 DAP.

Sesbania pea seeds were presoaked in 404.2 μm MGA_3 solution 6 h and sowed on 26 April 2017. At 21 DAP, all the plots were thinned to the target planting density. During the whole growing season, all the other field managements, including herbicide application, pest and disease control followed the local recommendations.

Observations and measurements

Growth characteristics: At four dates after planting, including 47, 77, 107 and 138 DAP, 10 representative plants were randomly sampled from each plot to measure growth parameters. The roots of each plant were dug out from the soil with a spade and washed with tap water to measure the root characteristics. Plant height and root length were measured with a standard metre ruler. All plant samples were oven-dried at 105°C first for 30 min to deactivate the enzymes and then at 80°C until they reached a constant weight for biomass determination.

Physiological characteristics: Leaf chlorophyll content was measured using a hand-held chlorophyll metre (SPAD-502) in the middle portion of a leaf at 47, 77, 107 and 138 DAP. Nine leaf SPAD readings were averaged to represent the mean SPAD readings of each leaf, and six leaves were measured at each plot. The dried samples were ground to measure plant N concentration using the micro-Kjeldahl method[31].

Seed yield and yield components: At maturity, 10 samples were hand harvested randomly from each plot. All plant samples were dried in an oven at 105°C first for 30 min to deactivate the enzymes and then 80°C until they reached a constant weight. The following observations and measurements were made for each plant: 1000-seed weight, seed number per pod and pod number per plant.

One square metre area was selected randomly to measure total seed yield.

Statistical analysis: The experiment was designed with two factors (two planting densities and three nitrogen application rates) and arranged in a completely randomized design with three replicates. The collected data of each variable were subjected to analysis of variance (ANOVA) using the statistical package of SAS 9.4. Significant differences in means between the treatments were compared by the protected least significant difference (LSD) procedure at 5% probability level, as described by Gomez and Gomez[32].

Results

Both planting density and nitrogen application rate had significant effects on plant height (Table 1). Plant height

Table 1. Effect of planting density and nitrogen application rate on the height, root length and SPAD reading of *Sesbania* plants grown in saline soils

Experimental factor	Experimental level	Height (cm)				Root length (cm)				SPAD reading			
		DAP				DAP				DAP			
		47	77	107	138	47	77	107	138	47	77	107	138
Density (plants ha^{-1})	120,000	25.0	93.7	218.9	270.7	5.8	13.9	27.7	31.5	46.6	50.8	57.2	53.9
	200,000	23.1	86.6	202.7	250.3	4.9	11.2	22.1	26.7	45.6	50.1	57.9	54.8
	$LSD_{(0.05)}$	1.2	7.1	12.2	6.0	0.6	1.5	1.6	1.8	1.6	1.4	1.3	3.1
Nitrogen (kg ha^{-1})	0	20.8	82.6	198.9	246.3	5.3	12.0	24.8	28.5	44.1	49.1	54.4	51.3
	180	24.4	92.1	210.2	261.5	5.7	12.3	25.1	29.5	47.0	50.9	57.8	54.5
	360	26.9	95.6	223.3	273.9	5.2	12.4	24.9	29.4	47.2	51.4	60.3	57.3
	$LSD_{(0.05)}$	1.5	8.7	15.0	7.3	0.8	1.8	2.0	2.2	1.9	1.7	1.6	3.8
Density	**	*	**	**	*	**	**	**	ns	ns	ns	ns	
Nitrogen	**	*	**	**	ns	ns	ns	ns	**	*	**	**	
Density × nitrogen	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	

DAP, Days after planting; *Significant difference at $P \leq 0.05$; **Significant difference at $P \leq 0.01$; ns, Non-significant difference.

Table 2. Effects of planting density and nitrogen application rate on the dry weight of leaf, stem and root of *Sesbania* plants grown in saline soils

Density (plants ha^{-1})	Nitrogen (kg ha^{-1})	Dry leaf weight (kg ha^{-1})				Dry stem weight (kg ha^{-1})				Dry root weight (kg ha^{-1})			
		DAP				DAP				DAP			
		47	77	107	138	47	77	107	138	47	77	107	138
120,000	0	17.7	180	1072	684	14.8	348	3000	4154	3.9	74	415	720
	180	20.7	317	1474	1072	19.7	590	4010	7159	5.2	127	623	928
	360	18.4	239	1262	794	16.7	439	3932	4886	4.8	102	519	772
200,000	0	28.4	205	1425	916	23.8	460	4075	6014	5.7	98	588	972
	180	30.2	369	1489	1316	25.2	632	4727	7575	6.4	127	758	1120
	360	31.1	460	1836	1605	30.4	791	5130	10,840	8.1	196	905	1233
	$LSD_{(0.05)}$	1.8	56.9	197.6	183.2	2.5	100.0	367.1	756.6	1.1	17.4	103.6	110.4
Density		**	**	**	**	**	**	**	**	**	**	**	**
Nitrogen		**	**	**	**	**	**	**	**	**	**	**	**
Density × nitrogen		*	**	**	**	**	**	ns	**	*	**	**	*

DAP, Days after planting; *Significant difference at $P \leq 0.05$; *Significant difference at $P \leq 0.01$; ns, Non-significant difference.

at 200,000 plants ha^{-1} was lower than that at 120,000 plants ha^{-1}. With increased nitrogen application rate, plant height generally increased and maximum height was recorded at 360 kg ha^{-1} level on most sampling dates. At 77 and 107 DAP, maximum plant height was obtained with N at 180 and 360 kg ha^{-1} levels. During the whole growth period, plant height increased rapidly before 107 DAP and then tended to be gentle. At 107 DAP, plant height was decreased by 7.4% at 200,000 plants ha^{-1} level, and increased by 12.3% with N at 360 kg ha^{-1} level.

Density had significant effects on root length (Table 1). At 120,000 plants ha^{-1}, root length was higher than that at 200,000 plants ha^{-1}. Although root length showed an increasing trend with the application of nitrogen fertilization, the effects of nitrogen on root length were not statistically significant. Similar to plant height, root length increased gradually throughout the whole growth period. At 107 DAP, root length decreased by 20.2% at 200,000 plants ha^{-1} level.

The effects of planting density on SPAD value were not significant, whereas nitrogen had significant effects on SPAD values (Table 1). SPAD values generally showed an increasing trend with increasing nitrogen application rate, but the increase had no significant difference between N at 180 and 360 kg ha^{-1} levels, except for that at 107 DAP. The highest SPAD value was observed with N at 360 kg ha^{-1} level, followed by 180 and 0 kg ha^{-1} levels. Before 107 DAP, SPAD value had an increasing trend and then decreased slightly. At 107 DAP, SPAD value increased by 10.8% with N at 360 kg ha^{-1} level.

The effects of planting density and nitrogen application rate on leaf dry weight, stem dry weight and root dry weight were significant (Table 2). They were all higher at 200,000 plants ha^{-1} than at 120,000 plants ha^{-1}. At 120,000 plants ha^{-1} level, with increase in nitrogen application rate all the above parameters generally increased first and then decreased. However, at 200,000 plants ha^{-1}

Table 3. Effect of planting density and nitrogen application rate on N uptake of leaf, stem, root and seeds of *Sesbania* plants grown in saline soil

Density (plants ha^{-1})	Nitrogen (kg ha^{-1})	Leaf N uptake (kg ha^{-1})	Stem N uptake (kg ha^{-1})	Root N uptake (kg ha^{-1})	Seed N uptake (kg ha^{-1})
120,000	0	9.2	22.3	3.7	13.8
	180	21.9	62.6	6.1	38.5
	360	17.8	45.8	5.7	30.8
200,000	0	10.9	28.6	4.3	14.7
	180	20.0	50.7	6.2	34.2
	360	32.4	90.2	8.2	50.3
	$LSD_{(0.05)}$	3.8	8.1	0.7	3.9
Density		**	**	**	**
Nitrogen		**	**	**	**
Density × nitrogen		**	**	**	**

*Significant difference at $P \leq 0.05$; **Significant difference at $P \leq 0.01$; ns, Non-significant difference.

level, leaf dry weight, stem dry weight and root dry weight increased with increasing nitrogen application rate. The maximum weight was observed at 200,000 plants ha^{-1} with N at 360 kg ha^{-1} level. Leaf dry weight increased before 107 DAP and showed a sharp decline after 107 DAP. However, stem dry weight and root dry weight increased during the growth period. Among the three sampling sites, maximum dry weight was observed in the stem, followed by leaf and root. At 107 DAP, leaf dry weight increased by 17.7%, stem dry weight by 31.1% and root dry weight by 25.1% at 120,000 plants ha^{-1} with N at 180 kg ha^{-1} level. While at 200,000 plants ha^{-1} with N at 360 kg ha^{-1} level, leaf dry weight increased by 28.8%, stem dry weight by 25.9% and root dry weight by 53.9%.

Planting density and nitrogen application rate had significant effects on N uptake (Table 3). Density at 200,000 plants ha^{-1} level resulted in higher N uptake than that at 120,000 plants ha^{-1} level. At 200,000 plants ha^{-1} level, N uptake increased with increase in nitrogen application rate and maximum N uptake was observed with N at 360 kg ha^{-1} level. At 120,000 plants ha^{-1} level, N uptake generally reached a maximum with N at 180 kg ha^{-1} and then decreased, except for root N uptake. The higher nitrogen application rate resulted in lower root N uptake, but the decrease was not statistically significant at 120,000 plants ha^{-1} level. At 120,000 plants ha^{-1} with N at 180 kg ha^{-1} level, leaf N uptake increased by 138.0%, stem N uptake by 180.7%, root N uptake by 64.9% and seed N uptake by 179.0%. While at 200,000 plants ha^{-1} with N at 360 kg ha^{-1} level, leaf N uptake increased by 197.2%, stem N uptake by 215.4%, root N uptake by 90.7% and seed N uptake by 242.2%. Planting density and nitrogen application rate had the greatest impact on stems, followed by seeds, leaves and roots.

The effects of density and nitrogen application on pod number per plant, seed number per pod and total seed yield were significant, but the effects on 1000-seed weight were not significant (Table 4). Pod number per plant and seed number per pod at 120,000 plants ha^{-1} were higher than those at 200,000 plants ha^{-1}, while total seed yield showed the opposite trend. At 120,000 plants ha^{-1} level, with increase in nitrogen application rate, seed numbers per pod and total seed yield reached maximum N at 180 kg ha^{-1} level and then decreased; nitrogen at 180 kg ha^{-1} level increased pod number per plant, whereas N at 360 kg ha^{-1} level had no significant effect on pod number per plant. At 200,000 plants ha^{-1} level with increasing nitrogen application rate, these traits increased. Higher seed number per pod and total seed yield were recorded at both 180 and 360 kg ha^{-1} N. The highest pod number per plant was recorded at 360 kg ha^{-1} N. The highest total seed yield was reached at 200,000 plants ha^{-1} level combined with N at 360 kg ha^{-1} level. Pod number per plant increased by 44.7%, seed number per pod by 19.3% and total seed yield by 92.9% at 120,000 plants ha^{-1} with N at 180 kg ha^{-1} level. Whereas at 200,000 plants ha^{-1} with N at 360 kg ha^{-1} level, pod number per plant increased by 66.3%, seed number per pod by 25.4% and total seed yield by 103.9%.

Discussion

Planting density is a main factor determining the ability of crops to obtain resources[11]. Height of plant can generally be regarded as one of the indicators of plant vigour, which depends on the vigour and growth habits of the plant[15]. In this study, increasing density decreased plant height and root length. The reduction in height and root length might be due to increased competition among plants for soil nutrients and climatic environmental resources[33,34]. Forbes and Watson[35] and Samih[36] explained that competition for light, mineral nutrients and available water increases as plant population density increases. This study showed that the application of nitrogen resulted in increase in plant height. Similar results were also obtained by Amanullah *et al.*[37], who found that plant

Table 4. Effect of planting density and nitrogen application rate on seed yield and yield components of *Sesbania* plants grown in saline soil

Density (plants ha^{-1})	Nitrogen (kg ha^{-1})	Pod number per plant	Seed number per pod	1000-Seed weight (g)	Seed yield (kg ha^{-1})
120,000	0	84.3	26.9	13.8	3217.3
	180	122.0	32.1	15.4	6207.7
	360	104.7	28.9	14.2	4456.5
200,000	0	68.0	24.0	14.2	3970.0
	180	99.0	28.1	15.2	7308.6
	360	113.1	30.1	13.9	8098.4
	$LSD_{(0.05)}$	11.0	3.2	2.0	1195.6
Density		**	*	ns	**
Nitrogen		**	**	ns	**
Density × nitrogen		**	*	ns	**

*Significant difference at $P \leq 0.05$. ** indicates significant difference at $P \leq 0.01$; ns, Non-significant difference.

height as well as biomass yield showed an increase with increasing nitrogen rate and the times of nitrogen application. Such results also confirm that N is essential for cell division and elongation[38].

Chlorophyll is the major light-harvesting compound for photosynthesis. The present study showed that application of nitrogen increased the SPAD value. The contribution of inorganic fertilizers to chlorophyll content might be because nitrogen is a constituent of the chlorophyll molecule. In addition, nitrogen is the main component of all amino acids in lipids and proteins, and they act as structural compounds of the chloroplast[39]. Our results are in agreement with those of Loecke *et al.*[40], who reported that chlorophyll meter readings of corn ear leaves responded positively to urea application rate at growth stage R1.

Regarding the effects of different nitrogen application rates and planting densities on agronomic traits and shoot development, Toghraei *et al.*[41] showed that increased vegetative growth and development of shoots were followed by increased biomass yield as a result of the application of more nitrogen. In the present study, we found that dry weight was higher with 120,000 plants ha^{-1}. Higher planting density limits light penetration as well as dry matter accumulation[33]. Our results showed that the application of nitrogen increased plant dry weight. The improvement in dry weight might be due to the combination of nitrogen and plant matter produced during photosynthesis, such as protein, ascorbic acid, glucose and amino acids[42]. Similar results were also reported by Lin *et al.*[43], who found that increased N concentration promoted biomass accumulation. In addition, the increased height and root length allowed crops to improve the exploitation of environmental resources; while the higher chlorophyll content enhanced photosynthesis, accumulated more photosynthetic products and resulted in an increase in plant dry weight. However, at 120,000 plants ha^{-1} level, the highest nitrogen application rate had lower plant dry weight but still higher than the control. This may be attributed to the supply of nitrogen in excess of plant demand and a possibility exists for losing nitrogen by leaching and denitrification[44].

Planting density and nitrogen application rate promoted pod numbers per plant and seed numbers per pod, and hence increased seed yield per plant. The significant effects of nitrogen on yield may be attributed to its cumulative stimulating effects on the vegetative growth characteristics that form the base for flowering and fruiting[33]. Khadem Hamzeh *et al.*[45] showed that as the density increased, the number of pods per plant decreased. This might be due to the fact that competition for growth demand factors increased with increase of planting density and hence, the growth and development of single plant was less. However, Zandi *et al.*[7] reported that different planting densities and nitrogen levels had no significant effects on the number of seed per pod of fenugreek. The difference might be due to the different species and planting density and nitrogen levels. Moreover, nitrogen levels and planting density did not affect 1000-seed weight. Our findings confirmed the results reported by Seghatoleslami and Ahmadi Bonakdar[46] that 1000-seed weight is considered as one of the genetic traits of cultivars which is less affected by environmental factors such as temperature, light and moisture. The highest total seed yield was reached at 200,000 plants ha^{-1} level combined with N at 360 kg ha^{-1} level. The total seed yield consists of the number of plants and seed yield per plant. This may be because high planting density produced more plant numbers and the effect of plant number on production was more than seed yield per plant.

The planting density at 200,000 plants ha^{-1} produced higher N uptake than at 120,000 plants ha^{-1} level. The competition for space, nutrients and light among plants grown at high density is always high. More nitrogen is needed to contribute to the competitiveness of plants. High planting density leads to overshadowing between different plants, hence much more N has to be assigned to light capture to compensate for the decrease in light intensity and to maintain photosynthesis[14,47,48]. Our results showed that at low planting density, higher nitrogen application rate had lower N uptake. It indicated that because the supply of nitrogen was in excess of plant requirement and possibility existed for loss of N by leaching and denitrification, *Sesbania* pea were unable to uptake N at the highest nitrogen application rate. Similar results were also reported by Khan *et al.*[44]. Furthermore, at high planting density N uptake increased as nitrogen application rate increased. Similar results were found by El-Gizawy[49], who reported that increasing nitrogen application rate up to 150 kg/fed decreased agronomic nitrogen efficiency, apparent nitrogen recovery and increased N uptake in maize. Although crops absorbed more N at high nitrogen application rate, the absorbed N was used to increase the competition among plants, rather than obtain high crop yield. Thus, the management strategy of nitrogen should be determined according to both plant demand and plant N uptake, which in turn are determined by variety, planting density, soil conditions and other factors[50].

Conclusion

Planting density and nitrogen application rate significantly affected growth and yield of *Sesbania* pea, but had no significant effects on 1000-seed weight. The planting density of 120,000 plants ha^{-1} promoted individual growth of *Sesbania* pea, while planting density of 200,000 plants ha^{-1} had higher dry weight and total seed yield. At 120,000 plants ha^{-1} level and with N at 180 kg ha^{-1} level showed the best effects on plant growth and seed yield of *Sesbania* pea. At 200,000 plants ha^{-1} level, the optimum rate of nitrogen was 360 kg ha^{-1} level.

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