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Effect of Different Levels of Nitrogen Fertilizer and Cultivars on Growth, Yield and Yield Components of Romaine Lettuce (*Lactuca sativa* L.)

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ABSTRACT

In order to determine the effect of nitrogen fertilizer rates and cultivars on growth and critical yield of lettuce, an experiment was conducted at Shahid Chamran University of Ahwaz, Iran during 2005-2006. This study was arranged in a spilt plot experiment based on a randomized complete block design, in three replications. The treatments included four nitrogen rates (0, 60, 120, and 180 kg N ha^{-1}) as the main plot and two lettuce cultivars ('Pich Ahwazi' and 'Pich Varamini') as the sub-plot. Sampling was done in 86, 100, 114, 126, 142, and 156 days after sowing. The criteria measured were plant length, fresh and dry weights of leaves, leaf area, number of leaves, crop growth rate (CGR), leaf area index (LAI) and yield. Results indicated that different levels of nitrogen fertilizer on all growth characteristics were significant at $P<0.01$. Nitrogen fertilizer caused head formation to accelerate and delayed the bolting date of lettuce. Cultivar had a significant effect on growth characteristics, on fresh and dry weights of leaves and on leaf number but not on plant length and leaf area. The highest yield was obtained with 120 kg ha^{-1} treatment by 'Pich Ahwazi'. Also, it took 'Pich Varamini' longer to form a head and to flower than 'Pich Ahwazi'.

Keywords: bolting, leaf area index

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is the most popular amongst the salad vegetable crops (Squire *et al*. 1987). In line with investigations carried out by Stevens (1974) in USA, lettuce is ranked 26th among vegetables and fruits in terms of nutritive value and 4th in terms of consumption rate highlighting the ever-increasing importance of this crop.

This vegetable requires a high rate of nitrogen for growth and development. In Iran, farmers who applied excesses nitrogen fertilizer to increase crop yield disturbed the equilibrium balance of nutrient elements in the soil, caused pollution, decreased crop quality and thus a great part of the nation's resources became useless (Tehrani and Malakouti 1997). The investigations carried out by Tittonell *et al*. (2003) on lettuce showed that increasing nitrogen fertilizer from 0 to 150 kg ha^{-1} increased the fresh weight of the crop. Rincon *et al*. (1998) reported that increasing nitrogen up to 100 kg ha^{-1} increased the yield of lettuce, reaching 53.4 t ha^{-1} (total fresh matter) while the application of 150 and 200 kg ha^{-1} caused a decrease in the biomass and yield index, respectively. Gulser (2005) observed that an increase in the fertilizer nitrogen level increased the yield, stem length and leaf surface area but not the number of leaves in spinach. Demir *et al*. (1996) reported that by increasing the nitrogen fertilizer rate the leaf area, stem length and yield of spinach increased. Zarehie (1995) reported that by increasing the nitrogen fertilizer rate to 200 kg ha^{-1} increased the yield of spinach but that by increasing the nitrogen level up to 200 kg ha^{-1} the corresponding increase in yield was not economical. The investigation conducted by Mahmoudi Kliber (2005) on lettuce (cv. 'Siah Karaj') showed that by increasing the rate of nitrogen fertilizer to 300 kg ha^{-1} increased the yield and dry matter of lettuce but between 100 and 200 kg N ha^{-1} was not significantly different. However, the yield response of lettuce to increasing N rate varies with different environmental variables, including weather, soil type, residual fertility, soil moisture, seasons and cultivar. This study aimed to evaluate the effect of different nitrogen levels applied as urea on the growth and productivity of two cultivars of lettuce, which can help to predict the optimal N fertilizer requirement and to improve the practice of lettuce production.

MATERIALS AND METHODS

The investigation was conducted during the 2005-2006 growing season at the experimental field of the Agricultural Faculty, Shahid Chamran University of Ahwaz (31°19′N and 48°40′E, elevation 12 m), Iran. The experimental field was cleared, ploughed, harrowed and divided into plots. The soil texture at the experimental site was loam clay silty with approximately 0.56% organic matter, pH 7.6, EC 2.9 ds m^{-1}. The 0-30 soil layers contained respectively 0.56% total nitrogen, 7.1 ppm available phosphorous, and 142 ppm available K. The experimental design was a split plot fitted to randomized complete block. The treatments included four nitrogen rates (N_1=0, N_2=60, N_3=120, N_4=180 kg N ha^{-1}) as the main plot and two Iranian lettuce cultivars ('Pich Awazi', 'Pich Varamini') as the sub-plot. Before sowing, phosphorous (P_2O_5) and potassium

Table 1 Average monthly temperature and precipitation during growing season.

Months	Temperature (°C)		Precipitation (mm)	
	2005-2006	Normal[a]	2005-2006	Normal
November	21.5	19.6	22.2	31.9
December	18.6	13.8	22.2	47.2
January	12.9	12.2	48.1	48.3
February	14.5	14.3	102.6	27.0
March	19.3	18.6	0.8	29.5
April	24.0	24.7	0.4	15.1
May	31.0	30.7	0.5	5.2
Mean or total	20.2	19.1	196.8	204.2

[a] Normal refers to the long-term (50 years) average.

Received: 24 August, 2007. Accepted: 4 December, 2007.

Original Research Paper

Table 2 Mean effects of different nitrogen levels and cultivar on yield and growth criteria of Romaine lettuce.

N doses (kg N ha^{-1})	Plant length (cm)	Leaf number	Leaf area (cm^2)	Leaf dry weight (g)	Leaf fresh weight (g)	Yield (kg m^{-2})
0	28 b*	56.12 b	6902.50 c	31.33 b	418.45 c	2.83 d
60	32.37 a	63.75 a	9437.50 b	41.07 a	673.30 b	5.14 c
120	33.37 a	64.37 a	12463.75 a	46.78 a	798.69 a	7.00 a
180	34.25 a	62.75 a	1160.75 ab	43.55 a	721.24 b	6.17 b
Cultivar						
'Pich Ahwazi'	32.44 a	65.06 a	10280.00 a	39.79 b	621.81 b	5.36 a
'Pich Varamini'	31.56 a	58.44 b	9923.75 a	41.57 a	697.02 a	5.21 a

*Means followed by the same letter did not differ significantly at P=0.05.

(K_2O_5) were applied at a rate of 150 and 100 kg ha^{-1}, respectively. On the 24th October 2005, lettuce seeds were sown chassis. Then seven weeks after emergence on the 18th December 2005, lettuce seedlings at the 5-6 leaf stage were planted out in a field. Plantation density was 12 plants m^{-2}. All recommended cultural practices such as irrigation, eradication of weeds and plant protection were adopted uniformly according to standard crop requirements. Weeds were manually controlled. Nitrogen fertilizer was applied and split into three applications (2, 4 and 8 weeks after lettuce transplanting). The form of nitrogen fertilizer used was urea (46% N). Lettuces were hand-harvested on the 27th March, 2006. Sampling was done on 86, 100, 114, 126, 142, and 156 days after sowing. During the study and after plant harvesting, some plant characteristics such as plant length, leaf number, fresh and dry weight of leaves, leaf area index (LAI), crop growth rate (CGR) and yield were measured. LAI was estimated by the following formula:

$$LAI = \frac{\text{Leaf area}}{SA}$$

where SA is the area covered by the plant. Also, crop growth rate (CGR) was determined by the following formula:

$$CGR = \left(\frac{W_2 - W_1}{T_2 - T_1}\right)\left(\frac{1}{SA}\right)$$

where W_1 is the first dry weight, W_2 is the secondly dry weight, T_1 is the time of first harvest, T_2 is the time of second harvesting in a two-stage sampling and SA is the area covered by the plant.

Statistical analysis of experimental data were conducted using the MSTATC software package and the means were separated following ANOVA by Duncan's multiple range test with at least $P<0.05$.

RESULTS AND DISCUSSION

The meteorological conditions during the growth period of lettuce are presented in **Table 1** showing the average air temperature and monthly rainfall from November 2005 to April 2006. The weather conditions during the growth period varied. The growing season (2005-2006) was relatively hotter than the 50-year average. The air temperatures were lower at the beginning of the growth season. The monthly temperature in the growth period between November 2005 and March 2006 was above average. The main rainfall received in the growing season (2005-2006) was relatively lower than in the 50-year average. The highest monthly precipitation during the growing period of lettuce occurred in February (102.6 mm).

Plant height

Nitrogen fertilizer application at all levels increased plant height by 4.3-6.2 cm. The highest level of nitrogen fertilizer (180 kg N ha^{-1}) produced the tallest plants and the shortest plants formed in the control (without N). However, no significant difference was found between three treatments: 60, 120, and 180 kg N ha^{-1} (**Table 2**). The effect of nitrogen rate on the changes in plant height was investigated. In the first stages of growth differences between nitrogen levels were not significant because plants are in the rosette stage and growth of plants is typically low at this stage. After this stage increased plant height and difference between treatments could be observed (**Fig. 1**). In both cultivars and from transplanting stage to plant harvesting an increase in plant height was observed. The effect of cultivar on plant height was not statistically significant, but the interaction nitrogen amount × cultivar was significant at the 5% level. The tallest 'Pich Ahwazi' and 'Pich Varamini' plants formed at 180 kg N ha^{-1} and the shortest in the control treatment (without N). Similar results have been reported in investigations conducted by Baloch *et al*. (1991) and Rostamforoudi *et al*. (1999).

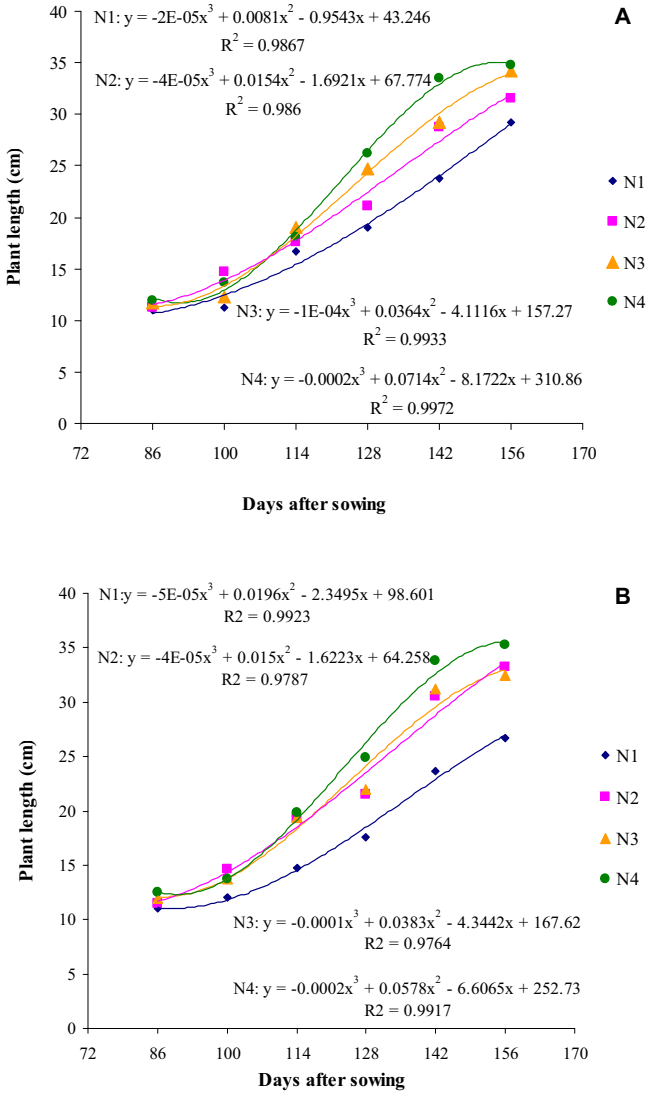


Fig. 1 Response of plant height to different nitrogen levels in two lettuce cultivars. 'Pich Ahwazi' (**A**); 'Pich Varamini' (**B**).

Fresh weight of leaves

The effect of nitrogen fertilizer level on the fresh weight of leaves was significant at the 1% level. The lowest leaf fresh

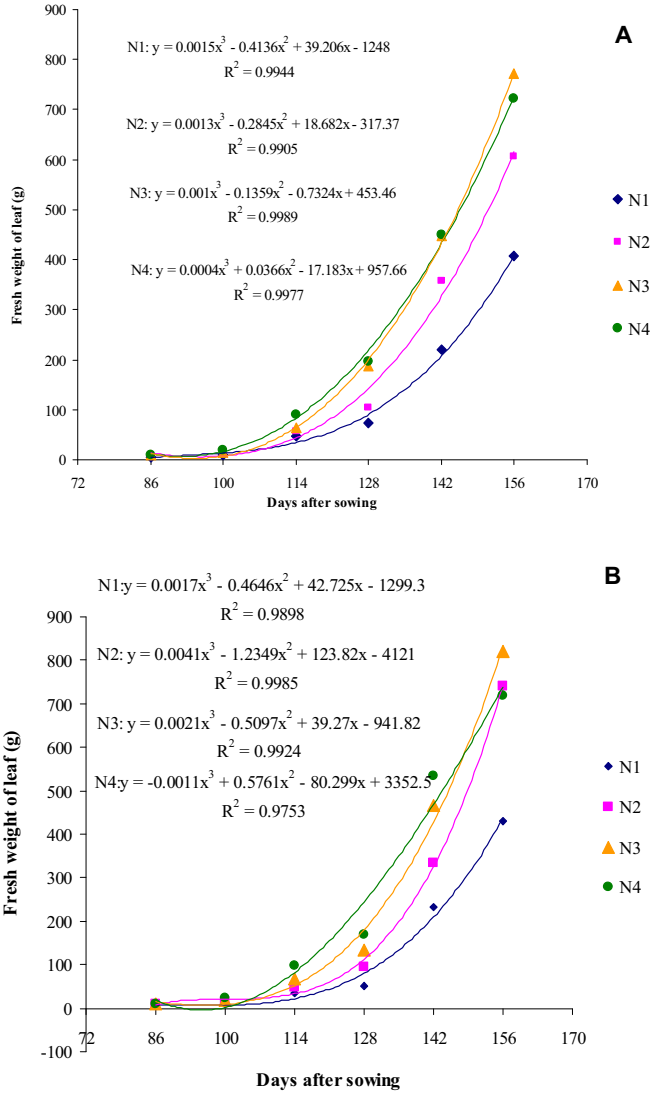


Fig. 2 Response of leaf fresh weight to different nitrogen levels in two lettuce cultivars. 'Pich Ahwazi' (**A**); 'Pich Varamini' (**B**).

weight (418.45 g) was obtained in the control treatment and the highest leaf fresh weight (798.69 g) at 120 kg N ha^{-1}. The application of N fertilizer up to 120 kg N ha^{-1} increased the fresh weight of leaves significantly but as nitrogen fertilizer dose increased above 180 kg N ha^{-1} leaf fresh weight decreased. Between 60 kg N ha^{-1} and 180 kg N ha^{-1} no significant difference was found. The studies carried out by Rincon *et al*. (1998) on lettuce showed that increasing nitrogen fertilizer level to 100 kg N ha^{-1} increased its value. Tei *et al*. (2000) applied N fertilizer at different levels (0, 50, 100 and 200 kg N ha^{-1}) for two lettuce cultivars (cv. 'Audran' and cv. 'Canasta') and estimated N fertilizer rate to obtain maximum fresh weight at about 155 kg N ha^{-1} for both cultivars. Similarly, Broadley *et al*. (2000) and Tittonell *et al*. (2003) on lettuce (cv. 'Saladian R_{100}' and cv. 'Grand Rapids', respectively) reported that fresh weight increased as N rate increased.

The effect of cultivar was significant on fresh weight. 'Pich Ahwazi', with an average of 679.02 g, had a higher fresh weight than 'Pich Varamini'. Shahbazi (2005) also reported that among four lettuce cultivars ('Pich Babol', 'Pich Nokandeh', 'Bogond Boston', 'Siah Lobnani') there was a were significant difference in fresh weight, the highest observed in 'Pich Babol'. Although this result agrees with the finding by Shahbazi (2005), that study different to ours in climatic conditions, growth season, cultivar and local situation.

The effect of nitrogen rate on changes in fresh weight was investigated (**Fig. 2**). From the first growth stage to 114 days after sowing little difference between nitrogen levels was found. This is because plants were in the rosette stage. However, plants gradually reached the head stage and reacted differently under different fertilizer treatments, with the fresh weight increasing rapidly in the four weeks before harvest. The interaction nitrogen fertilizer × cultivar on fresh weight was significant at the 1% level. The highest fresh weight was obtained at 120 kg N ha^{-1} for 'Pich Varamini' and the lowest fresh weight was produced by the control and 'Pich Ahwazi'.

Leaf dry weight

A significant difference among the nitrogen treatments was found on the dry weight of leaves ($P<0.01$). By increasing the nitrogen fertilizer rate the dry weight of leaves increased but the difference between 60, 120 and 180 kg N ha^{-1} treatments was not statistically significant. The highest dry weight of leaves was obtained at 120 kg N ha^{-1} application while the least leaf dry weight was obtained in the control (**Table 2**). Takebe *et al*. (1995) reported that increments in leaf dry weight may be due to a combination of nitrogen with plant matter produced during photosynthesis such as glucose, ascorbic acid, amino acids and protein. Also, Tei *et al*. (2000) reported that increasing the rate of nitrogen fertilizer significantly increased the dry weight of leaves. In the first stages of growth there was a small increase in the rate of dry weight formation but by increasing plant growth and leaf area the amount of dry matter increased proportionally (**Fig. 3**). Increasing the rate of nitrogen fertilizer affected leaf dry weight because nitrogen stimulates plant vegetative growth and increases leaf area; as a result increments in leaf area increase the rate of plant photosynthesis and thus high-

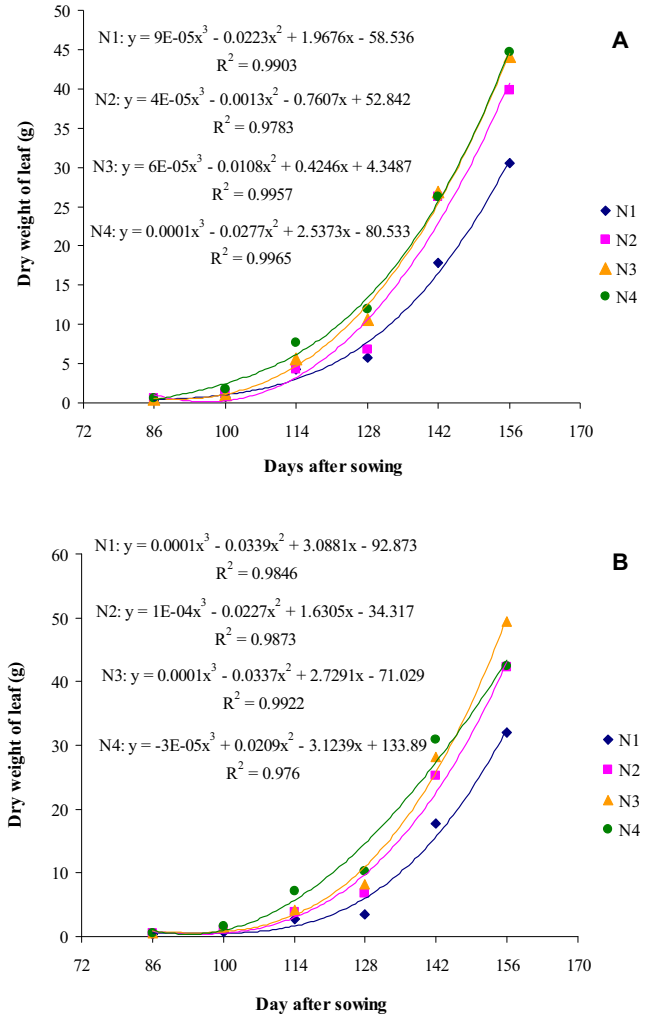
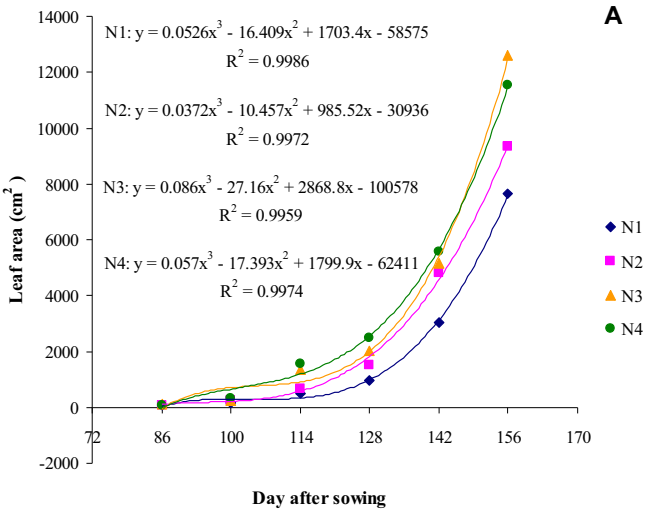
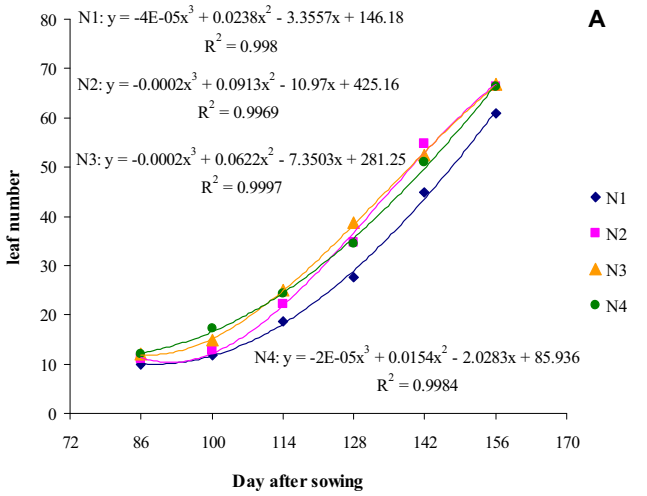
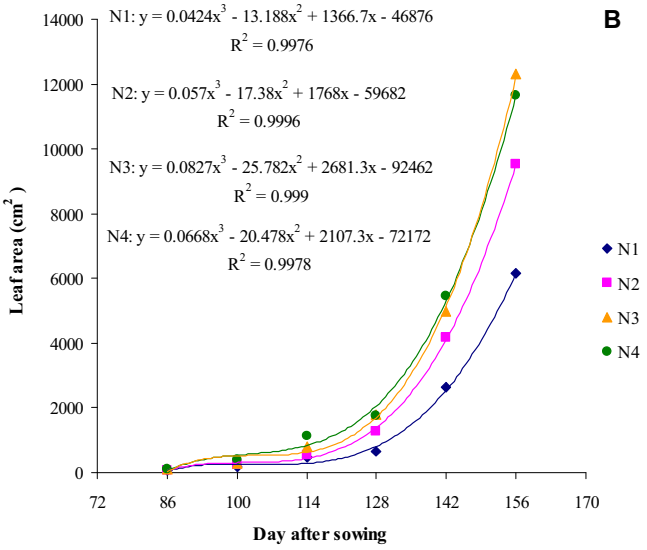


Fig. 3 Response of plant leaf dry weight to nitrogen different levels in two lettuce cultivars. 'Pich Ahwazi' (**A**); 'Pich Varamini' (**B**).







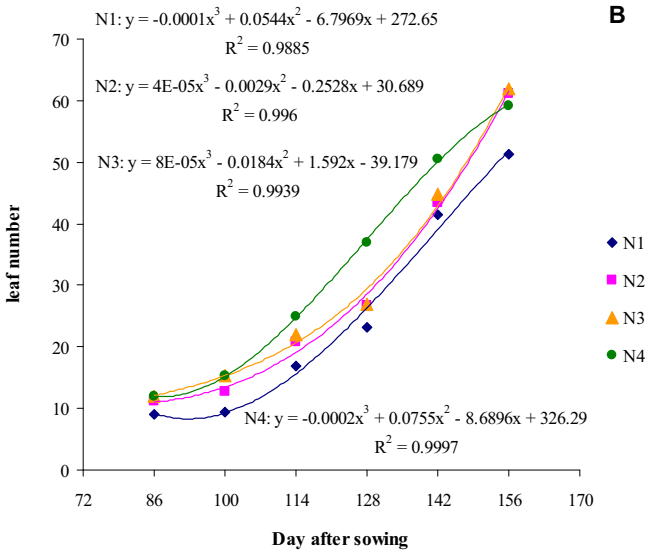


Fig. 4 Response of plant leaf area to different nitrogen levels in two lettuce cultivars. 'Pich Ahwazi' (**A**); 'Pich Varamini' (**B**).

Fig. 5 Response of plant leaf number to different nitrogen levels in two lettuce cultivars. 'Pich Ahwazi' (**A**); 'Pich Varamini' (**B**).

er dry matter production. There were significant differences in leaf dry matter and dry weight between cv. 'Pich Varamini' and 'Pich Ahwazi', being higher in the former. Also, the interaction between nitrogen fertilizer rate and cultivar on leaf dry weight was significant at P=0.05 and the highest leaf dry weight in the third treatment (120 kg N ha^{-1}) and cv. 'Pich Ahwazi', while the lowest leaf dry weight was obtained in the control treatment (without N) and 'Pich Ahwazi'.

Leaf area

The effect of nitrogen fertilizer level on leaf area was significant at P=0.01. The highest leaf area was related to the third treatment (i.e., 120 kg N ha^{-1}) and the lowest leaf area was related to the control treatment (**Table 2**). An investigation carried out by Gulser (2005) on spinach showed that increments in the nitrogen dose from 0 to 150 increased the leaf area and the highest leaf area was obtained as 203.7 m^2 ha in the highest nitrogen level, i.e. at 150 kg N ha^{-1}. Theses results are consistent with those reported by Demir *et al.* (1996), who also reported that increasing the rate of nitrogen fertilizers increased the leaf area of spinach. The results showed that nitrogen fertilizer, when applied up to 120 kg N ha^{-1}, enhanced leaf growth and photosynthesis, thus increasing leaf area. The effect of different nitrogen levels on leaf area showed that in the first stages of growth leaf area was low but gradually, by increasing leaves' growth, the leaf area increased and the difference between treatments could be assessed (**Fig. 4**). The effect of cultivar, also the interaction cultivar × level of nitrogen fertilizer did not affect leaf area significantly but in both cultivars, although as application of nitrogen fertilizer increased, so too did the average leaf area increase.

Leaf number

Nitrogen fertilizer level significantly affected leaf number, and the highest leaf number was related to the third treatment (120 kg N ha^{-1}) while the lowest was related to the control treatment. However, no significant differences were found between three levels, namely 60, 120 and 180 kg N ha^{-1} (**Table 2**). Gulser (2005) also reported that increments in the nitrogen rate of the fertilizers increased the number of leaves in spinach, but these increases were not statistically significant. Karic *et al.* (2005) applied four nitrogen levels (0, 50, 100 and 200 kg N ha^{-1}) to leek culture and reported that the application of 200 kg N ha^{-1} resulted in a maximum number of leaves per plant (14.4), but no effect was observed on the number of leaves up to 100 kg N ha^{-1}. Also, Shahbazi (2005) showed that there was a significant difference in the number of leaves among nitrogen levels (0, 50, 100, 150 and 200 kg N ha^{-1}), and that the highest leaf number was obtained with 200 kg N ha^{-1}. The application of nitrogen fertilizer stimulated vegetative growth by increasing the number of leaves (**Fig. 5**).

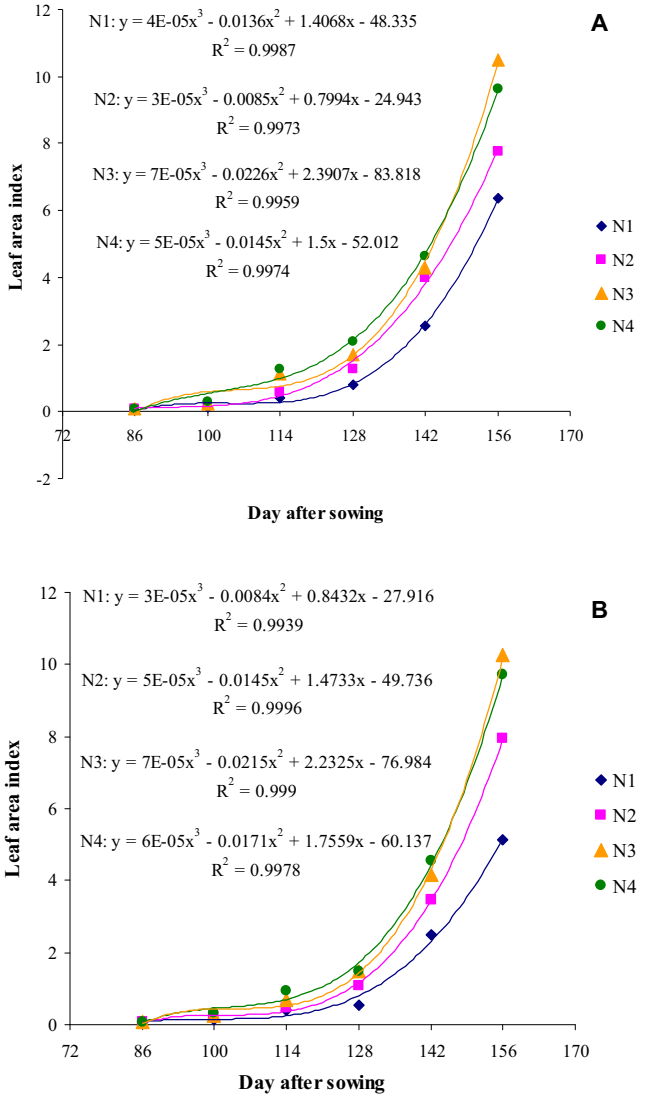


Fig. 6 Response of plant leaf area index to different nitrogen levels in two lettuce cultivars. 'Pich Ahwazi' (**A**); 'Pich Varamini' (**B**).

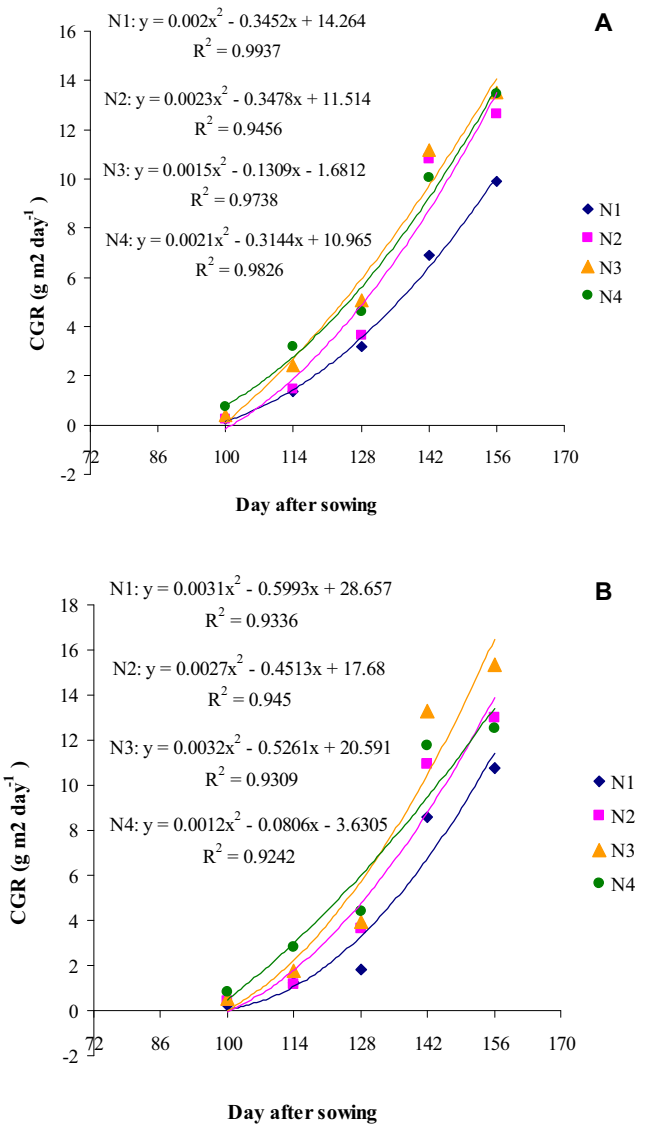


Fig. 7 Response of plant CGR to different nitrogen levels in two lettuce cultivars. 'Pich Ahwazi' (**A**); 'Pich Varamini' (**B**).

Leaf area index (LAI)

The effect of nitrogen fertilizer levels on LAI was determined in cvs. 'Pich Ahwazi' and 'Pich Varamini' (**Fig. 6**). In both cultivars, LAI in the first growth stage was low for all treatments, but rapidly increased and reached a maximum in the harvest stage. LAI increased as the nitrogen dose increased, particularly at fertilizer levels of 120 and 180 kg N ha^{-1} although the difference in LAI between these two treatments was insignificant. Allen and Morgan (1972) reported that LAI was significantly affected by nitrogen level. The increase in LAI in response to an increase in N fertilizer is probably due to enhanced availability of nitrogen which enhanced more leaf area resulting in higher photoassimilates and thereby in more dry matter accumulation. Also, Squire *et al*. (1987) established that the main effect of N fertilizer was to increase the rate of leaf expansion, leading to increased interception of daily solar radiation by the canopy.

Crop growth rate (CGR)

Nitrogen level altered the growth rate of cvs. 'Pich Ahwazi' and 'Pich Varamini' (**Fig. 7**). In the first stage of growth, CGR was low for all fertilizer levels but as time passed as the plant grew and leaf area increased (i.e. the rate of dry matter production increased) so the CGR increased.

In both cultivars, the highest CGR was obtained with the application of 120 kg N ha^{-1}. The results of some studies showed that high nitrogen levels increased leaf area, leaf number and vegetative growth of plants thus increasing the photosynthetic capacity; consequently the higher dry matter produced increasing CGR (Allen and Morgan 1972; Gulser 2005).

Yield

The effect of different levels of nitrogen on yield was significant at P=0.01. The highest yield in lettuce was obtained as 7 kg m^{-2} after application of 120 kg ha^{-1}; the lowest yield was obtained as 2.83 kg m^{-1} in the zero nitrogen application, i.e. the control (**Table 2**). Increasing the N levels of the fertilizers to 120 kg N ha^{-1} significantly increased the yield of lettuce while yield decreased at the highest nitrogen dose. This decrease in yield might be due to toxicity in the plant (Tabatabaie and Malakouti 1997). The relationship between nitrogen level and lettuce yield is shown in **Fig. 8**. The quadratic equation was:

$$y = -0.0002x^2 + 0.0591x + 2.7205$$

where Y is yield in t ha^{-1} and X is N applied in kg ha^{-1}.

This formula could be used when making a recommendation for lettuce N application, and when the conditions are the same or very close to those in this experiment.

Shahbazi (2005) reported that by increasing nitrogen level from 0 to 120 kg N ha^{-1} the yield of lettuce increased but between 100, 150 and 200 kg N ha^{-1} there were no

Table 3 correlation coefficients between characteristics investigated.

Characteristics	Plant length	Leaf fresh weight	Leaf dry weight	Leaf area	Leaf number	Yield
Plant length	1.000					
Leaf fresh weight	0.801**	1.000				
Leaf dry weight	0.734**	0.950**	1.000			
Leaf area	0.794**	0.877**	0.865**	1.000		
Leaf number	0.525**	0.351*	0.395*	0.438*	1.000	
Yield	0.813**	0.914**	0.859**	0.874**	0.463*	1.000

* Statistically significant at P=0.05.
** Statistically significant at P=0.01.

Table 4 Time of head formation in cvs. 'Pich Ahwazi' and 'Pich Varamini' affected by different levels of nitrogen fertilizer.

Cultivar	Rate of nitrogen fertilizer (kg ha^{-1})			
	0	60	120	180
'Pich Ahwazi'	After 10 weeks*	After 9 weeks	After 7 weeks	After 7 weeks
'Pich Varamini'	After 11 weeks	After 10 weeks	After 8 weeks	After 8 weeks

* Time duration after transplanting

Table 5 Time of bolting in cvs. 'Pich Ahwazi' and 'Pich Varamini' affected by different levels of nitrogen fertilizer.

Cultivar	Rate of nitrogen fertilizer (kg ha^{-1})			
	0	60	120	180
'Pich Ahwazi'	After 11 days*	After 16 days	After 20 days	After 23 days
'Pich Varamini'	After 17 days	After 24 days	After 29 days	After 32 days

* Time duration after harvesting

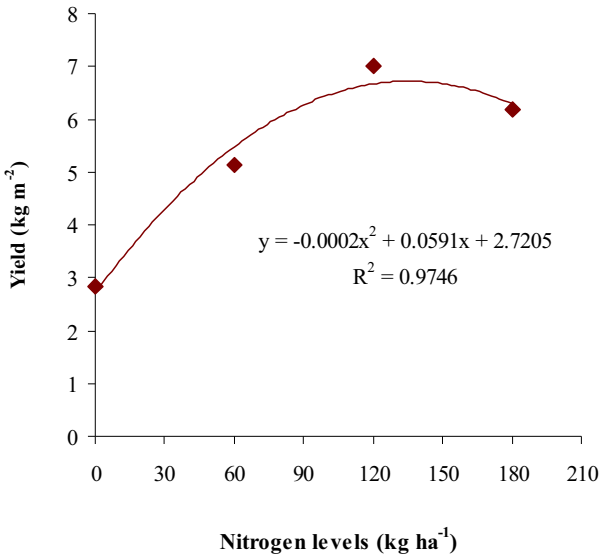


Fig. 8 Relationship between nitrogen level and lettuce yield.

observable significant differences. Also, Behtash (1995), during experiments on cabbage and celery, found that the application of nitrogen fertilizer increased yield more than the control treatment but the economically best yield (as 9.38 and 13.63 kg m^2 in celery and cabbage, respectively) and quality was obtained with an application of 100 kg N ha^{-1}. The effects of cultivar, fertilizer rate and interaction cultivar × fertilizer application were not significant.

Correlation among vegetative characteristics

In order to evaluate the correlation between characteristics following the application of different levels of nitrogen and cultivars and also to assess the connection of these characteristics with the yield of lettuce, all correlation coefficients between characteristics were investigated (**Table 3**). There were significant positive correlations between all characteristics investigated. Also, significant positive correlations were obtained among yield with all characteristics. Therefore, yield increased with increasing characteristics such as plant length, leaf number, fresh and dry weight of leaf, and leaf area. According to the correlation coefficients, the yield of lettuce caused by an increase in the fresh weight of leaves gave a higher correlation than others characteristics investigated. In contrast, the relationship between yield and leaf number was lower compared with other interactions.

Time of head formation

There were differences between cultivars in time of head formation and cv. 'Pich Ahwazi' formed a head about one week earlier than cv. 'Pich Varamini' in all treatments. Also, there were differences in the time to head formation between different fertilizer treatments: Earlier fertilizer treatments of 120 and 180 kg N ha^{-1}, more than other treatments (0 and 60 kg N ha^{-1}), caused a head to form earlier by about 2-3 weeks (**Table 4**).

Time of bolting

The effect of cultivar and rate of nitrogen application on bolting time is shown in **Table 5**. There were differences among cultivars in bolting time and cv. 'Pich Ahwazi' was more susceptible to bolting than cv. 'Pich Varamini'. Since the former is better adapted to variable weather conditions, the latter, which was recently cultivated in Ahwaz, is less sensitive to day-length. Consequently cv. 'Pich Ahwazi' reacted quickly to a change in photoperiod and temperature. The rate of nitrogen fertilizer affected bolting time. As the rate of nitrogen fertilizer increased, so flowering in lettuce became delayed since nitrogen stimulated the vegetative growth of the plant and increased the rate of leaf expansion. In both cultivars, the control treatment flowered earlier than all other treatments, whereas fertilizer treatment at 180 kg N ha^{-1} resulted in the last flowering among all treatments.

CONCLUSIONS

1. Plant length, fresh and dry weight of leaves, leaf area, number of leaves, leaf area index, crop growth rate, and yield increased as nitrogen fertilizer rate increased to 120 kg N/ha.
2. Cultivar had a significant effect on fresh and dry weight of leaves, and leaf number but not on plant length and leaf area. The local cultivar ('Pich Ahwazi') enters the heading and bolting stages earlier than the introduced cultivar ('Pich Varamini').
3. Cv. 'Pich Ahwazi' was more resistant than cv. 'Pich Varamini' to local, native diseases and physiological disorders such as tip burn.
4. In Conclusion, 120 kg N ha^{-1} is the optimum amount of N for lettuce production in this region and regions with similar climatic and soil conditions. However, this N ap-

plication level can not be generalized for all lettuce cultivars since the optimum amount of N fertilizer depends on the soil mineral N concentration and the amount of N mineralized from soil organic sources during the growth period.

ACKNOWLEDGEMENTS

The authors wish to thank Shahid Chamran University of Ahwaz, Iran for supporting this work.

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