The Effect of Ammonium Nitrate and Plant Density on Yield and Vegetative Characteristics of Burley Tobacco (*Nicotiana tabacum* L.) Variety No. 21

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In order to investigate effect of plant density and ammonium nitrate fertilizer rates on yield and vegetative characteristics of Burley 21 (*Nicotiana tabacum* L.) an experiment was conducted during the year 2012 on farm located at 10 km from the city of Salmas (Northwest of Iran) by the factorial experiment based on randomized complete blocks design (RCBD) with three replications. The factor A was three levels of ammonium nitrate (300, 200 and 100 kg/ha) and factor B was 3 levels of Plant density: I. Transplanted at 100 × 60 cm, leading to a density of 1.67 plants/m², II. Transplanted at 100 × 50 cm, leading to a density of 2 plants/m², III. Transplanted at 100 × 40 cm, leading to a density of 2.5 plants/m². Leaves were stripped from the stalk and harvested in four stages (four stalk positions) consisted of first, second, third and fourth priming. Leaf area (LA) in each of the four stalk positions (LA1, LA2, LA3 and LA4) data were collected after leaves detached from the stalk at the four times. Dry leaf yield (DLY1, DLY2, DLY3 and DLY4) g/m² and total dry leaf yield Kg/ha (TDLY) data were collected after leaves air-cured. The results showed that the effect of fertilizer rates on the leaf area (LA) and dry leaf yield (DLY) were significant at the all 4 harvesting stage of burley tobacco. Plant density affected LA2, LA3, LA4, DLY2, and DLY4 significantly but did not affect LA1 lower leaves area, DLY1, DLY3 and DLY4 significantly. Results indicated the burley tobacco produced optimum yield (2207 kg ha⁻¹) at the 300 kg ha⁻¹ rate of ammonium nitrate and 50 cm within row spacing (about 2 plants per m²).

Key words: Ammonium nitrate; Burley 21; Dry leaf yield; Plant density.

Introduction

Tobacco, *Nicotiana* spp., is a member of the Solanaceae family. Currently there are 70 naturally occurring species of tobacco [13]. Burley tobacco (*N. tabacum* L.) is the most common type of tobacco, grown in West Azerbaijan, Iran. Tobacco growing areas in this region are limited; however crop yield potential is high. Soil, climate, low presence of diseases and irrigation are factors responsible for high yields by the way nitrogen is the main yield driver, mainly when the economic yield is a vegetal organ like leaves. Due to increased prices of fuel, labor and other inputs, it is imperative that farmers manage production costs. One of the highest costs of production is nitrogen fertilizer. Also nitrogen is one of the most important inputs for growers to manage because nitrogen fertilizer affects burley tobacco yield and quality [9] and also it is highly susceptible to leaching into groundwater. Accurate assessment of N availability is important from economic, environmental, and health standpoints. Ammonium nitrate has been the dominate source of fertilizer nitrogen (N) for tobacco in recent years. It is relatively easy to transport, store and apply with equipment common on most tobacco farms. The mix of ammoniomical nitroger form and nitrate-N helps to balance nitrogen availability through the growing season, and matches well with the N uptake pattern of tobacco [16]. Dorn [10] showed that N is taken into plants in only two forms. These forms are NO₃⁻ (nitrate based) and NH₄⁺ (ammonium based). Rathbone research results showed the newer burley tobacco cultivars (TN 90, KT 204, NC 2000, and NC 7) produced maximum yield at the recommended 224 kg ha⁻¹ rate of nitrogen [18]. Also in North Carolina, researchers have recommended that 180 kg N ha⁻¹ to 224 kg N ha⁻¹ applied to burley tobacco [12]. Among growing practices, plant spacing can affect agronomic and chemical traits of tobacco [6]. Plant
spacing in tobacco production usually refers to distances between tobacco plants within rows and between rows in the field, but it can also be interpreted as total number of plants and leaves produced within a defined area [22]. In Croatia, most of the flue-cured tobacco is grown at plant spacing of 45 cm within and 100 cm between rows, i.e. at planting density of about 22,000 plants per ha [2]. In Northern Italy, the recommended number of flue-cured tobacco plants per hectare is 24,000 [12]. It is obtained by 115 to 120 cm between rows and approximately 35 cm within row spacing. Such spacing enhances leaf growth towards the row and eases mechanical harvesting and it gives high leaf yield. Yields and values of burley tobacco (from three fertility treatments with two plant spacing) at the Southwest Virginia Research Center showed that The 18” (45 cm) spacing within rows and 42”-48” (105-120 cm) between rows produced a higher acre yield of burley tobacco than the 24” (60 cm) spacing at each fertility level, with an average 4.15% increase for the closer spacing [17]. In the USA, plant populations in conventional flue-cured tobacco production were average 14,700 plants ha⁻¹ and row spacing is 122 cm with approximately 56 cm between plants [3]. In Iran burley tobacco is commonly grown at plant spacing of 100 cm between and 50 cm within rows (planting density of about 20,000 plants ha⁻¹). Closer spacing of plants generally results in a reduction of size, body, thickness, and weight per unit area of the leaf [22]. Wu et al. [23] observed a decrease in total leaf area per plant with increased plant population. The objective of this study was to compare yield and Vegetative Characteristics of burley tobacco in Salmas (Northwest of Iran) grown at three within-row spacing.

**Materials and Methods**

Field studies were conducted on the farm near Salmas city, North west of Iran on a loamy clay soil in 2012 year. Research plots were set up as a factorial randomized complete block design (RCBD) with three blocks and six plant rows per experimental plot. Tobacco seedlings for the trials were grown in a float greenhouse system. Seedlings were started the second week of April, and transplanted to the field during the second week of June. Burley 21 cultivar was used throughout the study. This cultivar was selected because of present or past use by growers throughout the tobacco growing region of the Northwest of Iran. Treatment factors were ammonium nitrate (AN) rate and density. The three AN treatments were 100, 200 and 300 kg AN ha⁻¹ and three density treatments were Plant spacing 100 cm between rows, and 40, 50 and 60 cm within rows, leading to a density of 16,667, 20,000, 25,000 plants/ha of or plant densities of about 1.67, 2 and 2.5 plants per m², respectively. Plants were grown in a bed-furrow irrigation system in 6 rows per plot that were spaced 1 m apart. Ammonium nitrate (34.5 % nitrogen) was applied pre-plant and as a side-dress application. Fertilization at the rate of 200 kg triple super phosphate and 300 kg potassium sulphate ha⁻¹ were applied, based on soil test recommendations (Table 1) and all treatments received half (1/2) amount of AN as a pre-plant broadcast application. Additional Ammonium nitrate (1/2) fertilizer was side-dressed at varying rates, 35 days after transplanting. Tobacco flowering is a physiological characteristic that differs among tobacco cultivars and topping is the process of cutting the blooms off the plants. By removing the flowers, plants are forced to stop growing taller and allocate more carbohydrates to roots and leaves. Plants are topped when 50% of the plants in the field are in bloom. Pesticides to control weeds and insects were applied as needed. Leaves were stripped from the stalk and harvested in four stages consisted of first priming leaves, second priming leaves, third priming leaves and fourth priming leaves. Five plants of the middle rows of plot randomly were measured for dry leaf yield (Weight of total dry leaf yield=DLY, dry first priming leaves=DLY₁, dry second priming leaves=DLY₂, dry third priming leaves=DLY₃ and dry fourth priming leaves=DLY₄). Also vegetative traits consisted of leaf length, leaf width and leaf area (LA₁, LA₂, LA₃, and LA₄) data were collected after leaves detached from the stalk at the four times. Air cured (the methods of curing consisted of conventional air-curing) and all practices that essential for curing were performed as usual in air-cured tobacco production by standard burley practices. Five number of leaves harvested in every 4 times of priming at 20 day intervals (27, 47, 67 and 87 days after transplanting). The relative leaf areas (LA₁, LA₂, LA₃, and LA₄), calculated by the Suggs formula, are as follows:

\[
\text{Leaf areas (cm}^2\text{)} = b \times \text{length (cm)} \times \text{width (cm)}
\]

In which the coefficient b was 0.7028 for tobacco leaves of 203 cm² or less, and was 0.6203 for tobacco leaves greater than 203 cm². After curing, total dry leaf yield (kg ha⁻¹) of each experimental plot was determined. All statistical analyses were performed using MSTAT-C computer program and Duncan’s multiple range test (α = 0.05) was used to compare means.

**Table 1: Soil analysis result.**

<table>
<thead>
<tr>
<th>Soil Texture</th>
<th>OC (%)</th>
<th>Cl meq/L</th>
<th>EC dsm</th>
<th>pH</th>
<th>N (%)</th>
<th>P ppm</th>
<th>K ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loamy clay</td>
<td>0.8</td>
<td>0.7</td>
<td>0.47</td>
<td>7.57</td>
<td>0.115</td>
<td>22</td>
<td>238</td>
</tr>
</tbody>
</table>
Results:

Leaf area of first priming (LA1):

Results of statistical analysis of first priming leaves of burley tobacco showed that ammonium nitrate fertilizer levels significantly affected leaf area of first priming in %1 level and plant density not affected leaf area of first priming in %5 level also the interaction between density and ammonium nitrate rate effects on leaf area of first priming was not significant at %5 level (Table 2). Table of means (Table 3) showed that the highest leaf area (5158 cm² per square meters) was obtained with the highest ammonium nitrate level (300 kg ha⁻¹) and the lowest leaf area with 3037 cm²/m² obtained from the lowest rate of ammonium nitrate (100 kg ha⁻¹). Leaf area of first priming was increased from 3037 to 4037 and 5158 cm²/m² with increasing ammonium nitrate rate level from 100 to 200 and 300 kg ha⁻¹, respectively. These increases represent 32.9%, 69.8% respectively. Means compare show that 300 kg/ha ammonium nitrate with 5158 cm²/m² was the highest mean that classed "a" level (Table 3) and 100 kg ammonium nitrate per hectare with 3037 cm²/m² mean classed at a lower level "c".

Leaf area of second priming (LA2):

Table 2 represented that ammonium nitrate fertilizer and plant density affected leaf area of second priming leaves in %1 level and interaction between density and ammonium nitrate rate effects on leaf area of second priming leaves was not significant. Means of leaf area of second priming leaves showed that 300 kg ammonium nitrate per hectare (AN₁ treatment) with 6961 cm²/m² was graded in "a" group (Table 3), 200 kg ammonium nitrate per hectare (AN₂ treatment) with 5472 cm²/m² was graded in "b" group and 100 kg ammonium nitrate per hectare (AN₃ treatment) with 4068 cm²/m² was graded in "c" group. Increasing leaf area of the second priming leaves from 4068 to 5472 and 6961 were 34.5% and 71.1% respectively. According to the table for comparison of average simple effects of density on leaf area, 2.5 and 2 plants/m² were determined as the highest leaf area in third priming with 5781 and 5406 cm²/m² respectively were classed "a" (Table 3), the level of 1.67 plants/m² with 3037 cm²/m² mean classed at a lower level "c". There was roughly 15.7% increase in leaf area as plant density increased from 2 to 2.5 plants/m².

Leaf area of third priming (LA3):

Using the variation analysis (Table 2), it was found that ammonium nitrate fertilizer and plant density affected leaf area of third priming at %1 level in burley tobacco and interaction between density and ammonium nitrate rate effects on leaf area in third priming was not significant. Means compare show that 300 kg/ha ammonium nitrate with 7681 cm²/m² was the highest mean that classed "a" level (Table 3) and 100 kg ammonium nitrate per hectare with 3352 cm²/m² mean classed at a lower level "c". According to the table for comparison of average effects of density on leaf area, 2.5 and 2 plants/m² were determined as the highest leaf area in third priming with 5781 and 5406 cm²/m² which were classed "a", the levels of 2 and 1.67 plants/m², with averages of 5406 and 4856 cm²/m² were classed at a lower level "b" (Table 3). There was roughly 19 percent increase in leaf area as plant density increased from 1.67 to 2.5 plants/m². It is notable that between 2 and 2.5 density levels were not significant differences (P>0.05).

Leaf area of fourth priming (LA4):

Analysis of variance in fourth priming showed that there were significant differences between ammonium nitrate treatments in %1 level and plant density at %5 level and interaction between density and ammonium nitrate rate effects on leaf area of fourth priming was not significant at %5 levels (Table 2). Table of means showed that ammonium nitrate with 300 kg ha⁻¹ produced the highest leaf area of fourth priming by 4346 cm²/m² was classed as "a" group (Table 3). And leaf area with 2640 and 2378 cm²/m² obtained from the 200 and 100 kg ha⁻¹ rate of ammonium nitrate respectively were classed at "b" level. Leaf area was increased from 2640 to 2378 cm²/m² as ammonium nitrate rate increased from 100 to 200 kg/ha but the difference was not significant (P>0.05). Increasing leaf area from 2378 to 2640 and 4346 were around 11% and 82.8% respectively. According to the table for comparison of average simple effects of density on leaf area, 2.5 and 2 plants/m² were determined as the highest leaf area with an average of 3504 and 3240 cm²/m² respectively were classed "a" (Table 3), the level of 1.67 plants/m², with averages of 2620 cm²/m² was classed at a lower level "b". There was roughly 23.6% and 33.7% increase in leaf area as plant density increased from 1.67 to 2 and 2.5 plants/m².

Dry leaf yield in first priming (DLY1):

Dry leaf yield in first priming with consideration of statistic results, was under the influence of ammonium nitrate rate in %1 level but dry leaf yield in first priming was not affected by plant density and interaction effects between ammonium nitrate and plant density (Table 2). Using ammonium nitrate could increase the dry leaf yield of first priming from 22.78 gr/m² (Table 3) in 100 Kg ha⁻¹ to 44.91 gr/m² in 300 Kg ha⁻¹. AN₁ treatment (100 kg ammonium nitrate per hectare) with 22.78 gr/m² was the lowest dry leaf yield in first priming classed in III, AN₂
Nitrogen fertilizer levels significantly affected the weight of dry leaves in %1 level and plant density not affected dry leaf yield of fourth priming in 5% level also the interaction between density and ammonium nitrate rate effects on dry leaf yield of fourth priming was not significant at 5% level (Table 2). Using ammonium nitrate could increase the dry leaf yield of fourth priming from 16.54 gr/m² (Table 3) in 100 Kg ha⁻¹ to 37.15 gr/m² in 300 Kg ha⁻¹. AN₃ treatment (100 kg ammonium nitrate per hectare) with 16.54 gr/m² and AN₂ treatment (200 kg ammonium nitrate per hectare) with 20.87 gr/m² which were the lowest dry leaf yield in fourth priming, classed in II and AN₁ treatment (300 kg ammonium nitrate per hectare) with 37.15 gr/m² which increases by 124.6% classed in I (Table 3).

**Total weight of dry leaf yield (kilo gram per hectare):**

Total weight of dry leaf yield was another trait that evaluated in this experiment. Result showed that there are significantly differences between ammonium nitrate fertilizer and plant density levels in this experiment with the probability of 1 percent and the interaction of plant density and ammonium nitrate level effects on total dry leaf yield was not significant (Table 2). Table of means showed that total weight of dry leaf yield was increased from 979 to 1480 and 2186 kg ha⁻¹ with increasing ammonium nitrate level from 100 to 200 and 300 kg ha⁻¹, respectively. Means compare show that 300 kg/ha ammonium nitrate with 2186 kg ha⁻¹ dry leaf yield was the highest mean that classed "a" level (Table 3), 100 kg ammonium nitrate per hectare with 979 kg ha⁻¹ dry leaf yield mean classed at a lower "c" level and 200 kg ammonium nitrate per hectare with 1480 kg ha⁻¹ dry leaf yield mean classed at "b" level. The "a" class was about 47.7% and 123.5% more than "b" and "c" classes respectively. According to the table for compare means, 2.5 and 2 plants/m² were determined as the highest dry leaf yield with 1624 and 1565 kg ha⁻¹ which were classed "a", the levels of 2 and 1.67 plants/m² with averages of 1565 and 1456 kg ha⁻¹ were classed at a lower level (Table 3). There was roughly 11.5 percent increase in dry leaf yield as plant density increased from 1.67 to 2.5 plants/m². It is notable that there was not statistically significant difference between D₂ and D₃ (2.5 plants/m²) treatments and D₂ (2 plants/m²) by D₁ (1.67 plants/m²) treatments so.

**Discussion:**

Nitrogen plays a key role in making important vegetable protein and in the formation of plant cells. Reducing nitrogen or its non-use causes a delay in the formation of plant cells that leads to a decrease in yield [11]. Nitrogen is available as an important part of all protein compounds, compounds in construction
and energy transfer and even in the structure of DNA, which is responsible for transferring inherited traits. Ammonium nitrate rates have important effects on vegetative characteristics and reproductive development in burley tobacco. Leaf area in 4 harvesting stages (LA1, LA2, LA3, and LA4) cm²/m², weight of dry leaf in 4 harvesting stages (DLY1, DLY2, DLY3, and DLY4) gr/m² and total weight of dry leaf yield kg/ha were significantly increased as N fertilizer levels increased (Table 3). Similar results were reported by Tejeda in Chile. Nitrogen deficiency prevents plant growth processes and cause shortness, yellowing and reduction in performance of dry materials. Table of means showed that LA1, LA2, LA3, and LA4 with highest amounts (5158, 6961, 7681, and 4346 cm²/m² respectively) were graded in A group that were obtained with the highest ammonium nitrate level (300 kg AN ha⁻¹). The significant positive relations between leaf area and high nitrogen fertilization were observed in this research. DLY1, DLY2, DLY3, and DLY4 with 44.91, 67.81, 68.73, and 37.15 gr/m² respectively (Table 3) were graded in A group that were obtained with the highest AN level (300 kg AN ha⁻¹). Dry leaf yield kg/ha was increased from 979 to 1480 and 2186 kg ha⁻¹ with increasing AN level from 100 to 200 and 300 kg AN ha⁻¹, respectively (Table 3). These increases represent 51% and 123% respectively. The increase in LA area, dry leaf and total yield characters as the increase in AN levels might be due to the role of nitrogen in stimulating vegetative growth. Therefore the results were similar at all 4 harvesting stage of burley tobacco as the increase of AN rates at trial plots. Dry leaf yield was greater in the higher AN rate treatment, indicating that more nitrogen is needed to obtain optimum yield than lower rate due to the lower N rates did not supply enough N for the yield potential of burley tobacco. This result has observed in other trial. The significant positive relations between dry leaf yield and high nitrogen fertilization were observed in this study and AN rate was increased quantity features in burley tobacco. Plant spacing significantly affected LA2, LA3, LA4, DLY2 and total dried leaf yield. Also it was apparent that plant spacing has not an effect on LA1, DLY1, DLY3, and DLY4 (Table 2) due to decrease of photosynthesis content in high density per plant was compensated by the increase in number of plant per unit area. Data in Tables 2 and 3 showed that LA2, LA3, LA4, DLY2 and total dried leaf yield were significantly increased from 5370 to 5971 cm², 4856 to 5781 cm², 2620 to 3504 cm², 47.98 to 52.73 gr/m² and 1456 to 1624 kg ha⁻¹ respectively as the space between plants decreased from 60 to 40 cm with the highest at 2.5 plant density (D=2.5 plants/m²). Similar results were reported by others. Results of statistical analysis showed that dry leaf yield increased from 1456 to 1565 kg ha⁻¹ when plant density was increased from 2 to 2.5 plants/m² but the difference was not significant (P>0.05). In other words even though statistically significant effect was found in DLY2 and total dried leaf yield but were not affected to a large degree by differential plant population treatments. Therefore the increase in dry leaf yield kg ha⁻¹ with sowing burley tobacco at 2.5 plants/m² might not be suitable due to the compensation cost of planting additional number of plants per hectare and additional dry leaf yield kg ha⁻¹. Similar results were reported by others. The increase in dry leaf yield kg/hectare with increasing plant density might be attributed to the increase in number of plants ha⁻¹ which were increased with the increase in plant density as reported by Ashkesh and Hodjati. Similar results were also reported by other researchers: Reducing the density of plants per hectare reduced yield by 14.2% (365.45 kg/ha) compared with the standard density. The increase in plant density level was associated with an increase in LA1, but the difference in leaf area did not reach the level of significance by reducing plant space to 40 cm with the same AN level. Increasing in TDLY ha⁻¹ with the increase in density up to 2.5 plants/m² despite the decrease of photosynthesis content per plant was due to the increase in number of leaf and plant per unit area. At higher planting densities an increase of yield has been reported by several authors. The interaction between density and AN rate was not significant. The highest dry leaf yield (2211 kg ha⁻¹) was produced with sowing burley tobacco on 40×100 cm and adding 300 kg AN ha⁻¹, but the difference in dry leaf yield did not reach the level of significance by increasing plant space to 50×100 cm with the same AN rate. Therefore we have to sow in Plant spacing 100 cm between rows and 50 cm within rows due to the cost of planting additional (25000 – 20000 = 5000) number of plants per hectare. Also the lowest dry leaf yield (876 kg ha⁻¹) was obtained from 100 kg AN ha⁻¹ fertilizer at 1.67 density.

Dry leaf yield in tobacco is a quantitative trait largely influenced by the environment and hence has a low heritability. Therefore, the response to direct selection for dry leaf yield may be unpredictable unless there is good control of environmental variation. So, according to the results of this investigation, the highest yield was obtained by the highest amount of AN, highest plant density. Because total photosynthesis increases by development of leaves area and lead to increases yield. Also yield will be reduced when soil nitrogen is depleted, as shown by the lower yield at lower AN rates. The best dry leaf yield (2207 kg ha⁻¹) was obtained from treatment of (AN=300 kg ha⁻¹, D=2 plants/m²). The results of this experiment have demonstrated the importance of nitrogen fertilization in the production of burley tobacco as shown by yield increases at the higher AN rate.
It is apparent that LA, DLY and total dry leaf yield were affected more by nitrogen rate than density that nitrogen rate play an important role. These observations led to the final conclusion that the spacing for obtaining the highest dry leaf yield is sowing burley 21 in 50 × 100 cm (2 plants per square meters) and adding 300 kg AN ha⁻¹ to the soil of this region.

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