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DIFFERENCES IN THE NITROGEN ASSIMILATION AMONG GENOTYPES OF *BITUMINARIA BITUMINOSA* (L.) C.H. STIRTION

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Abstract

Bituminaria bituminosa is an herbaceous, perennial legume that use as feed crop and have ability to remain green in dry summer. This research was conducted to determine the differences in nitrogen assimilation ability among genotypes of *B. bituminosa*. The nitrate assimilation capacity in different plant parts (roots and leaves) of selected genotypes has been determined with Nitrate Reductase (NR) activity (μmol NO_2^- $gDW^{-1}h^{-1}$). Also, N contents (%) of root and leaves together with changes (%) in the soil N contents and dry plant yield (g/plant) were detected. Among the investigated genotypes there were the changes in nitrate assimilation capacity. The highest NR activity was determined in the leaves of *B. bituminosa*, especially in genotype numbered by A1 being from Spain and in genotypes numbered by 2 and 11 belong to Turkey. These results reveal about the nitrogen assimilation ability of genotypes of *B. bituminosa*, which is separated with its many characteristics from other forage crops.

Key words: Nitrogen assimilation, Nitrate reductase (NR) activity, Forage crops, *Bituminaria bituminosa*.

Introduction

The genus Bituminaria (Psoralea) in the Leguminosae family (or Fabaceae) contains approximately 120 species (Boardley *et al*., 1986). *Bituminaria bituminosa* is a perennial species within this genus and is common in the Mediterranean basin (Mendez and Fernandez, 1990; Mendez *et al*., 1991). Although its homeland is Mediterranean, it shows a wide distribution in natural vegetation of countries such as Turkey, South Europe, Crimea, western Syria, Cyprus, the Caucasus, Israel, North Africa, Portugal and Spain (Davis, 1965). In Turkey, it is located in the native flora of Adana, Antalya, Çanakkale, Bursa, Istanbul, Izmir, Bursa, Samsun, Sinop, Tekirdağ, Trabzon, Yozgat and Zonguldak. Sternberg *et al*., (2006) have stated that *Bituminaria bituminosa* is an alternative forage source for animals due to its rich nutrient content. It is also mentioned that *B. bituminosa* can grow in soils which are inefficient for other plants such as acidic soil conditions (Mendez *et al*., 1991). *B. bituminosa* is one of the forage plants that have been the most discussed on the global scale because of not only growing in this kind of marginal areas without irrigation, but also it has resistant to drought and heat, and has sustain its growth during the summer months and protect its greenery. It was even determined that *B. bituminosa* is highly resistant to intensive grazing pressure (Gutman *et al*., 2000).

Today, various research programs are being developed in countries such as Spain, Israel and Australia about the use of *B. bituminosa* in arid lands or as a feed plant (Gintzburger & Le Houerou, 2002; Sternberg *et al*., 2006; Fernandez *et al*., 2010; Real *et al*., 2014). Turkey is one of the most important natural distribution area of this species, has gained pace recently with researches in this direction (Ventura *et al*., 2004; Gulumser, 2011; Gulumser *et al*., 2010; Gulumser & Acar, 2012; Kumbasar, 2015; Sahin, 2016; Kaymak, 2017; Orenç, 2017; Kumbasar *et al*., 2018). Because the cultivation of forage crops is of vital importance in influencing the national economies of the countries. It also has an important ecological input in terms of the abundance of organic matter in the soil. For example, there are in a variety of studies that have increased in the rate of organic matter and nitrogen in the soil after the planting among 3-5 years (Açıkgöz, 2001). It is a wiser method to allow the cultivation of such feed plants instead of adding mineral nitrogen to the soil. Because, the addition of mineral nitrogen enhances the quality of the product, but when it comes to long term it is becoming one of the pollution factors.

As well as increasing the content of nitrogen in the soil, forage crops like this belonging to Leguminous family can live by using nitrogen efficiently in N-poor soils. The plants take nitrogen in the form of nitrate or ammonium from soil. The nitrate reductase (NR) enzyme present in the plant is very sensitive to nitrate and responsible from converting of nitrate to nitrite. Subsequently, nitrite reductase (NiR) enzyme take part to transformation and nitrite is metabolized to ammonium. These enzyme activity values are one of indicator of the nitrogen assimilation capacity of plants. Plants have variation in their nitrogen assimilation ability, that variations may sometimes be between species, sometimes within one species (Högberg *et al*., 1986). There are even differences between the organs of plants (roots, leaves, shoots etc.) (Jiang & Hull, 1999; Arslan *et al*., 2009). Therefore, more information is needed about the nitrogen assimilation ability of *B. bituminosa,* which is separated from other forage crops by many characteristics. But, there is not yet enough information on this subject, especially about its genotypes. In this study, it was aimed to determine the changes in nitrogen assimilation among different plant parts and genotypes of *B. bituminosa* collected from different places (11 of them is being from Turkey and other from Spain).

Materials and Methods

Field experiment and design: In this study, nitrogen assimilation ability in different plant parts and genotypes of *Bituminaria bituminosa* (L.) C.H. Stirtion were investigated. The genotypes were collected from 11 different locations belong to Samsun, Kastamonu and Sinop provinces in the Middle Black Sea Region of Turkey. In addition to, there was also a genotype originated from Spain (Table 1).

The experiment was conducted on a controlled area in Samsun/Turkey ecological conditions. The content N of the soil in this area was kept very low (Average 0, 16%). First of all, seeds were cleaned, then they were dried at 30ºC (Walker *et al*., 2007) in laboratory conditions. Each one genotypes were firstly sown into seed trays and then they were transplanted to experimental area with 70 x 70 cm spaces in autumn during 2015-2016 (Fig. 1). After one vegetation period, plants were harvested at flowering stage (Fig. 2).

Table 1. Location (latitude and longitude) data of *Bituminaria bituminosa* (L.) C.H. Stirtion genotypes taken from Turkey and Spain.

Genotypes	Location	Latitude	Longitude
1	Kastamonu-İnebolu	41° 58' 32.8" N	33° 46' 10.4" E
2	Samsun-Çatalzeytin	41° 57' 48.4" N	34° 09' 07.8" E
3	Samsun-Kanlıçay	41° 40' 40.3" N	35° 22' 22.8" E
4	Samsun-Kozağzı	41° 28' 05.1" N	35° 49' 56.8"E
5	Samsun-Çarşamba	41° 04' 35.1" N	36° 40' 09.0" E
6	Samsun-Central	41° 18' 39.0" N	36° 20' 02.5" E
7	Samsun-Central	41° 19' 08.5" N	36° 19' 13.6" E
8	Samsun-Nebyan	41° 23' 35.9" N	35° 59' 06.2" E
9	Samsun-Central	41° 22' 16.0" N	36° 11' 46.7"E
10	Sinop-Tıngıroğlu	41° 47' 41" N	35° 00' 23" E
11	Samsun-Kavak	41° 03' 14.35" N	35° 56' 59.84" E
A1	Spain originated location		

Fig. 1. General view of controlled experimental area and close-up.

Fig. 2. Appearance of *Bituminaria bituminosa* at flowering stage.

Determination of nitrogen (N) content in plant and soil samples: Plant samples harvested at the flowering stage were divided into sections such as root and leaf for analysis. N content of all plant parts was analyzed and the changes in Nitrate Reductase (NR) activity were determined. At the same time, N analysis in the soil samples was done.

For determination of nitrogen (N) content in plant and soil samples, plant samples harvested at the flowering stage were dried at 65°C temperature until constant. Then, samples were grounded through hammer mill particle size of about 0.5 to 1 ml. Also, soil samples of the experiment field were taken from 30 cm depth for each a genotype. Then, these soil samples were dried, and they sieved through particle size of about 4 ml. N contents of plant and soil samples were determined using Kjeldahl apparatus (FOSS 984.13) and expressed as percent (%).

Determination of dry plant yield: Dry plant yield were determined in plant samples dried at 65 0C until they have a constant weight according to methods of Kumbasar *et al*., (2018).

Determination of nitrate reductase (NR) activity: The nitrate reductase (EC 1.7.99.4) activity was determined according to the in vivo test described by Hageman & Hucklesby (1971) and Jaworski (1971) and modified by Gebauer *et al*., (1984). This method is based on the spectrophotometric absorbance of the nitrite (NO_2^-) formed as the product of the reduction of nitrate in the incubation medium. NR Activity (NRA, µmol NO_2^- g^{-1} D.W. h^{-1}) was calculated using the absorption value and dry weight.

Statistical analysis: All experiments were performed using a completely randomized design and each treatment included three replicates. The obtained values for N contents in both soil and plant parts, values belong to dry matter yield and NR activities were analyzed by repeated measure analysis in SPSS version 16.0 and means were separated by Duncan's Multiple Range test. All the statistical tests were performed at a significance level of 0.05.

Results and Discussion

The soil N content during the sampling period of 12 different genotypes of *Bituminaria bituminosa* (Fig. 3). It also shows the percentage of soil nitrogen content belong to the beginning and sampling period of the area where *Bituminaria bituminosa* is located. According to this, there was found statistically significant differences ($p<0.05$) on nitrogen content of soils belong to genotypes.

While the average soil N content of the area was 0.160% at the beginning, there was 0.4257% at the sampling time. This suggests that all genotypes increase nitrogen content in the soil. This is an expected result for *B. bituminosa* being a species belong to the Leguminosae family. As a matter of fact, it was stated that this plant increased soil organic matter and N content in the study conducted by Açıkgöz (2001). But the difference between the genotypes of *B. bituminosa* has never been studied until now.

Between soil samples, the highest N content was statistically determined in soil of the genotype numbered by 10 (0.500%), while the lowest N content was detected in soil of the genotype numbered by 6 (0.326%). In point of nitrogen contribution to the soil, this is indicating that there is a difference between the genotypes of *B. bituminosa.* It is also understood that genotype numbered by 10 taken from Sinop city in Turkey has the highest contribution in terms of N binding capacity to soil according to others. Also, A1 genotype taken from Spain has been observed to increase the nitrogen content in the soil similarly.

The dry plant yield of *B. bituminosa* was given in Fig. 4. The dry plant yield was significantly ($p\leq 0.05$) different between genotypes and it ranged from 126.31 g/plant to 170.19 g/plant. The highest dry plant yield was determined in genotype number 3 was same statistical group with genotypes number of A1, 1, 2, 4, 5, 6, 8 and 9. According to previous studies, dry plant yield in *B. bituminosa* was reported as 6.9 – 205.0 g/plant (Gulumser, 2011; Gulumser and Acar, 2012; Kumbasar *et al*., 2018). This was consistent with obtained data in our study.

At the same time in our study, the N contents in root and leaves of plants were determined (Fig. 5). The results were statistically significant ($p<0.05$). Accordingly, the highest N content in roots was detected in the genotype numbered by 11.

The N content in the root of the genotype numbered by A1 (originated from Spain) is close to the value obtained in genotype numbered by 11. The lowest N content in roots was found at the genotype numbered by 7. The high N content in the leaf parts was observed in genotype numbered by 11 again. Also, the N content in the leaves of genotypes originated from Spain like in the root parts is very close to the results obtained in genotype numbered by 11. It can attribute that there were differences in growth rate of genotypes. Especially genotype numbered by 11 has higher metabolic machinery and a higher growth costs for the emergence of young leaves. Similar expressions can be said for genotype numbered by A1. Hernández-Montes *et al*., (2018) have expressed that high N content is associated with high respiration rates. Increasing in respiratory rate accelerates metabolic machinery of plants. These are important physiological results belong to genotypes of *B. bituminosa.* N for a plant is one of the major factors in determining growth rate. Nitrogen assimilation ability gives the plants an opportunity to survive under adverse conditions.

Plants generally take nitrogen in the form of NO_3^-. Once taken by plants, NO_3^- is rapidly metabolized and converted to protein. Nitrate accumulation have poison effects for especially feed crops. The NR enzyme is sensitive to the presence of nitrate because it uses nitrate as the substrate (Olsonn & Falkengren-Grerup, 2003; Arslan & Guleryuz, 2005). It inhibits NO_3^- accumulation in the plants. *B. bituminosa* is one of important feed crops and it is not desirable to have its nitrate accumulation. NR enzyme is sensitive to the presence of nitrate and does not allow the accumulation of nitrate in plants or plant parts. Thus, detection of NR enzyme activity is important to determine whether nitrate accumulation is present in *B. bituminosa* consumed as a feed crop. In this matter, researches on N content in feed crops in our country are limited (Sulak & Aydın, 2005).

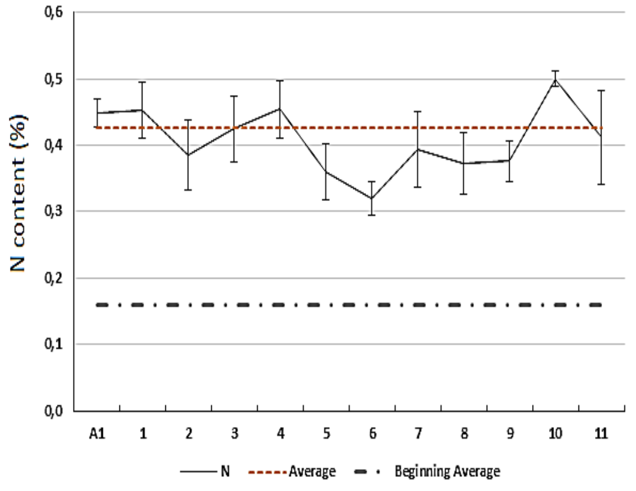

Fig. 3. Nitrate content (%) in the soil of experimental area

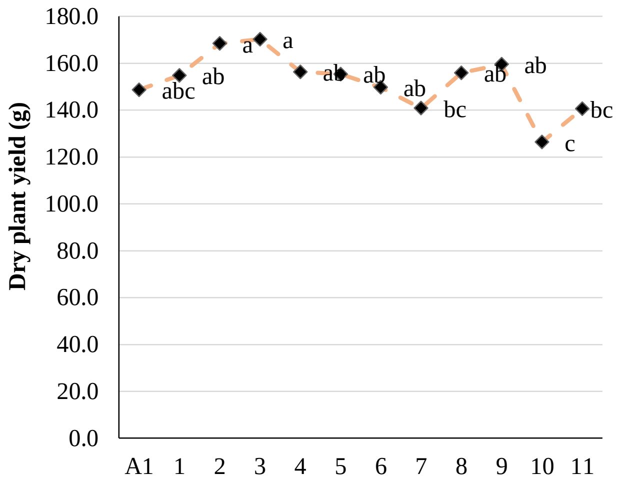

Fig. 4. Dry plant yield of *B. bituminosa* genotypes.

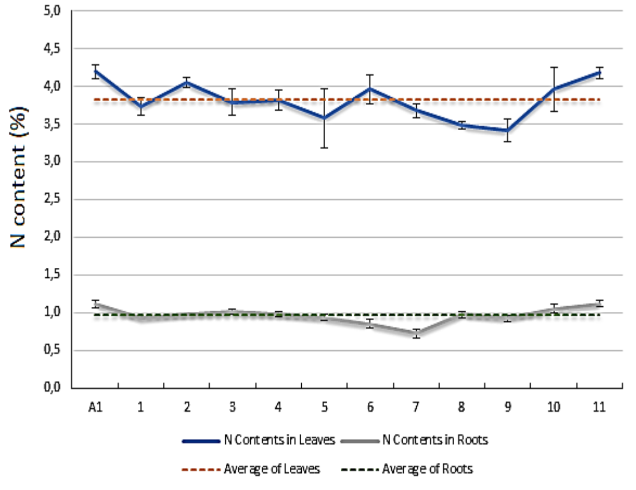

Fig. 5. N contents (%) determined in leaves and roots of 12 different genotype of *Bituminaria bituminosa.* Values are mean of three replicates ± SE and bars indicate standard error (SE). All values are significant at $p<0.05$ compare to control.

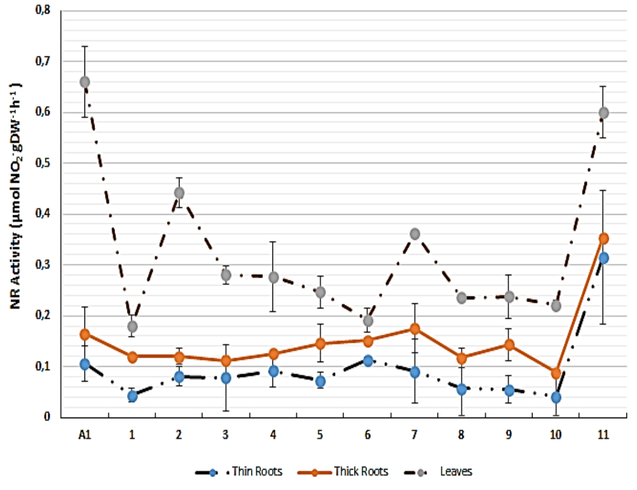

Fig. 6. Nitrate Reductase (NR) Activity in both of leaves and roots of 12 different genotype of *Bituminaria bituminosa*. Values are mean of three replicates ±SE and bars indicate standard error (SE). All values are significant at $p<0.05$ compare to control.

We have identified NR activity in root and leaves of the above-identified genotypes of *B. bituminosa* (Fig. 6). The NR activity was examined in two different parts as thin and thick root because of the different root morphology of *B. bituminosa*. The results obtained from the NR analysis show that the thin roots are more effective in nitrogen assimilation of *B. bituminosa*. The differences are found statistically significant ($p<0.05$).

While the highest NR activity in thin roots is detected in genotype numbered by 11, it is seen that the NR activity in the thick roots of the same genotype is very low. Similarly, it has been found to have a higher NR activity in the thin roots than thick roots in A1 genotype originated from Spain. In only genotypes numbered by 1 and 10, it was determined to have higher NR activity in thick roots.

The NR activity values determined in this study show that the nitrogen assimilation in the roots and leaves of *B. bituminosa* is different and the leaves of *B. bituminosa* are more effective in the nitrogen assimilation capacity (Fig. 6). The highest NR activity in the leaves was determined in genotype numbered by A1. Among genotypes taken from Turkey, genotypes numbered by 2 and 11 come into prominence ($p<0.05$). The presence of NR activity in the leaf indicates that the nitrate taken with the roots is carried to the leaves and contained to the nitrogen metabolism. Because nitrogen metabolism is directly related to plant growth, our results are compatible with highly resistant of *B. bituminosa* to intensive grazing pressure. This results also support that *B. bituminosa* is a high quality feed.

Conclusion

In this comprehensive study, differences in nitrogen assimilation among genotypes of *B. bituminosa* were comparatively investigated. Results have demonstrated that there are significant differences between genotypes of *B. bituminosa*. And the leaf parts of *B. bituminosa* are effective in nitrogen assimilation. It is observed that *B. bituminosa* is a resistant feeding plant against intensive grazing. These results reveal about the nitrogen assimilation ability of genotypes of *B. bituminosa*, which is separated with its many characteristics from other forage crops. It is recommended that genotypes numbered by A1 from Spain and 11 from Turkey should be cultivated as feed crops. Further studies are needed to determination of genotypic differences in gene level in order to contribute to the breeding studies for feed crops.

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