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Nodulation Study of Natural Forage Legume in Semiarid Region, Turkey

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Abstract: In this study, we investigated the natural nodulation of legume forage crops were widely grown in the natural pastures in Şanliurfa, Turkey. This legume forage crops are *Vicia sativa* L. subsp. *sativa* L., *Vicia narbonensis* L. var. *narbonensis* L., *Vicia palaestina* Boiss., *Vicia hybrida* L., *Vicia lutea* L. var. *lutea* Boiss. ET Ball., *Pisum sativum* L. subsp. *sativum* L. var. *sativum* L, *Cicer echinospermum* P.H. Davis, *Trifolium tomentosum* L., *Trifolium retusum* L., *Trifolium campestre* SCHREB., *Medicago truncatula* GAERTN. var. *truncatula* Schultz Bip., *Trigonella mesopotamica* Hub.-Mor., *Lens culinaris* Medik., *Onobrychis crista-galli, Lathyrus cassius* Boiss., *Melilotus officinalis* (L.) DESR., *Coronilla scorpioides* (L.) W.D.J. Koch. Nodulation, nodule colors and shapes were examined at the blooming period of forage legumes. In this study, the colour of the interior of nodules are pink-red colour and may be related to high rates of nitrogen fixation in legume crops.

Key words: Nodule, legume forage crops, pasture, semi arid region

INTRODUCTION

The pastures are an important part of farming systems (Albayrak and Sevimay, 2005). Forage legumes are commonly grown to provide a seed and hay crop in many different farming systems in Turkey (Avcýoðlu *et al*., 2009). Forage legumes have a symbiosis life with specific bacteria called *Rhizobium* (Hansen, 1994).

Rhizobium bacteria are genetically diverse and physiologically heterogeneous group of symbiotic nitrogen fixing bacteria that the bacteria which fix atmospheric nitrogen within nodules in the roots (Alexander, 1984). Root nodulating isolate of *Rhizobium* bacteria provide a significant amount of nitrogen. For instance; in pastures, about 80% of the total nitrogen with legume plants is provided (Hansen, 1994).

Şanliurfa's natural pastures are approximately 234.537 ha. *Vicia*, *Trifolium* species are the primary legumes used in pasture in Şanliurfa, Turkey (Sayar *et al*., 2010). Environmental conditions negative effects root nodulation and nitrogen fixation of legumes. Drought and low temperature is detrimental to nitrogen fixation (Hungria and Vargas, 2000). Present research aimed to study the shape of nodule, color of nodule and morphology of nodules of forage crops were investigated in semiarid region in Turkey.

MATERIALS AND METHODS

Region of study: Şanliurfa which states in Turkey's Southeast Anatolia region lies between 37°49'12"-40°10'00" east of the meridian 36°41 '28"-37°57'50" north latitude. The altitude of Şanliurfa is about 500 m and surrounded by places 600-800 m in elevation. The city Şanliurfa (Fig. 1) is situated in semi-arid region of Mediterranean climate. In this region, the days are hot and arid in summer, mild and rainy in winter.

The climate of the region was evaluated according to the records of Şanliurfa Meteorology Station (Table 1). As shown according to the records, the climate is dry for a long period from June to October (Anonymous, 2001).
Legume forage crops from Şanliurfa's natural pastures were collected Atatürk forest, Kýrbadi, Ýkizce, Bozova, Birecik, Kulaflý, Osmanbey Campus, Osmanbey village, AKB status, Hilvan, Airport vicinity, Karaköprü (Table 2). The fodders were collected and dried. Plant identifications were made according to Davis (1988), Donner (1990).

Table 1: Climate and rainfall of the research field

Elevation (m)	P (mm)	M (°C)	m (°C)	Q	PE	S	Biyoclima type
547	457.8	46.8	-6.8	42.94	7.2	0.18	Semi-arid, cold winter

P: The average annual rainfall (mm), M: The average maximum temperature of the warmest month (°C), m: The average minimum temperature of the coldest month (°C), PE: Summer rainfall (mm), S: Drought index S: PE/M, Q: Rainfall-temperature precedent Q: 2000xP/(M+m+546.6) (M-m)

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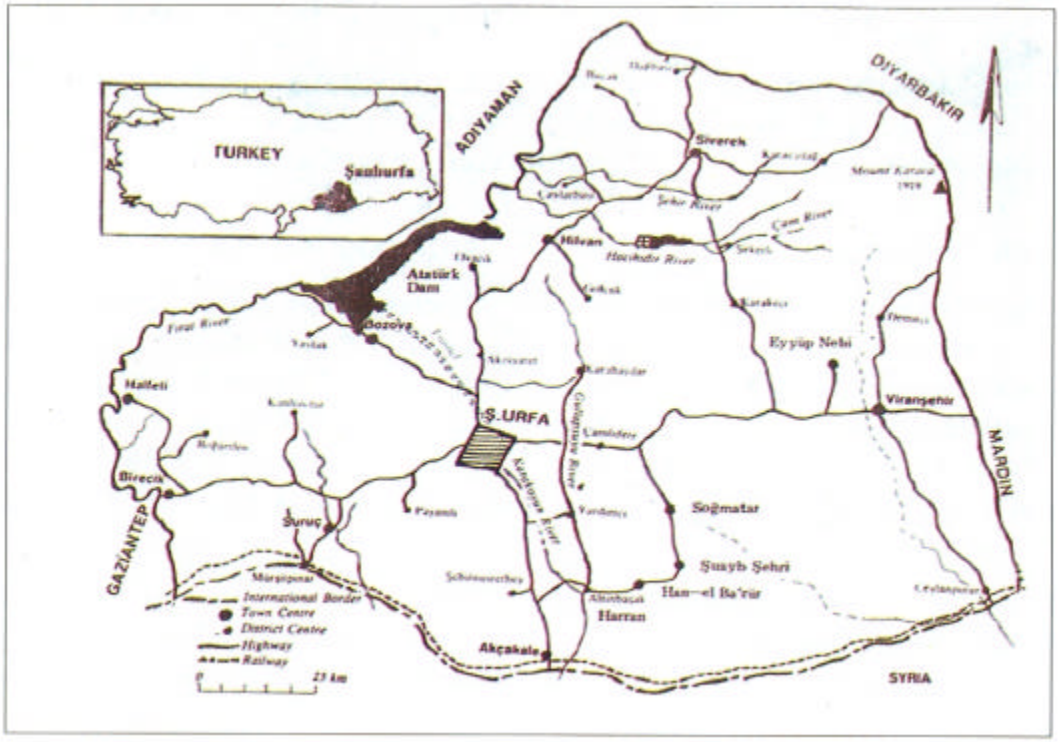

Fig. 1: Location map of study area

Table 2: Locations of forage legumes and some soil properties

		Soil properties					
Legume forage crops	Collected region	EC	pH	$CaCO_3$ (%)	P_2O_5 (kg da^{-1})	K_2O (kg da^{-1})	O.M (%)
Vicia sativa L. subsp. *sativa* L.	Airport vicinity	1.53	7.52	41.31	13.80	276.50	3.6
Vicia narbonensis L. var. *narbonensis* L.	Osmanbey village	1.24	7.78	35.7	7.70	88.00	2.9
Vicia palaestina Boiss.	Bozova	1.18	7.67	21.60	36.30	209.50	2.4
Vicia hybrida L.	Hilvan	1.39	7.77	26.20	11.45	94.04	3.1
Vicia lutea L. var. *lutea* Boiss. ET Ball.	AKB status	1.40	7.76	4.90	8.80	133.90	4.7
Pisum sativum L. subsp. *sativum* L. var. *sativum* L.	AKB status	1.51	7.67	8.90	7.20	152.50	4.3
Cicer echinospermum P.H. Davis	AKB status	1.47	7.70	1.50	9.36	110.10	3.8
Trifolium tomeutosum L.	Karaköprü	1.41	7.65	6.00	8.20	151.20	4.7
Trifolium retusum L.	Osmanbey campus	1.38	7.51	17.60	12.03	27.40	3.6
Trifolium campestre Schreb.	Karaköprü	1.41	7.65	6.00	8.20	151.20	4.7
Medicago truncatula Gaertn. var. *truncatula* Schultz Bip.	Osmanbey campus	1.39	7.40	2.60	11.80	164.10	1.6
Trigonella mesopotamica Hub.-Mor.	Kulaflý	1.04	7.53	31.50	4.50	17.20	2.3
Lens culinaris Medik.	Birecik	1.60	7.53	18.10	28.32	124.20	2.7
Onobrychis crista-galli	Bozova	1.59	7.44	28.50	14.70	110.10	2.5
Lathyrns cassius Boiss.	Ýkizce	1.62	7.58	22.40	26.20	182.60	1.3
Melilotns officinalis (L.) Desr.	Kýrbadi	1.46	7.69	3.40	16.10	156.60	3.9
Coronilla scorpioides (L.) W.D.J. Koch	Atatürk forest	1.53	7.72	6.90	18.69	12.30	3.9

OM: Organic matter

These plants are kept in Herbarium of Biology Department, Harran University. Leguminous fodder crops are identified in Table 2.

Nodulation in plants, shape, size, color were investigated. Soil samples of plant samples were taken and some soil physical and chemical properties (pH, EC, Lime, P_2O_5, K_2O, OM) of soil samples were made. Some characteristics of the soil samples used in this study were determined in samples taken from 0-15 cm depth. After soil samples were air dried and passed through a sieve with 2 mm size, some soil properties were determined as follows; soil reaction pH 1:1 (w/v) soil water suspension

by pH meter (Rowell, 1996). Electrical conductivity (EC) in the soil samples suspension by EC meter (Rowell, 1996). $CaCO_3$ contents were determined by volumetric method (Martin and Reeve, 1955). Organic matter contents were determined by the wet oxidation method with $K_2Cr_2O_7$ (Walkley and Black, 1934). P content was determined according to Olsen *et al.* (1954). Some soil chemical properties are given in Table 2.

RESULTS AND DISCUSSION

In the studied soils, pH changes between 7.18-7.79, amounts of organic matter (%) are between 1.3-4.7%. The Electrical Conductivity (EC) is between 1.18-1.62 (Table 2).

In Şanliurfa and the surrounding natural pasture, legume forage were collected. In this study, *Vicia sativa* L. subsp. *sativa* L., *Vicia narbonensis* L. *narbonensis* L., *Vicia palaestina* Boiss., *Vicia hybrida* L., *Vicia lutea* L. *lutea* Boiss. Meat Ball., *Pisum sativum* L. subsp. *Sativum* L. *Sativum* L., *Cicer echinospermum* P.H. Davis, *Trifolium tomentosum* L., *Trifolium retusum* L., *Trifolium campestre* Schreb., *Medicago truncatula* Gaertn. Schultz Bip *truncatula*, *Trigonella mesopotamica* Hub.-Mor., *Lens culinaris* Medik., *Onobrychis crista-galli*, *Lathyrus cassius* Boiss., *Melilotus officinalis* (L.), *Coronilla scorpioides* (L.) wdj Koch have grown extensively determined (Table 2, 3). Legume forage crops distribution was studied in relation to the level of disturbance. During flowering of plants were taken root samples and the nodule shape, nodule color, formation of nodulation were obtained (Table 4). The colors of nodule exterior varied from white to white-brown (Table 4). Ott *et al.* (2005) reported that the color of nodule exterior isn't related to nitrogen fixation. The nodule interior color is related to leghemoglobin (Ott *et al.*, 2005). In our results, the pink-red color in the nodules related to the presence of leghemoglobin. Its presence in the nodule seems to be essential to the nitrogenase activity (Ott *et al.*, 2009). The efficiency of the assimilation of the fixed nitrogen by the plant could play an important role productivity and effective isolate. In the simplest, activity of nodule isolates is determined by the number of nodules formed by plants (Freiberg *et al.*, 1997; Hungria and Stacey, 1997; Krishnan and Bennett, 2006). Effective of *Rhizobium* strains specific in the various legume crops were formed dark red nodules (Atallah *et al.*, 2008; Rejili *et al.*, 2009). Fourteen species were presented with cylindrical nodules determined. The remaining species had multi-branched structure, long-thin structure and long elipse nodules (Table 4). The nodules shape depends upon the plant host (Zahran, 1998).

The number of nodule were differentiated according to plant species. This difference may be associated with the soil adaptation of *Rhizobium* bacteria and the plant species. Lowest nodulation examined the roots of *Vicia narbonensis* L. var. *Narbonensis* L. Plant species are differentiated according to the number of nodule. This difference may be associated with the adaptation of bacteria and the plant species.

Zahran (1998, 1999) determined that *Trifolium resupinatum*, *Melilotus indica*, *Medicago intertexta*, *Trigonella hamosa*, *Alhagi murarum* grows naturally in Egypt, and the number of nodules and nodule weight of the studied plants were obtained. Researcher has determined nodule number and shape, former vary according to plant species. In our study, we determined different shapes and sizes in forage legume nodule Similar results were obtained with Zahran (1998)'s findings.

Zahran (1999) reported that a successful symbiotic relationship with leguminous crops-*Rhizobium* bacteria are sensitive high soil temperature in semiarid and arid regions. For most *Rhizobium* bacteria, the optimum temperature range for growth in culture is 28-31°C

Table 3: Legume forage characteristics

Genus	Species	Plant property
Vicia L.	*Vicia sativa* L. subsp. *sativa* L.	Annual
Vicia L.	*Vicia narbonensis* L. var. *narbonensis* L.	Annual
Vicia L.	*Vicia palæstina* Boiss.	Annual
Vicia L.	*Vicia hybrida* L.	Annual
Vicia L.	*Vicia lutea* L. var. *lutea* Boiss. ET Ball.	Annual
Pisum L.	*Pisum sativum* L. subsp. *sativum* L. var. *sativum* L.	Annual
Cicer L.	*Cicer echinospermum* P.H. Davis	Annual
Trifolium L.	*Trifolium tomeutosum* L.	Annual
Trifolium L.	*Trifolium retusum* L.	Annual
Trifolium L.	*Trifolium campestre* Schreb.	Annual
Medicago L.	*Medicago truncatula* Gaertn. var. *truncatula* Schultz Bip.	Multi year
Trigonella L.	*Trigonella mesopotamica* Hub.-Mor.	Annual
Lens Miller	*Lens culinaris* Medik.	Annual
Onobrychis Adans.	*Onobrychis crista-galli*	Annual
Lathyrns L.	*Lathyrns cassius* Boiss.	Annual
Melilotns L.	*Melilotns officinalis* (L.) Desr.	Annual
Coronilla	*Coronilla scorpioides* (L.) W.D.J. Koch	Annual

Table 4: Some morphological features of leguminous fodder crops nodules

Legume forage	Nodule shape	Nodule exterior color	Nodule interior color	Nodule size
Vicia sativa L. subsp. *sativa* L.	Elongated-cylindrical	White	Pink-red	Large
Vicia narbonensis L. var. *narbonensis* L.	Elongated-cylindrical	White-brown	Pink-red	Small
Vicia palaestina Boiss.	Elongated-cylindrical	White	Pink-red	Small
Vicia hybrida L.	Cylindrical	White	Pink-red	Small
Vicia lutea L. var. *lutea* Boiss. ET Ball.	Cylindrical	White	Pink-red	Small
Pisum sativum L. subsp. *sativum* L. var. *sativum* L.	Spherical	White	Pink-red	Large
Cicer sp.	Cylindrical	White-brown	Pink-red	Large
Trifolium tomentosum L.	Cylindrical	White-brown	Pink-red	Large
Trifolium retusum L.	Cylindrical	White	Pink-red	Small
Trifolium campestre Schreb.	Cylindrical	White	Pink-red	Küçük
Medicago truncatula Gaertn. var. *truncatula* Schultz Bip.	Cylindrical	White	Pink-red	Small
Trigonella mesopotamica Hub.-Mor.	Multi-branched structure	White-brown	Pink-red	Large
Trigonella sp.	Multi-branched Structure	White	Pink-red	Small
Lens culinaris Medik.	Cylindrical	White-brown	Pink-red	Small
Onobrychis crista-galli	Long, thin structure	White-brown	Pink-red	Large
Lathyrns cassius Boiss.	Long ellipse	White	Pink-red	Large
Melilotns officinalis (L.) Desr.	Cylindrical	White	Pink-red	Large
Coronilla scorpioides (L.) W.D.J. Koch	Cylindrical	White	Pink-red	Small

(Hungria and Vargas, 2000). Present study must be more depended to improve the output of nitrogen fixation in the semi-arid region.

In our study, the number of rhizobial nodulation in leguminous forage crops detected to be different, such as result from differences in isolates that infect plant species and microorganisms in the soil density may be the competitiveness between. *V .narbonensis* in the territory of the region *V .narbonesis* lack of nodule formation in the lack of nodule isolates thought to be forming. The climate data of the study area, especially soil temperature is also believed to affect some of the negative development of rhizobial isolates. Grover *et al*. (2011) and Zahran *et al*. (1999) have been reported that especially in free-living and symbiotic bacteria living in conditions of precipitation is less than the majority negatively affected by high soil temperature. The optimum temperature development of *Rhizobium* bacteria is between 28-31°C in broth culture (Graham, 1992). Hungria and Stacey (1997) reported that cellular polysaccharides secreted by *Rhizobium* bacteria play an important role in the nodulation formation.

The most important function of leghemoglobin in the cells of the nodule is that the diffusion of oxygen (Appleby, 1984; Gordon *et al*., 1999; Ott *et al*., 2009). Present study also the root nodules of the plant species identify to be red colors, indicate the presence in Şanliurfa pasture soil of natural and effective *Rhizobium* bacteria. Atallah *et al*. (2008) was studied the formation of nodulation wild grown *Astragalus*, *Bituminara*, *Calicotome*, *Cicer*, *Hippocrepis*, *Hymenocarpos*, *Lathyrus*, *Lotus*, *Lupinus*, *Medicago*, *Melilotus*, *Onobrychis*, *Ononis*, *Physanthyllis*, *Scorpiurus*, *Securigera*, *Spartium*, *Trifolium* and *Vicia* species. Nodulation of *Medicago coronata* was reported for the first time in France (Atallah *et al*., 2008). Host specificity and certain symbiotic genes of nodule formation was examined (Hungria and Stacey, 1997).

CONCLUSIONS

Nitrogen fixing ability of legume forage crops lies environmental conditions such as temperature. In this study, we investigated natural nodulation of forage crops in semiarid region, Şanliurfa, Turkey. Pastures in Şanliurfa (semi-arid region of Turkey), *Rhizobium* bacteria show a host specificity for the species of forage crops that they infect. Therefore, isolation of bacteria from nodules and the amount of fixed nitrogen has not determined. Thus, selected *Rhizobium* bacteria, increasing the rate protein of legume forage crops would be expected to. The bacteria will be isolated from fodder crops, effectively isolate and/or isolates will be selected and effectiveness rhizobial isolates were identified by comparison of greenhouse and field trials.

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