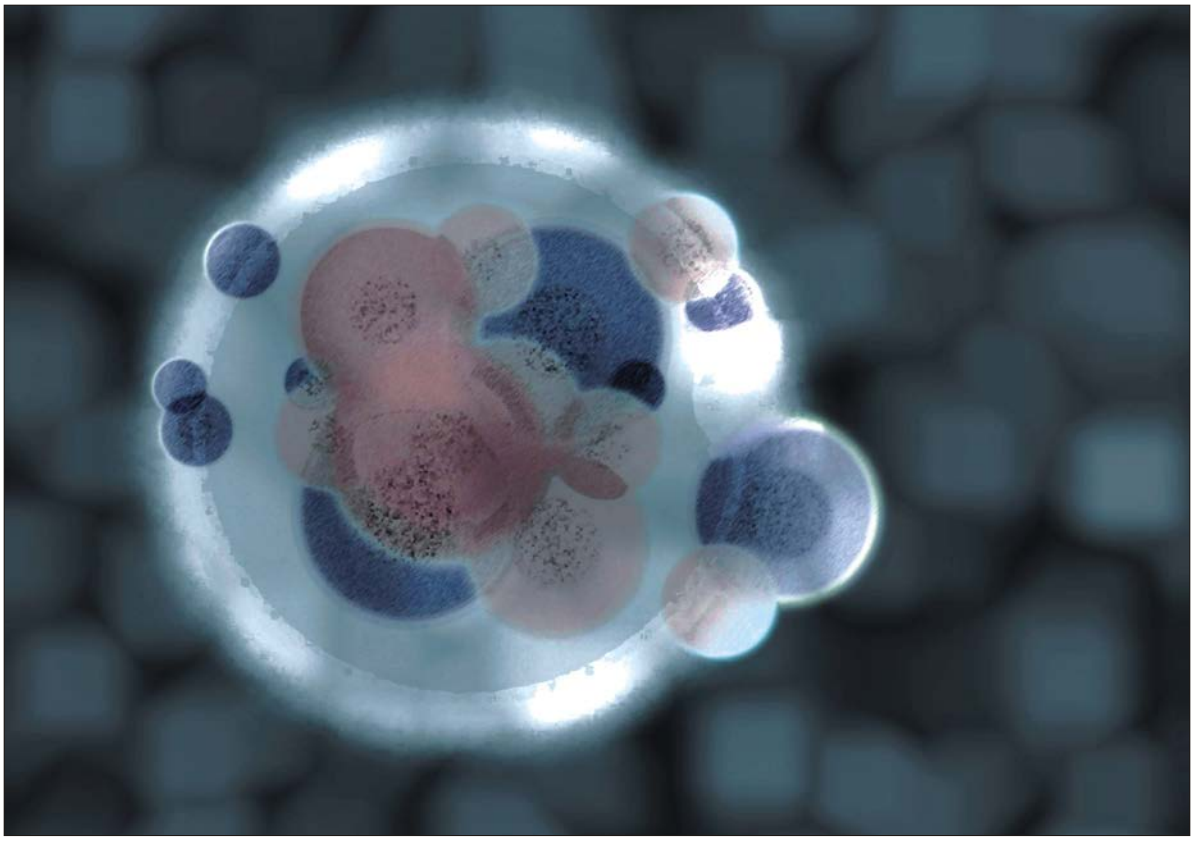

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Comparison of Leaf and Stomatal Characteristics in Faba Bean (*Vicia faba* L.)

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Abstract: In this study, faba bean genotypes which are sown in 2003-2004 winter season were compared for the number of stomata per mm^2, stomata width and length on both surface of leaflet, the number of leaflet per leaf, leaflet width and length, and leaf area. Ten genotypes from Black Sea Region of Turkey, two lines from ICARDA, two registered Turkish cultivars and one commercial faba bean cultivar were examined. The stomata numbers per mm^2 on the lower and upper epidermis of the leaflets ranged from 259.52-305.88 and 236.66-288.26, respectively. Faba bean genotypes differed only for the number of stomata per mm^2 on upper epidermis of the leaflet ($p<0.05$) and leaflet number $leaf^{-1}$ ($p<0.01$). The number of stomata per mm^2, stoma width and length on the lower epidermis of the leaflet were greatly higher than that on the upper epidermis ($p<0.01$). There was a positive and significant correlation between the number of stomata on lower and upper epidermis of the leaflets. Increases in the number of stomata on the lower epidermis reduced stomata length on both lower and upper epidermis. The number of stomata on both surface of the leaflets positively correlated with the number of leaflet $leaf^{-1}$. Correlations between the leaf area and all investigated characteristics were found to be non significant.

Key words: Faba bean, *Vicia faba* L., stomata, leaf characteristics, genotypes

INTRODUCTION

Cultivated faba bean (*Vicia faba* L.) is used as human food in developing countries, generally in Asia and Africa and as animal feed, mainly for pigs, horses, poultry and pigeons in industrialized countries. It can be used as a vegetable, green or dried, fresh or canned. It is a common breakfast food in the Middle East, Mediterranean region, China and Ethiopia (Bond *et al*., 1985). Nowadays, although faba bean is the second widely grown dry grain legume in European Union (FAO, 2003), little is known about the growth and development of this crop.

Stomatal function is important on the physiology, adaptation and productivity of plants. Adaptation ability of the plants is closely associated with transpiration and photosynthesis process occurred in their leaves. The number and distribution of the stomata in unit leaf area have an important role in these processes by adjusting CO_2, O_2 and moisture exchange between the leaves and the atmosphere (Brownlee, 2001).

Leaves of some species have stomata on both lower (abaxial) and upper (adaxial) epidermis. These pores are more numerous over the lower epidermis than the upper epidermis in most leaves (Anonymous, 2005). They occur on both sides of the broad bean leaf. Stomatal structure varies greatly in concert with species, habitat and leaf architecture. The number of the stomata in per unit area may be varies among plant species, cultivars and clones (Yanmaz and Eriş, 1984; Abak and Yanmaz, 1985; Yanmaz and Abak, 1985; Gülcan and Mısırlı, 1990; Rana and Chadha, 1990; Şahin and Soylu, 1991; Mısırlı and Aksoy, 1994; Mısırlı *et al*., 1998; Çağlar and Tekin, 1999) and it can be related to the plant ecotype (between 300 and 800 stomata per mm^2 (Kimball *et al*., 1986; Woodward, 1987 and 1993; Rowland-Bamford *et al*., 1990). There are large heritable differences between species in stomatal dimensions, distribution and morphology. Leaf characteristics and structure of stomata could be used for micro taxonomic classification of *Vitis* species (Racz, 1973; Swanepoel and Villiers, 1987).

The objectives of the present study were to determine leaf and stomatal characteristics of faba bean (*Vicia faba* L.) genotypes, to determine whether there are differences for these characteristics, and to investigate the relationships among them.

MATERIALS AND METHODS

The study was carried out in the experimental area of Faculty of Agriculture, Ondokuz Mayis University of Samsun, Turkey, during the 2003-2004 growth season. The experimental area located at the north of the Black Sea Region of Turkey (41.3°N longitude, 36.3°E altitude, 120 m above sea level). Ten faba bean genotypes from

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Table 1: Faba bean genotypes and their collection sites

Genotypes	Collection sites
Vezirköprü 1	Vezirköprü/Samsun
Vezirköprü 2	Vezirköprü/Samsun
Sinop	Sinop
Yenice	Yenice/Amasya
Merzifon	Merzifon/Amasya
Avren	Avren-Merzifon/Amasya
Gemenez	Gemenez-Merzifon/Amasya
Seyhyeni	Seyhyeni-Merzifon/Amasya
Lara	May-Agro Seed Company/Bursa
FLIP 86-116FB	ICARDA
FLIP 85-172FB	ICARDA
Eresen-87*	Aegean Agricultural Research Institute, Menemen/İzmir
Filiz-99*	Aegean Agricultural Research Institute, Menemen/İzmir
Gümüşhacıköy	Gümüşhacıköy/Amasya
Turhal	Turhal/Tokat

*Registered by Aegean Agricultural Research Institute, Menemen/İzmir

diverse location in Black Sea Region of Turkey, two lines from ICARDA, two registered Turkish cultivars and one commercial cultivar from May-Agro Seed Company were used in the study (Table 1).

Field experiment was arranged in Randomized Complete Block Design with three replications. Leaves samples were taken from the sixth/seventh nodes of the main stem at the full flowering stage between the hours of 9:00 and 10:00 am. The number of stomata was evaluated by preparing slides from the clear nail polish impression on both lower and upper epidermis of the leaflets. Stomata were counted and measured using a 10×20 magnification. In each replication, counts were done in randomly selected five plants in four different fields from either side of the mid-rib of the leaflets. Stoma length and width was measured in three stomata in four different fields for five plants (as a mean of 3×4×5 = 60 for each genotype in each replication) with ocular micrometer. In addition, leaflet length and width (cm) and the number of leaflet per leaf were also determined. Leaf width was measured from tip to tip at the widest part of the lamina and leaf length was measured from lamina tip to the point of petiole intersection along the lamina midrib. Finally, leaflet samples were scanned by a flatbad scanner. Delta-T SCAN (Ver. 2.04) image analyzer program was used to determine leaflet area (Anonymous, 1996).

Data were subjected to analysis of variance and correlation by using TARIST program. Means showing statistically differences for stomata and leaf characteristics were compared according to Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Means for some stomatal characteristics of faba bean genotypes are shown in Table 2. The number of stomata on the lower epidermis of leaflets ranged from 259.52 to 305.88 per mm^2, and on the upper epidermis of leaflets ranged from 236.66 to 288.26 per mm^2. The number of stomata per mm^2, stoma width and length on the lower epidermis of the leaflet were greatly higher than that on the upper epidermis ($p<0.01$) (Table 2).

Our results for the number of stomata were in agreement with Artık (2005), who stated that the number of stomata on the lower surface of leaflet varied between 228.60 and 304.37 per mm^2, and on the upper surface of leaflet varied between 218.80 and 290.65 per mm^2 in faba bean cultivars/line for the different gamma irradiation doses.

The number of stomata per unit leaf area is special to cultivars (Marasalı and Aktekin, 2003). In numerous studies, it was revealed that there were great variation in stomatal density among plant species, cultivars, clones and also plant types (Yanmaz and Eriş, 1984; Abak and Yanmaz, 1985; Düzenli and Ergenoğlu, 1991; Mısırlı and Aksoy, 1994; Sabo *et al*., 2001). Although some

Table 2: Means of faba bean genotypes for some stomatal characteristics

	No. of stomata per mm^2		Stoma width (μm)		Stoma lenght (μm)	
Genotypes	Lower epidermis	Upper epidermis	Lower epidermis	Upper epidermis	Lower epidermis	Upper epidermis
1	284.77	244.93bc*	30.38	29.36	49.85	48.49
2	279.98	269.10a-c	30.19	29.5	49.32	47.67
3	293.27	241.88bc	32.16	30.38	49.83	47.75
4	283.99	273.45a-c	31.86	29.98	50.15	48.47
5	294.35	267.35abc	32.15	29.95	49.67	48.39
6	305.88	288.26a	31.22	31.33	50.49	50.06
7	291.31	278.02ab	30.82	30.15	49.84	49.01
8	295.22	265.40a-c	31.73	30.37	50.00	47.80
9	259.52	236.66c	31.82	30.74	50.72	49.53
10	305.02	249.50bc	30.51	29.28	49.47	49.47
11	283.47	273.89a-c	32.23	30.33	50.43	49.10
12	272.15	245.37bc	31.56	30.54	50.10	48.72
13	266.70	248.63bc	30.23	30.24	51.37	49.25
14	295.22	237.31c	30.72	28.77	49.88	47.88
15	277.59	257.99a-c	31.35	30.58	49.54	48.63
Mean	285.90a**	258.52b	31.26a**	30.10b	50.04a**	48.68b

*: Significant at 0.05 level. **: Significant at 0.01 level

Table 3: Means of faba bean genotypes for some leaflet and leaf characteristics

Genotypes	Leaflet width (cm)	Leaflet length (cm)	Leaflet number per leaf	Leaf area (cm^2)
1	3.85	7.67	6.02ab**	78.76
2	3.80	7.85	5.60a-c	81.99
3	3.45	7.06	5.50a-c	69.06
4	3.65	6.19	6.07a	83.89
5	3.79	7.68	6.28a	81.43
6	3.58	7.30	6.46a	95.48
7	4.17	8.07	6.10a	80.35
8	3.78	7.35	6.21a	75.19
9	3.57	7.55	4.67bc	69.95
10	3.41	6.70	5.50a-c	86.80
11	3.46	6.82	6.65a	61.87
12	3.67	7.34	4.25c	69.89
13	3.30	6.69	5.75ab	64.45
14	3.55	7.32	5.65a-c	70.50
15	3.32	6.70	5.93ab	85.24

**: Significant at 0.01 level

researchers informed that the number of stomata shows low variability, some others reported that it is affected by ecological condition and physiological process (Düzenli and Ergenoğlu, 1991). Compared with optimum soil moisture both water logging and water stress generally decreased the number of stomata on both leaf surfaces and decreased stomatal opening (Youmis *et al*., 1993). Environmental factors also influence stomata size and density (Çağlar *et al*., 2004).

No significant differences were found among genotypes in terms of stoma width and length on both lower and upper epidermis of the leaflets, and the number of stomata on lower epidermis for the present study. Genotypes differed only for the number of stomata per mm^2 on upper epidermis of the leaflet ($p<0.05$). Considerable genotypic variation was found for stomatal frequency and dimensions in faba bean by Ricciardi (1989). Means for the present study in terms of stoma length were similar to Ricardi (1989), who stated that stomata length varied markedly, ranging from 47.4 μm in Vesuvio (minor) to 52.2 μm in Violetta di Policoro (major). The effects of seasonal changes in water availability on leaf morphology (area, stomatal density, number, length and width of stomata and trichome density and number) showed no difference among faba bean varieties (Ricciardi and Steduto, 1988). Plant density did not influence stomatal characteristics such as length, width, number and frequency but these characters were affected by faba bean genotypes, year, leaflet position and leaflet surface (upper or lower) (Ricciardi *et al*., 1986).

In the present study, leaflet width and length, and leaf area were not affected by the genotypes. Means ranged from 3.30 to 4.17 cm for leaflet width, 6.19-8.07 cm for leaflet length and 61.87-95.48 cm^2 for leaf area. The number of leaflet $leaf^{-1}$ was found to be lower in Genotype 9 and 12 than the others ($p<00.1$) (Table 3).

There was a positive and significant correlation between the number of stomata on lower epidermis and on upper epidermis of the leaflets. Length of stomata on both lower and upper epidermis reduced with increase in the number of stomata on the lower epidermis of the leaflets for the present study. Positive and significant relationships were found between stoma length and stoma width for each surface of the leaflet and vice versa. Increases in leaflet length resulted in increases in leaflet width. The number of stomata on both lower and upper epidermis of the leaflets positively correlated with the number of leaflet $leaf^{-1}$. No significant correlations were found between the leaf area and all investigated characteristics (Table 4).

Jones (1977) found a negative correlation between the number of stomata and leaf width. Significant interactions were found between leaf positions and cultivars for stomata number, stomata length and leaflet area. It is concluded that stomatal frequency can be altered by breeding to improve water use (Tanzarella *et al*., 1984). Kara and Özeker (1999) found non significant relationships between the number of stomata and leaf width, fresh and dry leaf weight in grape.

Results of some preceding studies have revealed that the number of stomata per unit area is important in drought resistance (Henzell *et al*., 1976; Potts and Herrington, 1982; Şahin and Soylu, 1991; Düzenli and Ergenoğlu, 1991), cold resistance (Knecht and Orton, 1970), net photosynthesis production (Bierhuizen *et al*., 1984) and vegetative growth (Rana and Chadha, 1990; Çağlar and Tekin, 1999). A positive relationship was

Table 4: Correlations among leaf and stomatal characteristics in faba bean genotypes

	LSW	LSL	USN	USW	USL	LW	LL	LN	LA
LSN	-0.078	-0.476**	0.324*	-0.148	-0.367*	-0.173	-0.243	0.295*	0.154
LSW	-	0.402**	0.244	0.689**	0.308*	0.131	0.139	0.072	0.106
LSL		-	0.011	0.496**	0.591**	0.066	0.093	0.097	0.004
USN			-	0.304*	-0.049	0.013	-0.069	0.456**	0.213
USW				-	0.589**	0.145	0.049	0.015	0.254
USL					-	0.265	0.240	-0.042	0.153
LW						-	0.760**	0.047	0.174
LL							-	-0.033	0.087
LN								-	0.174

*: Significant at 0.05 level. **: Significant at 0.01 level

LSN: stoma number on lower epidermis; LSW: stoma width on lower epidermis; LSL: stoma length on lower epidermis; USN: stoma number on upper epidermis; USW: stoma width on upper epidermis; USL: stoma length on upper epidermis; LW: leaflet width; LL: leaflet length; LN: leaflet number; LA: leaf area

found between high stomata density and vegetative growth under controlled conditions in lettuce (Eenink *et al*., 1984) and eggplants (Daunay *et al*., 1986). Cultivars more resistant to cold had less stoma when compared with the cold susceptible ones (Mısırlı and Aksoy, 1994).

Study results showed that faba bean genotypes differed only for the number of stomata per mm^2 on upper epidermis of the leaflet and leaflet number $leaf^{-1}$. Relationships among leaf and stomatal characteristics, seed yield, vegetative growth, cold and drought tolerance of genotypes should be investigated in more detail.

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