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Stomatal size and frequency in wild (*A. webbii*) and cultivated (*A. communis*) almonds

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SUMMARY – Stomatal size and frequency are features commonly related to plant water stress tolerance. In general, size and number are negatively correlated and may vary greatly among species and genotypes. The number of stomata per leaf area unit is considered a peculiar characteristic of species and plant varieties. In order to provide information concerning this topic, a study was carried out on the stomata size and frequency of 15 cultivated almonds (*A. communis*) and 5 Apulian wild almonds (*A. webbii*). The varieties of *A. communis* were chosen taking into consideration their country of origin (extra Mediterranean/Mediterranean/Apulian), shell hardness (paper/hard) and kernel taste (sweet/bitter). The only evident difference between the cultivated and wild almonds concerned the leaf area; stomata frequency and size were independent of other characteristics, such as origin, country of origin, shell hardness and kernel taste of the twenty varieties/seedlings investigated.

Key words: Wild almond, cultivated almond, stomata.

RESUME – *"Taille et fréquence stomatales chez des amandiers sauvages (*A. webbii*) et cultivés (*A. communis*)". La taille et la fréquence stomatales sont des caractères généralement rattachés à la tolérance de la plante aux conditions hydriques adverses. En général, la taille et le nombre sont négativement corrélés et peuvent varier fortement entre espèces et génotypes. Le nombre de stomates par unité de surface foliaire est considéré comme une caractéristique particulière des espèces et des variétés végétales. Afin d'apporter de l'information sur cette question, une étude a été entreprise sur la taille et la fréquence stomatales de 15 amandiers cultivés (*A. communis*) et de 5 amandiers sauvages des Pouilles (*A. webbii*). Les variétés de* A. communis *ont été choisies en tenant compte de leur pays d'origine (hors Méditerranée/Méditerranée/Pouilles), de la dureté de leur coque (extrafine/dure) et du goût de leur amandon (doux/amer). La seule différence évidente entre les amandiers cultivés et les amandiers sauvages concernait la surface foliaire ; la fréquence et la taille stomatales s'avérant indépendantes d'autres caractéristiques, telles que l'origine, le pays d'origine, la dureté de la coque et le goût de l'amandon pour les vingt variétés/porte-graines étudiés.*

Mots-clés : *Amandier sauvage, amandier cultivé, stomates.*

Introduction

The mechanisms developed by plants as a response to water stress may be found in adaptations by roots, stems, leaves and fruits; the leaf modifications include the size and number of stomata (Kozlowski, 1976). Stomata are the morphological structures which control photosynthesis and transpiration. In general, there is an inverse relationship between the stomata number and size, characteristics which vary greatly among species and genotypes (Miller, 1938). Moreover, the stomata frequency and size can be significant both in plant genetics and ecology (Fregoni and Roversi, 1968).

With reference to the stomata characteristics of the cultivated almond [*Amygdalus communis* L.= *Prunus amygdalus* Batsch = *Prunus dulcis* (Mill.) D.A. Webb], considered a drought-tolerant fruit crop (Grasselly and Crossa-Raynaud, 1980) and of the wild almond (*Amygdalus webbii* Spach.), only scarce information is available. In studies performed in Apulia on young trees grown individually in containers under stress and non-stress conditions, the leaf area, stomatal density and overall size of *A. communis* were found to be greater than that of *A. webbii* (Fanizza and Reina, 1990). However, it has been demonstrated that some environmental factors, like water stress, may influence the stomata density (Miller, 1938; Ciha and Brun, 1975); therefore, the stomatal characteristics of leaves from trees grown under field conditions could differ from those cultivated as described above. In Egypt,

sweet almonds were found to have a stomata frequency and size lower than that of bitter almonds (Guirguis *et al.*, 1995). Since the above studies have been performed on a very limited number of genotypes, the aim of the present study is to provide additional information on the almond stomata characteristics by analysing a large representative and composite genetic sample of *A. communis* and *A. webbii*.

Material and methods

The study was carried out in late spring 2003 on adult trees of 15 almond varieties and 5 seedlings of *A. webbii* grown under field and rainfed conditions in the germplasm collection of the Department at Valenzano (Apulia, Southern Italy) (Table 1). The 20 genotypes belonging to the genus *Amygdalus* were chosen taking into consideration their origin (wild/cultivated almond), the country of origin (extra Mediterranean/Mediterranean/Apulian), shell hardness (paper/hard) and kernel taste (sweet/bitter). All almond varieties were grafted on 'Don Carlo' sweet almond, with a tree spacing of 6.0 x 6.0 m. The main pedoclimatic data of the site are reported in Table 2.

Table 1. The germplasm under investigation

Cultivar and seedling	Country of origin	Shell hardness	Kernel taste
Group A - *A. communis*			
'Baxendale'	Australia	Paper shell	Sweet
'Johnston's Prolific'	Australia	Paper shell	Sweet
'Ne Plus Ultra'	USA	Paper shell	Sweet
'Nonpareil'	USA	Paper shell	Sweet
'Thompson'	USA	Paper shell	Sweet
Group B - *A. communis*			
'Ferragnès'	France	Hard shell	Sweet
'Genco'	Italy	Hard shell	Sweet
'Pizzuta d'Avola'	Italy	Hard shell	Sweet
'Tuono'	Italy	Hard shell	Sweet
'Marcona'	Spain	Hard shell	Sweet
Group C - *A. communis*			
'Andria'	Italy	Hard shell	Bitter
'Gaetanuccia'	Italy	Hard shell	Bitter
'Padula di Ruvo'	Italy	Hard shell	Bitter
'Pasola'	Italy	Hard shell	Bitter
'Pizzuoantonio'	Italy	Hard shell	Bitter
Group D - *A. webbii*			
A2	Italy (Apulia)	Hard shell	Bitter
B2	Italy (Apulia)	Hard shell	Bitter
B3	Italy (Apulia)	Hard shell	Bitter
C2	Italy (Apulia)	Hard shell	Bitter
C3	Italy (Apulia)	Hard shell	Bitter

Samples of fully expanded leaves chosen at random from the basal portion of shoots all around the crown were gathered for each variety/seedling. The leaf blade area of 100 leaves for each variety/seedling were measured by means of a leaf area meter (LI COR 3100 - USA).

Preliminary observations allowed us to ascertain that the leaves of *A. communis* and *A. webbii* are hypostomatic with elliptic stomata. Therefore, leaf prints of the lower page were taken of 10 leaves for each variety/seedling by means of the adhesive tape and nail polish technique according to Gülcan and Misirli (1990).

Table 2. Main climatic and pedological data of the experimental site

Latitude	41° 03' 16'' N
Longitude	16° 52' 45'' E
Altitude	99 m.a.s.l.
Weather	
Average annual temperature (°C)	15.4
Average maximum temperature (July; °C)	29.2
Average minimum temperature (January; °C)	3.9
Average annual rainfall (mm)	601.0
Soil	
Sand (% d.w.)	63.4
Silt (% d.w.)	16.0
Clay (% d.w.)	20.6
pH	6.7

The stomatal frequency (n/mm^2) was studied on 4000 fields (20 fields x 10 leaves x 20 cultivars/seedlings) chosen at random in the middle of the leaf blade, each field having a surface of 0.043 mm^2. The stomatal size (length and width in m) was measured on 4000 stomata (1 stoma x 20 fields x 10 leaves x 20 cultivars/seedlings) randomly chosen inside each field. The stomatal frequency and size were examined with optical microscope by means of ARKON image analysis software.

Results obtained were statistically analysed by ANOVA using the SAS V8 software for MS Windows (SAS Institute, Inc., USA); the significant parameters of the F test (P = 0.01) were evaluated by the SNK protected test and the variability expressed as standard error.

Results and discussion

Figure 1 and Table 3 illustrate that within the *A. communis*, the mean leaf area ranged from a minimum of 16.4 cm^2 and a maximum of 19.9 cm^2 without any significant difference among the three groups.

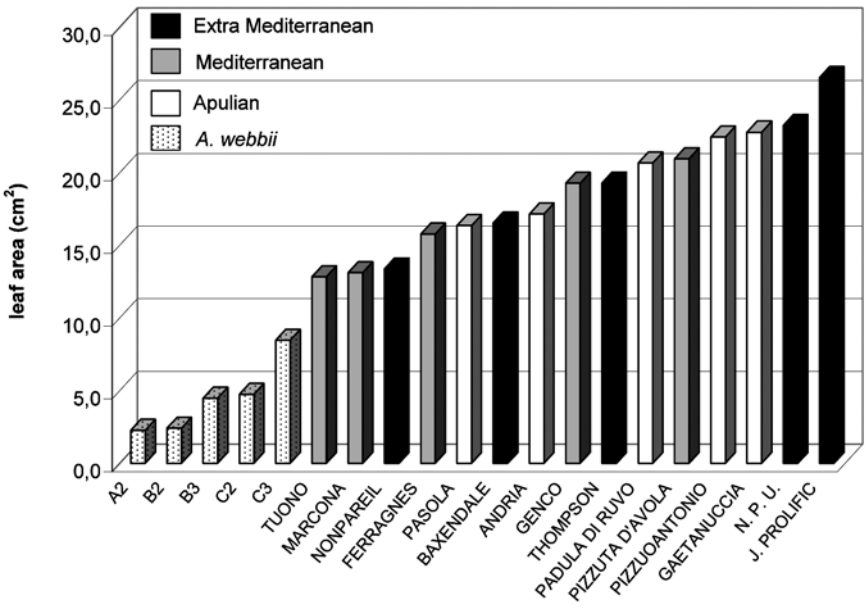


Fig. 1. Leaf area of wild and cultivated almonds.

Table 3. *A. communis* and *A. webbii* leaf area and stomatal characteristics

Cultivar and seedling	Leaf area (cm^2)	Stomata		
		Frequency (n/mm^2)	Length (m)	Width (m)
Group A				
'Baxendale'	16.5 ef	326.6 a	19.3 j	10.5 eg
'Johnston's Prolific'	26.5 a	287.3 b	20.4 ij	9.8 gh
'Ne Plus Ultra'	23.2 b	197.1 g	24.1 de	9.4 gh
'Nonpareil'	13.4 g	247.0 e	24.7 d	11.7 cd
'Thompson'	19.3 d	216.2 f	22.5 eh	10.4 ef
Group B				
'Ferragnès'	15.8 f	168.9 h	21.8 gh	9.0 h
'Genco'	19.3 d	272.0 bc	22.3 eh	11.3 ce
'Pizzuta d'Avola'	21.0 c	259.5 ce	22.2 eh	9.8 gh
'Tuono'	12.8 g	143.4 i	27.8 b	12.8 b
'Marcona'	13.1 g	251.4 de	22.1 eh	11.0 df
Group C				
'Andria'	17.2 e	285.2 b	20.8 hi	9.7 gh
'Gaetanuccia'	22.8 b	252.3 de	24.0 de	11.6 cd
'Padula di Ruvo'	20.7 c	266.9 cd	22.5 eh	10.1 gh
'Pasola'	16.4 ef	183.1 g	23.8 df	10.3 eg
'Pizzuoantonio'	22.4 b	199.2 g	26.7 bc	13.0 b
Group D				
A2	2.3 j	154.9 hi	26.3 c	12.2 bc
B2	2.4 j	200.9 g	22.7 eg	10.3 fg
B3	4.5 i	192.4 g	24.4 d	12.0 c
C2	4.7 i	162.7 h	30.0 a	14.9 a
C3	8.5 h	222.1 f	24.9 d	11.0 df
Mean				
Group A	19.8 b	254.8	22.2	10.4
Group B	16.4 b	219.1	23.2	10.8
Group C	19.9 b	237.4	23.6	10.9
Group D	4.5 a	186.6	25.6	12.1
Mean	15.1	224.5	23.6	11.0

Significant differences within the same column are indicated by different letters (P = 0.01, SNK test).

Among *A. communis* varieties, the character varied significantly from a minimum of 12.8 cm^2 for the Apulian 'Tuono' to a maximum of 26.5 cm^2 for the Australian 'Johnston's Prolific'. In the *A. webbii* seedlings, the leaf area was very small (average 4.5 cm^2) and significantly much smaller than for *A. communis*. In fact, all cultivated almonds exhibited a leaf area larger than 10 cm^2 whereas all wild almonds presented leaf blades smaller than 10 cm^2.

The average stomatal frequency was 224.5/mm^2. In *A. communis* this parameter ranged from a minimum of 143.4/mm^2 ('Tuono') to a maximum of 326.6/mm^2 ('Baxendale'). The stomatal frequency appeared to be quite constant within the *A. webbii* population, normally lower than 225/mm^2. Among 15 almond varieties, once again 'Tuono' showed the lowest stomata frequency and exhibited values similar to those of some *A. webbii* seedlings.

The average stomatal size was 23.6 m in length and 11.0 m in width. Since length and width are directly related, only data referring to the former will be discussed. On an average, the stomata length

did not vary significantly among the four groups. Again, 'Tuono' (27.8 m) exhibited characteristics quite close to those found in *A. webbii*. Besides 'Tuono', large stomata were also observed in the Apulian bitter kernel 'Pizzuoantonio' (26.7 m) whereas the lowest values were found in the Australian 'Baxendale' (19.3 m) and 'Johnston's Prolific' (20.4 m). Among the *A. webbii*, the largest absolute stomata was shown by 'C2' seedling.

As shown in Fig. 2, all *A. webbii* seedlings were concentrated in the graph area under 230/mm^2 for stomatal frequency and over 23 m for stomatal length, while about 50% of *A. communis* varieties were spread along the inverse trend between the two morphological parameters.

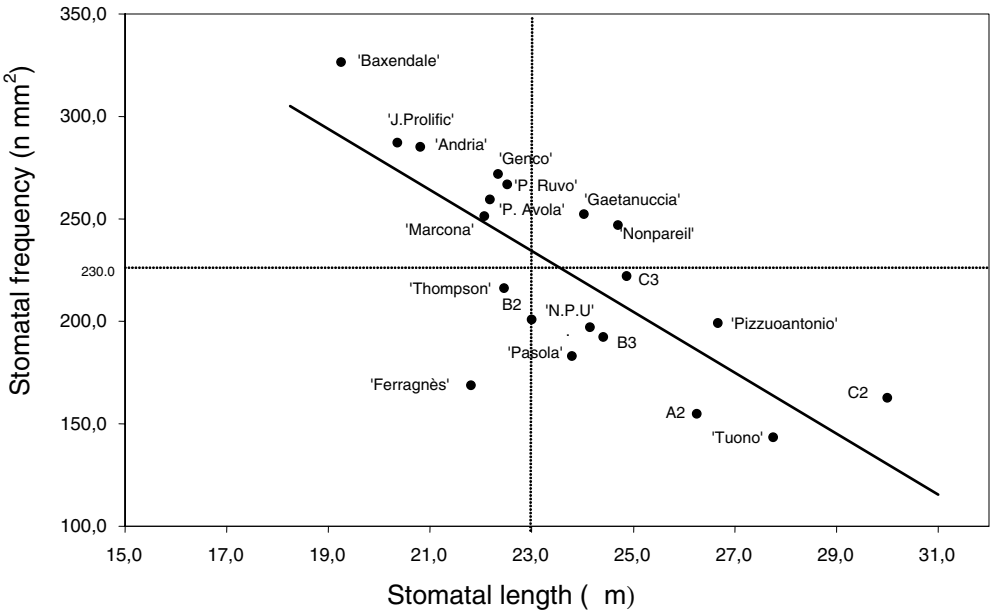


Fig. 2. Wild and cultivated almonds distribution in relation to stomatal length and frequency.

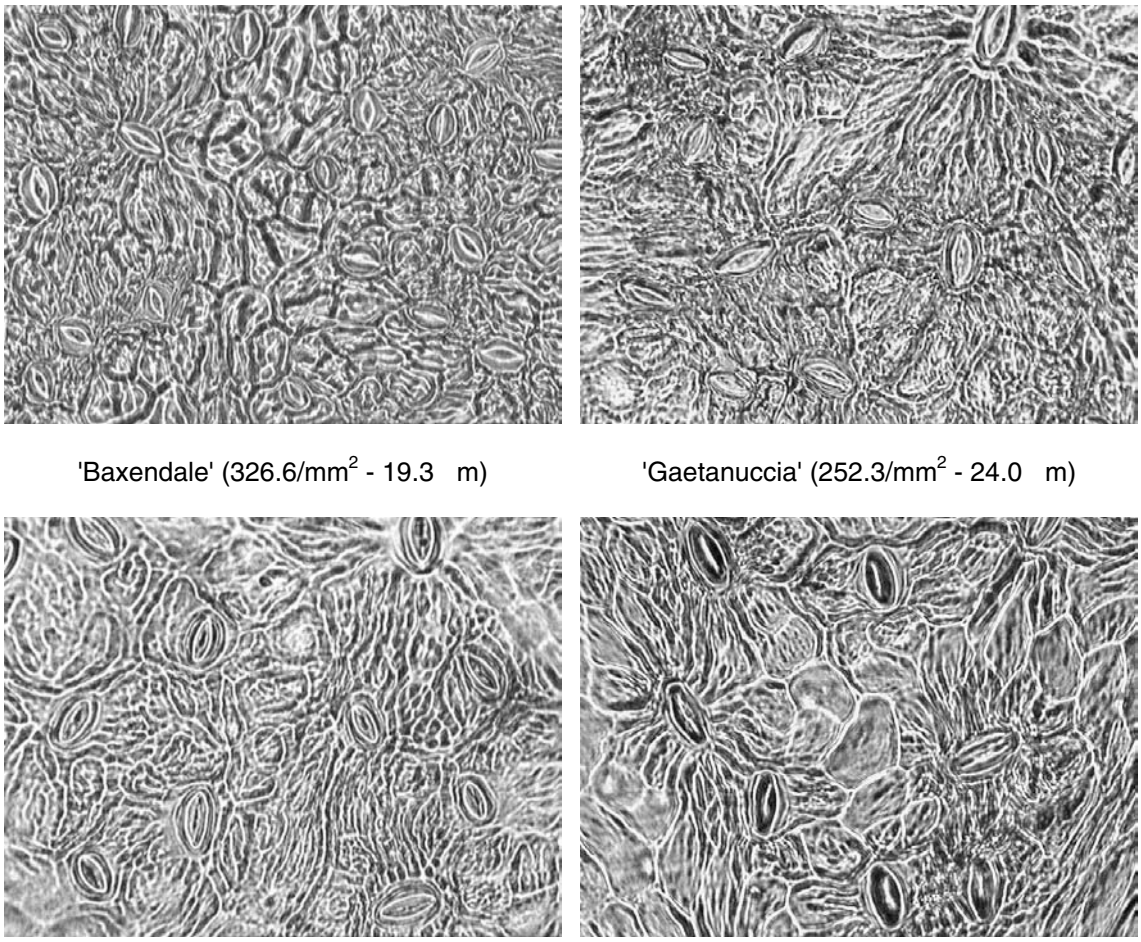
Conclusions

The results of this study confirm significant differences among genotypes of cultivated and wild almonds with regard to stomatal frequency and size. In *A. communis*, these parameters were independent of other characteristics, such as country of origin, shell hardness and kernel taste. Moreover, *A. communis* possessed leaf blades three to four times larger than those of *A. webbii*. It is worthwhile pointing out that stomata and leaf characteristics of the well-known Apulian self-compatible 'Tuono' were intriguingly similar to those of the *A. webbii* population.

As concerns the results obtained by other authors, our data agree with those of Fanizza and Reina (1990) only for stomatal frequency but not for difference in stomatal size between wild and cultivated almonds (Fig. 3). Moreover, our results are not in agreement with those of Guirguis *et al.* (1995) since we did not find any significant difference in stomatal frequency and size between sweet and bitter almonds; in addition, no almond variety/seedling exhibited such a low stomatal frequency, such as the types studied in Egypt, at a latitude definitely lower than that of Apulia.

The only evident difference between the studied cultivated and wild almonds concerns the leaf area, which was considerably smaller in the latter; all other characteristics depended only on genotype.

In conclusion, it is possible to classify the populations evaluated into four classes as follows: (i) stomata sparse and large: A2, B2, B3, C2, C3, 'Ne Plus Ultra', 'Pasola', 'Pizzuoantonio', 'Tuono'; (ii) stomata sparse and small: 'Ferragnès', 'Thompson'; (iii) stomata dense and large: 'Gaetanuccia', 'Nonpareil'; and (iv) stomata dense and small: 'Andria', 'Baxendale', 'Genco', 'Johnston's Prolific', 'Marcona', 'Padula di Ruvo', 'Pizzuta d'Avola'.



'Baxendale' (326.6/mm^2 - 19.3 m) 'Gaetanuccia' (252.3/mm^2 - 24.0 m)

'Tuono' (143.4/mm^2 - 27.8 m) *A. Webbii* 'C2' (162.7/mm^2 - 30.0 m)

Fig. 3. Stomata pictures of some wild and cultivated almonds (200X). Stomatal frequency (mm^{-2}) and length (m) are also indicated.

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