# Preliminary Survey on Stomatal Density and Length of Grapevine

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# Preliminary Survey on Stomata1 Density and Length of Grapevine Shin-ichi Shiraishi, Tung chuan Hsiung and Mikio Shiraishi

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For stomata1 density and length, a preliminary survey of 40 grapevines and its wild relatives, *Muscadinia rotundifolia* was conducted. Within genus *Vitis*, the stomata1 density and length of the diploid cultivars ranged between 140 and 300/mm² and between 20 and 30 pm, respectively. On the other hand, the stomata1 density and length of the tetraploid cultivars ranged between 80 and 120/mm² and between 32 and 37 pm, respectively. Stomata1 density of relatives of genus *Vitis*, *Muscadinia rotundifolia* was about 400/mm², and the stomata1 length was about 19 µm. These results suggest that genetic differentiation in stomata1 density and length may occurred within family *Vitaceae*.

## INTRODUCTION

Photosynthesis, the process by which light energy coming from the sun is intercepted by the plant canopy and transformed into chemical energy needed for further biosynthetic activity, comprises an extremely series of biochemical, physiological, anatomical, and morphological features, which have to be precisely matched and timed if biomass production of the crop is to increase and become consistent (Kuckuck et al., 1991). In fruit trees, with recent progresses of the photosynthesis measurements, data of changes in photosynthetic rates can be easily collected throughout the process of growth (Crews et al., 1975; Kriedmann and Smart, 1971; Barden, 1971; Sams and Flore, 1982). These data are of vital importance, both for the improvement of cultural conditions and for increasing biomass production.

Photosynthetic production depends on three basic features: (1) the size and spatial orientation of the light-intercepting green area (leaves as well as other green plant parts), (2) the duration of this area in active state, and (3) the specific rate of photosynthesis per unit green area. Of these features, the first two exhibit, the greatest genetic variation, and they have also been most successfully exploited in practical breeding. Furthermore, the specific rate of photosynthesis varies considerably between species and among cultivars within a species. However, in grapevine, little is known of the variation in photosynthetic activity such as light saturation level, transpiration coefficient, stomata conductance and intercellular CO, concentration.

From the earlier works by many ampelographists (cf. Mullins et al., 1992), morphological traits of grapevine differ considerably with genetic background and geographical origin of an accession. With respect to photosynthesis, in general, variation in leaf morphology is closely related to photosynthetic activity (Ceulemans et al., 1980, 1984; Gosiewski et al., 1982; Sasahara, 1984). In order to elucidate relationship between leaf morphology and photosynthetic activity, the present study was undertaken as the first step to survey leaf structures of grapevine, especially in stomatal density and length.

# MATERIALS AND METHODS

Two-year old, own-rooted cuttings from 40 grapevine accessions (12 *Vitis* species, 11 *vinifera* cultivars, 12 American hybrid cultivars, 4 rootstock cultivars) and 1 clone of *Muscadinia rotundifolia* were grown in 12-liter containers at a green house of Faculty of Agriculture, Kyushu University. Growth was limited to two or three shoots per vine, and mature leaf sample was collected from actively growing shoots between May and June. Stomata1 density (average of 3 leaves) and length (average of 3 leaves) were measured by microscopy. Furthermore, density of prostrate and erect hairs of lower leaf surface, shape of base of petiole sinus and number of lobes were examined according to the description system for grapevine (IBPGR, 1983).

#### RESULTS

As shown in table 1, there were considerable differences in both stomata1 density and length of accessions tested, especially between *Vitis* species and its wild relatives, *Muscadinia rotundifolia*. The stomata1 density in *Vitis* species ranged from 143.6 /mm² (*V. berlandieri*) to 302.6 /mm² (*V. cordifolia*), averaging 198.3 /mm". The stomata1 length in *Vitis* ranged from  $22.2\,\mu$ m (*V. amurensis*) to  $30.3\,\mu$ m (*V. rupestris*) with an average of  $25.2\,\mu$ m. The stomata1 density and length of *M. rotundifolia* were 407.7 /mm² and 19.1  $\mu$  m, respectively.

Table 1. Stomatal	density, stomata1	length and seve	eral morphological	traits of leaves in V	7itis
species and	its wild relatives	. Muscadinia	rotundifolia.		

Species	Stor	nata				
2,1111	density (mm²)		Prostrate hair lower surface'	Erect hair of lower surface <sup>2</sup>	Shape in leaf basis	Number of lobes
Vitis species (diploid)	, ,	u,				
V. vulpina	136.1	24.1	+	++	U	3
V. coriaceae	245.4	22.4	+++++	++++	U	1
V. cordifolia	302.6	24.9	++++	++++	V	
V. longii	173.2	28.0	+	+	U	3
V. aestivalis	233.0	26.1	+	+	U	1
V. rupestris	156.0	30.3	+	+	U	
V. arizonica	170.3	27.0	++	+	U	1
V. caribaea	237.0	22.8	++++	+	U	5
V berlandieri	143.6	23.3	+++	++++	V	
V. amurensis	223.1	22.2	+	+	U	1
V. coignetiae	180.6	26.0	++++	++	V	1
V. ficifolia	178.5	24.7	+	+	U	5
Muscadinia (diploid)						
M. rotundifolia	407.7	19.1	++	++++	U	

Z: +++++ (very high), ++++ (high), +++ (medium), ++ (low), + (very low)

For other morphological traits, differences were observed between *Vitis* species and among cultivars within *Vitis* species. Within *Vitis* species, density of prostrate hairs of lower leaf surface was high in V. coriaceae, V. cordifolia, V. caribaea, V. berlandieri, and V. coignetiae. High density of erect hairs of lower leaf surface was observed in V. coriaceae, V. cordifolia, V. berlandieri and M. rotundifolia. As for the shape of base of petiole sinus, type U was abundant in the accessions, and type V was found in V. cordifolia, V. berlandieri and V. coignetiae. Number of lobes of the accessions was either one or three with an exception of five for V. caribaea and V. ficifolia.

With respect to *Vitis* cultivars, varietal differences in stomata1 density and length were observed, especially between diploid and tetraploid cultivars (Table 2). For diploid cultivars, the stomata1 density ranged from 143.9/mm² ('Rizamat') to 239.3/mm² ('Muscat of Alexandria'), averaging 182.4 /mm". The stomata1 length ranged from 21.9  $\mu$  m ('Portland') to 30.7  $\mu$ m ('Teleki 5A') with an average of 26.9  $\mu$ m. The stomata1 density of tetraploid cultivars ranged from 84.2 /mm" ('Black Olympia') to 122.9/mm² ('Kuroshio'), averaging 111.4/mm². The stomata1 length ranged from 32.4  $\mu$ m ('Kyoho') to 36.5  $\mu$ m ('Kyogei') with an average of 34.5  $\mu$ m.

Large variation in other morphological traits of *Vitis* cultivars was found (Table 2). Density of the prostrate hairs of American hybrid (e.g. 'Catawba', 'Concord' and 'Niabell')

Table 2. Stoma	al density.	stomata1	length	and	several	morphological	traits	of
leaves	in <i>Vitis</i> cu	ltivars.						

Species	Stomata					
	density	length	Prostrate hair	Erect hair of	Shape in	Number of
	$(mm^2)$	(µm)	lower surface <sup>z</sup>	lower surface'	leaf basis	lobes
(V. vinifera : diploid)						
Chasselas Rose	196.1	26.1	+		V	5
Rizamat	143.9	26.9	+		U	5
Neo Muscat	165.9	26.5	++	+	V	5
Italia	194.4	26.8	++	+	U	5
Muscat of Alexandria	293.3	27.2	+	+	V	5
Royal	181.6	28.5	+++	++++	V	7
Melon	185.8	28.6	+		U	3
Queen	157.5	30.3	+		U	5
Muscat hamburg	181.1	28.6	+	+++	U	5
Orange Muscat	183.1	29.9	+	+	V	3
Flame Tokay	162.4	30.2	+	+	U	5
(American hybrid: dipl	oid)					
Portland	176.5	21.9	++++		V	1
Catawba	184.3	25.2	++++		V	3
Delaware	234.5	25.2	tt		U	3
Concord	198.4	24.1	++++		V	1
Scarlet	210.3	26.7	+++	+	$\mathbf{V}$	3
Isabella	214.4	24.0	++	+	U	1
Niagara	158.5	24.2	ttt	+	$\mathbf{U}$	3

Z: ++tt+ (veryhigh), ++++ (high), ttt (medium), t+ (low), t(very low)

Species	Stor	nata				
	density	length	Prostrate hair	Erect hair of	Shape in	Number of
	$(mm^2)$	(µm)	lower surface <sup>2</sup>	lower surface <sup>z</sup>	leaf basis	lobes
(American hybrid:	tetraploid)					
Kyoho	120.5	32.4	+++	+	U	3
Kyogei	117.5	36.5	+++	+	U	3
Kuroshio	122.9	33.8	+++	+	U	3
Black Olympia	84.2	34.9	+	+	V	1
Niabell	111.9	35.0	+++++	+	V	3
(Rootstock: diploid	)					
LN Amst	171.0	27.5	+	+++	V	5
Couderc	i75.6	24.1	+++	+	V	1
Teleki 5A	169.0	30.8	+	++++	U	1
s o 4	180.6	27.0	+	+	U	1

**Table 2.** (Continued). Stomata1 density, stomata1 length and several morphological traits of leaves in Vitis cultivars.

Z: +++++ (very high), ++++ (high), +++ (medium), ++ (low), + (very low)

was high, whereas that of *V.vinifera* cultivars was low. Density of the prostrate hairs of rootstocks was either very low or medium. High density of erect hairs was observed in 'Royal' and 'Teleki 5A'; that of the other cultivars was generally very low. For the shape of the base of petiole sinus, type V and U were found in equal frequencies among the accessions. Excepting 'Melon' number of lobes of *V.vinifera* cultivars was either five or seven, while American hybrids showed either one lobe or three lobes.

#### **DISCUSSION**

For stomata1 density and length, significant differences were observed in the accessions between Vitis and Muscadinia species. The stomata1 density and length of the Vitis species were between 100 to 200 /mm" and 20 to  $40\,\mu$ m, respectively, while in Muscadinia rotundifolia, the corresponding values were 408 /mm' and  $19\,\mu$  m. From the review of Mullins et al. (1992), the genera Vitis and Muscadinia can be easily distinguished on the basis of morphological, anatomical and karyological characters (e.g. subepidermal phloem fibers, continuous pith and simple tendrils). Although the number of Muscadinia accessions tested was quite small, the results obtained suggest that genetic differences in the stomata1 density and length exist within family Vitaceae. Moreover, great differences in the stomata1 density and length between diploid- and tetraploid-cultivars may be of interest for considering cultivar differentiation. It is thus possible that the stomata1 density and length can be useful indices for ampelographic study on the family Vitaceae.

Of the morphological traits examined, density of prostrate and erect hairs of lower leaf surface should be mostly considered from a viewpoint of environmental physiology of grapevine. There were large differences in the characters among the accessions used. In general, the density of prostrate hair of grapevine on the Northern Hemisphere is higher at high latitudes than at low latitudes. However, the density of prostrate hair of V. vulpina and V. amurensis grown at high latitude was low. The density of prostrate hair of V. vinifera grown in semi-arid areas was generally low, while that of American hybrids and V.coignetiae grown in humid areas was high. These results suggest that the density of prostrate hair of grapevine may be associated with humidity in the places of geographical origin. Furthermore, the density of prostrate hairs of American hybrids increases at short-day condition, indicating that day length influences the density of prostrate hairs of grapevine.

In general, transpiration coefficient of grapevine cultivars with prostrate hairs is higher than that of the cultivars without prostrate hairs. It is, therefore, possible that the density of prostrate hairs affects the photosynthetic rate and transpiration coefficient. Photosynthetic capacity of plants correlates with leaf area, mesophyll structure, chlorophyll content and density of prostrate hairs (Gosiewski et *al.*, 1982; Sasahara, 1984). Both stomata1 density and length correlate closely with photosynthesis (Ceulemans *et al.*, 1980, 1984) and tree vigor (Beakbane and Majumder, 1975; Pathak *et al.*, 1976). Considering the results obtained and reference reviews, morphological variations in stomata and prostrate hair are much interesting for physiological study on grapevine, especially in photosynthesis.

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