A Numerical-Taxonomic classification of *Vitis* spp and Cultivars Based on Leaf Characteristics

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Grapevine leaves collected from three positions on the shoot during véraison were investigated for taxonomic characteristics. Numerical analyses were employed on 13 ampelographical and 13 morphological characteristics. Characteristics contributing to the variation between taxa were mainly quantitative. Although not clearly perceptible specimens tended to cluster in American (*Vitis* spp and hybrids) and European (*Vitis vinifera*) groups. Using the ampelographical characteristics hybrids tended to cluster with at least one of their parents.

Prior to the nineteenth century botanical descriptions of *Vitis* spp were very limited and were mainly concerned with the aptitudes of the various cultivated varieties. In order to combat the increase in pests and diseases during the late nineteenth and early twentieth centuries, it became necessary to study resistant species and cultivars and to develop more complete botanical descriptions. Although numerous proposals were made according to Galet (1979), there was no serious attempt at classification of cultivars. Since the early twentieth century works were published on the ampelography (Viala & Péchoutre, 1910; Galet, 1952, 1976), morphology and anatomy (Metcalfe & Chalk, 1950; Esau, 1965; Pratt, 1974) of the grapevine.

Unlike most of his predecessors who predominantly described the grape cluster for identification purposes, Galet (1952, 1964, 1976) emphasised the growing tips and leaves of the vine in his ampelographical studies. Orffer (1966, 1979) used similar criteria in descriptions of important South African scion and rootstock cultivars. Both authors presented dichotomous keys with the aid of which a taxon could be identified.

Morphological characteristics such as frequency, index and diameter of stomata, (Denisov, 1970; Sievers, 1971; Hegedüs, 1974; Düring, 1980) and appearance, frequency and length of trichomes (Hegedüs, 1968; Schanderl, 1968) have been studied in detail. Although differences in these characteristics were found between cultivars grown in the same area, no attempt was made to employ these characteristics in the classification of the studied cultivars.

In this study numerical taxonomy based on certain ampelographical and morphological characteristics was used to calculate similarity indices of 15 species and/or cultivars of *Vitis* grown in the same geographical region.

MATERIALS AND METHODS

Leaves of four *Vitis* species, four scion cultivars (representing *V. vinifera* L.) and seven rootstock cultivars (representing hybrids and selections of different species) (Table 1) were used for the ampelographical and morphological investigations. All cultivars studied were grown on the Nietvoorbij Experimental Farm, Stellenbosch.

TABLE 1
Species and hybrids employed as experimental material

SPECIES	CULTIVAR
Vitis Berlandieri Planch.	:
V. champini Planch.	:
V. champini	: Ramsey
V. labrusca L.	:
V. longii Engelm.	:
V. riparia Michx.	: Riparia Gloire de Montpellier
V. rupestris Scheele	: Le Roux du Lot
V. vinifera L.	: Cinsaut
V. vinifera	: Muscat d'Alexandrie
V. vinifera	: Pinotage
V. vinifera	: Pinot noir
V. Berlandieri x *V. riparia*	: 420A Mgt
V. Berlandieri x *V. rupestris*	: 99R
V. vinifera x *V. riparia*	: 143B Mgt
V. aestivalis Michx. x *V. cinerea* Engelm. x *V. vinifera*	: Jacquez :

Five replicates each consisting of 20 five-year-old vines were used. Vines were grown on similarly trellised systems for scion (Slanting) (Zeeman, 1981) and rootstocks (Greiner-Decker) (Van der Westhuizen, 1981). Leaves were collected from five shoots per vine during véraison (mid January in the Southern Hemisphere) from three positions on a shoot:basal and apical leaves were sampled at the fifth nodes from the basal and apical ends of the shoot respectively, while the middle leaves were sampled at the middle node.

Initially 18 ampelographical and 23 morphological characteristics were measured. A pattern recognition computer programme was used to identify the most important characteristics (Table 2) contributing to the variability amongst taxa. Certain features could not readily be quantified and are therefore given qualitatively.

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TABLE 2
Characteristics used for the numerical classification.

NO	CHARACTERISTIC		UNIT
	Ampelography		
1	Leaf area		cm^2
2	Ratio: L2: L1 vein lengths		
3	L3: L1 vein lengths		
4	L4: L4 vein lengths		
5	Total leaf length: Total leaf width		
6	Total leaf length		
7	Total leaf width		
8	Depth:		
	Superior sinus		
9	Inferior sinus		
10	Angle between L1 and L3 veins		
11	Angle between L1 and L4 veins		
12	Indument:		
	Glabrous	=1	
	Cobwebby	=2	
	Downy	=3	
	Felty	=4	
	Thornlike hairs	=5	
	Pubescent	=6	
13	Petiolar sinus		
	Open	=1	
	V Shape	=2	
	U Shape	=3	
	Closed	=4	
	Morphology		
14	Stomatal frequency		$number/mm^2$
15	Stomatal index		
16	Stomatal pore diameter		µm
17	Stomata on adaxial epidermis:		
	Absent	=0	
	Sunken	=1	
	Raised	=2	
	Level	=3	
18	Stomata on abaxial epidermis:		
	Absent	=0	
	Sunken	=1	
	Raised	=2	
	Level	=3	
19	Stomata type:		
	Anomocytic	=1	
	Anisocytic	=2	
	Paracytic	=3	
	Diacytic	=4	
20	Indument:		
	Trichome frequency on veins		$number/mm^2$
21	Trichome length on veins		µm
22	Trichome frequency on inter veinal area		$number/mm^2$
23	Trichome length on inter veinal area		µm
24	Trichomes on adaxial side of leaf:		
	Absent	=0	
	Present	=1	
25	Presence of long hair on veins:		
	Absent	=0	
	Adaxial	=1	
	Abaxial	=2	
	Both sides	=3	
26	Presence of long hairs on inter veinal area:		
	Absent	=0	
	Adaxial	=1	
	Abaxial	=2	
	Both sides	=3	

Ampelography:

The leaf area was determined with an area meter. Characteristics 2–11 (Table 2) were measured with a Galet ruler and protractor and classified using the system proposed by Galet (1952). According to Galet (1979) the petiolar sinus can be an important ampelographic characteristic and therefore this characteristic as well as indument was also employed. The shape of the petiolar sinus is related to the angle S between the midvein and L4. The larger the angle S, the narrower the petiolar sinus.

Morphology:

Epidermal tissue for optical microscopy was prepared according to the method described by Swanepoel & De la Harpe (1982). Measurements were made using a microscope equipped with an eyepiece micrometer.

Leaf material (0,5 cm^2) was fixed in 6% glutaraldehyde, dehydrated in an acetone series, critical-point dried in carbon dioxide and sputter coated with gold after which it was studied with an ISI-100A scanning electron microscope.

Stomatal frequency (characteristic 14, Table 2) was calculated by counting the stomata on the abaxial epidermis and expressed as number per square mm. According to Düring (1980) stomata are evenly distributed over the abaxial epidermis, therefore the position of stomatal counting was not important.

Stomatal index (characteristic 15, Table 2) was calculated by counting the stomata and epidermal cells on the abaxial epidermis in a given unit area and using the formula as proposed by Gupta (1961):

$$\frac{\text{Number of stomata} \times 100}{\text{Number of stomata} + \text{Number of epidermal cells}}$$

Statistical analysis:

Significance of differences between cultivars was calculated for the quantitative characteristics by means of a factorial analysis based on Tukey's formula (Snedecor & Cochran, 1974).

Cluster analysis:

A cluster analysis was performed using a programme supplied by J Coetzee (1982, personal communication). The programme calculates the percentage similarity between individuals and for the purpose of this investigation the equation of Canberra (Snedecor & Cochran, 1974) was employed. The formulae used in these calculations for both quantitative and qualitative characteristics are given by Du Plessis & Van Wyk (1982). The percentage similarity is used to cluster the individuals by means of centroid linkage and a table from which a dendogram can be constructed is given.

RESULTS AND DISCUSSION

Preliminary numerical analyses executed on data from the three leaf positions indicated that leaves collected from the middle of the shoot closely resembled the arithmetic mean. Therefore, for the purpose of this paper, results from leaves collected from this position are utilised. Only quantitative results are presented (Table 3) and discussed in more detail.

Ampelography

Although leaf area (characteristic 1, Table 2) can be influenced by the environment, differences between species and cultivars grown under the same climatic conditions do occur (Orffer, 1966). Significant differences in the size of leaves of specimens studied were observed with Jacquez having the largest and *V. longii* and Le Roux du Lot the smallest leaves.

TABLE 3
Values for quantitative ampelographical and morphological characteristics of 15 *Vitis* spp and cultivars.

TAXON	Characteristics* 1	14	15	16	20	21	22	23
V. Berlandieri	76,0	190	6,1	16,5	12	355	8	204
V. champini	81,1	298	9,1	22,7	2	272	0	—
Ramsey	65,5	250	8,1	22,3	4	301	2	172
V. labrusca	72,3	261	7,9	16,3	38	421	19	390
V. longii	54,0	269	8,2	24,0	3	254	0	—
Riparia Gloire de Montpellier	108,2	343	11,3	19,6	8	304	4	173
Le Roux du Lot	62,5	223	10,1	18,3	0	—	0	—
Cinsaut	103,7	228	10,2	21,5	6	433	5	352
Muscat d'Alexandrie	89,4	175	11,9	21,0	8	250	4	117
Pinotage	112,8	253	8,6	21,9	11	271	4	169
Pinot noir	76,3	229	9,1	22,0	2	266	2	145
420A Mgt	75,6	241	8,2	23,4	13	413	0	—
99R	93,3	325	10,7	20,7	13	220	7	177
143B Mgt	117,1	241	8,6	25,2	6	401	0	—
Jacquez	139,6	273	11,2	20,7	24	378	20	371
P≤0,05	12,2	13,3	0,52	1,65	1,99	26,5	1,3	14,01

* 1. Leaf area (cm^2)
14. Stomatal frequency (number/mm^2)
15. Stomatal index
16. Stomatal pore diameter (µm)
20. Trichome frequency on veins (number/mm^2)
21. Trichome length on veins (µm)
22. Trichome frequency on inter veinal area (number-/mm^2)
23. Trichome length on inter veinal area (µm)

A cluster analysis indicates two distinct phenons at a 35% level (Fig. 1) viz an European group (*V. vinifera* cultivars) and an American group (*Vitis* spp and hybrids). The only exceptions are Jacquez, which clusters with the European group, and Muscat d'Alexandrie, which clusters with the American group. It is thus evident that according to the measured ampelographic characteristics Jacquez is dominated by *V. vinifera*. The reason for Muscat d'Alexandrie clustering with the American group can be attributed to the depth of lateral sinusses and the angles between the veins. At the 75% level four distinct phenons are perceptible. From this it is apparent that a hybrid clusters with at least one of its gene parents eg. Ramsey and *V. champini* (84% resemblance).

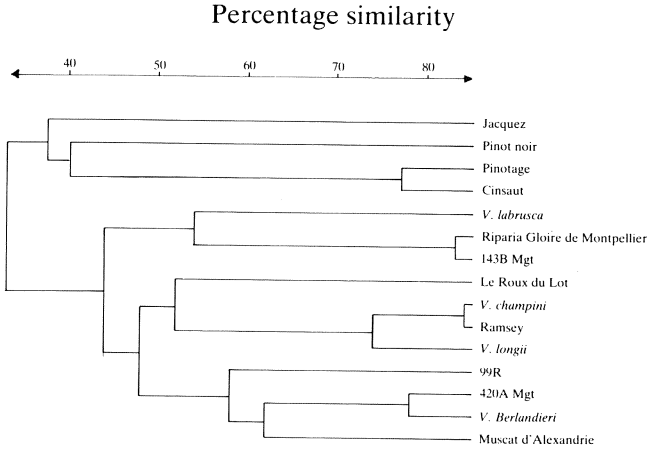

FIG. 1
Dendogram for 15 species and cultivars with 13 ampelographical characteristics.

Morphology

Stomatal frequency:

Stomatal frequency (characteristic 14, Table 2) is determined by environment (Liv *et al.*, 1978) or by genetics (Sievers, 1971; Düring, 1980). The frequency varies from 175/mm^2 (Muscat d'Alexandrie) to 343/mm^2 (Riparia Gloire de Montpellier). As environmental influences were excluded in this study this can be attributed to genetic variability. The frequency for *V. vinifera* cultivars is similar to the results reported by Sievers (1971).

From Table 3 it is also evident that leaves from American spp have, with the exception of *V. Berlandieri*, a significantly higher stomatal frequency than *V. vinifera* cultivars. It was also found that the number of stomata per mm^2 decreases from the apical to the basal leaves of the American spp while the opposite trend emerged in *V. vinifera* (data not shown).

Stomatal index:

(Characteristic 15, Table 2). This characteristic can be of importance in the morphological description of the leaf. While stomatal frequency is influenced by the environment, the index always remains constant for a given cultivar. The index as such does not give a clear distinction between the two groups but the higher the frequency, the higher the index tends to be in the American spp (r = + 0,71) while the opposite (r = -0,96) is observed in the *V. vinifera* cultivars.

Stomatal pore diameter:

If leaves are collected at the same time of the day, the diameter of the pore can be indicative of the leaf-water potential and drought resistance of a cultivar (Van Zyl, 1984). The pore size of American spp varies considerably with *V. Berlandieri* and *V. labrusca* having small pores (≤ 16,5 µm) and 143B Mgt (25,2 µm) larger ones. Those of *V. vinifera* cultivars tend to be more

constant.

Indument:

Trichomes, with the exception of Le Roux du Lot, are present on the veins of all the cultivars studied while they are not so frequently observed on the inter veinal area. Trichome length varies from 220 µm (99R) to 433 µm (Cinsaut) and if present, trichomes on the inter veinal area are shorter than those situated on the veins.

Long hairs are present on both the ad- and abaxial surfaces of the leaf. In *V. vinifera* cultivars the hair were more frequent on the abaxial than on the adaxial surface. When situated on the adaxial surface of the leaf the hairs are connected to the adaxial epidermis opposite a vein.

Qualitative features:

Stomata are mainly present on the abaxial surface of the leaf. According to Pratt (1974) the adaxial epidermis of mature leaves contains almost no stomata. In all the cultivars investigated, few stomata were observed next to the primary and secondary veins on the adaxial surface, while no stomata are present in the inter veinal area.

Stomata on the same leaf can be sunken, raised or level (Fig. 2). Where more than 34% of the stomata were of one type, that type was regarded as being typical. The stomata of *V. vinifera* cultivars are sunken, level in *V. Berlandieri* and 99R and raised in the other species and hybrids. Stoma type is commonly anomocytic (Fig. 3) although paracytic stomata were observed in *V. Berlandieri.* Glands were also frequently present on the leaves of *V. Berlandieri.*

Numerical Analysis:

A dendogram constructed from a numerical analysis (Fig. 4) shows a greater resemblance between cultivars than was observed with the ampelographic characteristics. Principally because of the absence of indument in Le Roux du Lot it forms a distinct group. Although in some cases hybrids do cluster with species (eg. 99R and *V. Berlandieri)* these clusters are not as distinct as those based on the ampelographical characteristics. With the exception of Ramsey, a distinct difference between the American spp and *V. vinifera* cultivars is perceptible. This can be ascribed to the indument characteristics of Ramsey being similar to that of the *V. vinifera* cultivars.

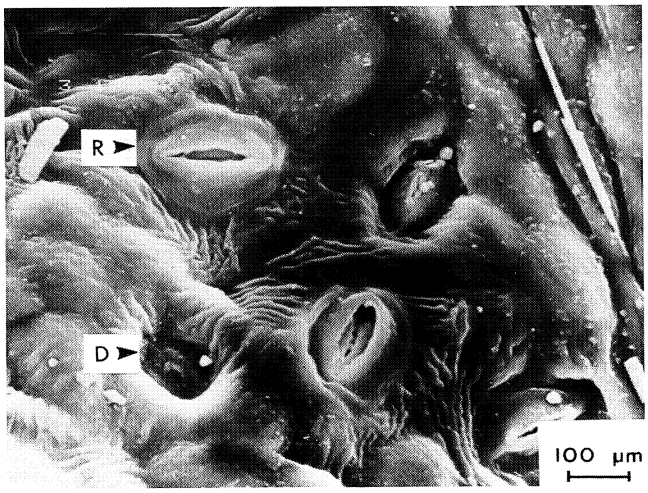

FIG. 2

Scanning electron micrograph showing sunken (D) and raised (R) stomata on the abaxial surface of *V. labrusca.*

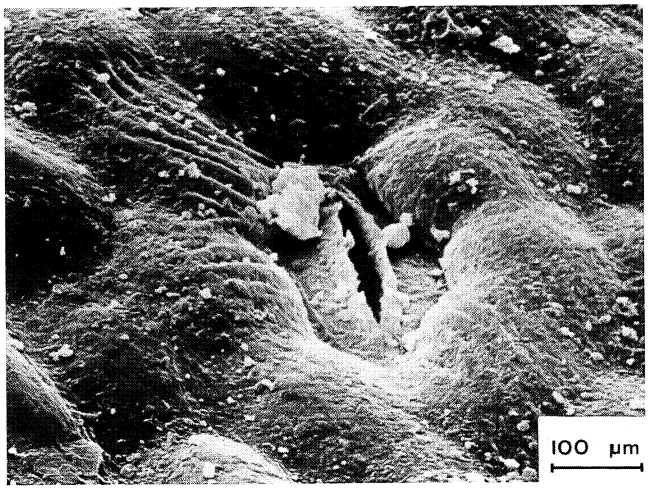

FIG. 3

Scanning electron micrograph of an anomocytic stoma of Pinotage.

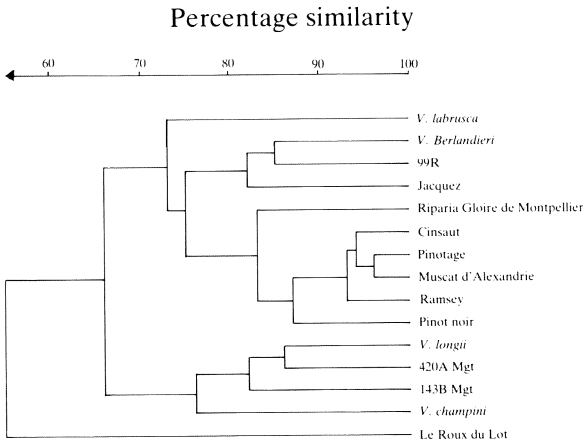

FIG. 4

Dendogram for 15 species and cultivars with 13 morphological characteristics.

CONCLUSION

The most important morphological characteristics causing the separation of a *vinifera* and non-*vinifera* group are stomatal frequency, stomatal index and indument distribution. Stomatal index is positively correlated to stomatal frequency in the non-*vinifera* cultivars while a negative correlation was observed in the *vinifera* group.

Numerical analyses based on ampelographical and morphological characteristics showed distinct differences between American spp and hybrids and *V. vinifera* (European) cultivars. With respect to the ampelographic characteristics the hybrids tended to cluster

with at least one of their parents. With the morphological characteristics however, this tendency was not as pronounced. This could be mainly ascribed to the differences in indument.

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