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Palynological study of the pollen grain of *Vitis vinifera* L. cultivars. Some aspects of sculpturing and pollination

by

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S u m m a r y : SEM analysis of pollen grains of 21 clones from 8 cultivars of *Vitis vinifera* L. has revealed no marked differences between them, except for some cultivars with heteropolar grains and the cultivar Jaén from Toledo and Tinto Fino from Madrid, which had a small type. Some details of ornamentation of the pollen grains and pollination are discussed.

K e y w o r d s : palynology, pollen, Vitis, morphology.

Introduction

Characterization of wild and cultivated plant species and clones by pollen grains has been relatively frequent. For example, Maas (1977) identified different strawberry and other small berry fruits; Westwood and Challice (1978) did similar studies in species of pear trees; Martens and Fretz (1980) on wild apple trees; and Ahmedullah (1983) characterized different grape cultivars based on pollen morphology.

In our country the pollen morphology of *Vitis vinifera* L. has been studied by Fernández (1987) in an atlas of pollen grains of western Andalusian plants, and Bueno (1989), who worked with plants from northern Spain and disagreed to some extent with Fernández.

Some other recent studies in *Vitis* spp., also with taxonomic aims, should be mentioned. Lombardo and Cargnello (1978) made observations on the ultrastructure of pollen grains in grapevines with low yields. Castelli *et al.* (1985) studied the characteristics of pollen grains in 20 clones belonging to 8 different cultivars, and did not detect marked differences in the measurements of the polar and equatorial axes, nor in the ornamentation, nor in the presence of pore arrangement. From these results he concluded that pollen morphology plus other morphological and biochemical observations, provided useful information for identification of grapevine clones. Uzun and Ilter (1987) and Kharitonashvili *et al.* (1989) studied pollen grains in different types of flowers of *Vitis vinifera* L., using SEM. Ben Slimane (1990) characterized 30 grapevine varieties, 10 wild and 20 cultivated, based on pollen size and established one group with small sized, one large, and the third a transition group with medium values. Martens *et al.* (1989) studied pollen size variability within genotypes of *Vitis*.

Materials and methods

Plants from which the material was sampled, are located in a plot at the township of Alcalá de Henares (Madrid), in the second terrace of the Henares river. The soil was loamy in texture with high levels of active calcium. Pedological classification according to the USDA soil taxonomy criteria is order Alfisols group Haploxeralfs. The climate is dry mediterranean, with average temperature of 13.1 °C and 469 mm precipitation per year. This study began in 1989. The plantation was 28 years old at the beginning of study. All the plants had a vase type of pruning, with 3-4 shoots per vinestock. Four plants of each cultivar were available. All plants were submitted to the same cultural practices for the whole period.

Table 1

List of the cultivars studied

KEY	COMMON NAME	TYPE *)	GERMPLASM BANK CODE	ORIGIN
TELO	TEMPRANILLO	T	22-A-23	LA RIOJA
CECR	CENCIBEL	T	22-I -25	CIUDAD REAL
TFM	TINTO FINO	T	22-J-02	MADRID
TMTO	TINTO MADRID	T	22-J-49	TOLEDO
GNLO	GARNACHA	T	22-A-14	LA RIOJA
GNZ	GARNACHA	T	22-D-50	ZARAGOZA
GNM	GARNACHA	T	22-J-17	MADRID
NGM	NEGRAL	TR	22-J-30	MADRID
GTV	GARNACHA TINTORERA	TR	22-O-49	VALENCIA
MOLU	MOURATON	T	22-N-05	LUGO
ARCR	AIREN	B	22-I -42	CIUDAD REAL
MNAB	MANCHEGA	B	22-I -03	ALBACETE
ARM	AIREN	B	22-J-47	MADRID
MVGU	MALVAR	B	22-I -56	GUADALAJARA
MVCU	MALVAR	B	22-I -37	CUENCA
MVM	MALVAR	B	22-J-29	MADRID
JNGU	JAEN	B	22-I -51	GUADALAJARA
JNTO	JAEN	B	22-J-44	TOLEDO
ABAV	ALBILLO	B	22-I -16	AVILA
ABM	ALBILLO	B	22-J-15	MADRID
ABVA	ALBILLO	B	22-L-23	VALLADOLID

*) T: red; TR: red pulp; B: white

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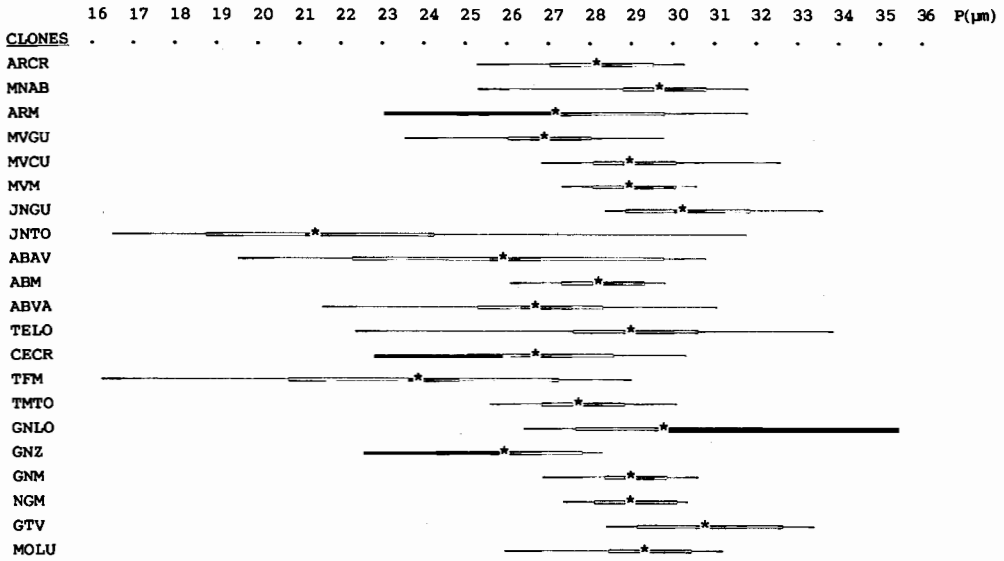

Fig. 1: Simpson & Roe test for P (polar axes).

The collection plot is included in the Grapevine Germplasm Bank at El Encin (Alcalá de Henares), belonging to the "Servicio de Investigación Agraria de la Comunidad de Madrid". 21 clones of the following 8 cultivars (with parentheses synonymies) were studied: Tempranillo (Cencibel, Tinto Fino or Tinto Madrid), Garnacha, Negral (Garnacha Tintorera), Mouratón, Airén (Manchega), Malvar, Jaén and Albillo. The rest of the cultivars belongs to different origins for the above mentioned varieties (see Tab. 1). Accession codes are indicated too in Tab. 1.

In order to prevent contamination with foreign pollen grains, inflorescences were covered with bags before opening of the flower buds, this corresponds phenological stage "H" of BAGGIOLINI (1952). Sampling of bagged flowers was carried out when it was observed about 40 % of the flowers in the uncovered inflorescences had opened; that ist the phenological stage "I" of BAGGIOLINI (1952). Flowers soon to open were sampled, put in glass vials and left until than had opened.

Observation of pollen grains was made with a Hitachi S-2500 SEM. Non acetolyzed grains were used, coated in a "Diode Sputtering System Type P-S1" with a 400 Å thick platinum layer. Photomicrographs were taken with a Mamiya camera.

Descriptions of pollen grains were based on morphological observations and include: shape (symmetry and polarity), size, apertures (type, number and arrangement) and exine sculpturing. The polar (P) and equatorial (E) axes and the P/E relationship (Tab. 2) were measured. In Figs. 1, 2 and 3 maximum (M), minimum (m) value, mean (X) and confidence interval (CI) are presented. Measurements were always recorded as the average of 16 randomly sampled pollen grains for each sample, and measured on the micrographs, in a computerized Summagraphics image analyzer, model MM 1103, following ORTIZ *et al.* (1990).

Results

Observation of the pollen grains in the 21 clones gave the following description for *Vitis vinifera* L.: The pollen grain is three-zonocolporate, with radial symmetry, generally isopolar, although some heteropolar pollen grains were observed in Negral from Madrid (NGM, Fig. 4 C), Garnacha Tintorera from Valencia (GTV) and Airén from Ciudad Real (ARCR) (Fig. 4 G). In Fig. 4, the most representative pollen grains of the chosen cultivars are shown. Also it can be observed that the shape in Jaén from Toledo (JNTO) (Fig. 4 D) is less elliptical and smaller in size, and Tinto Fino from Madrid (TFM) is smaller than in the rest of studied clones (Fig. 4 A).

In equatorial view the pollen is elliptical, and in polar view it is triangular angle-aperturated with the sides slightly concave (Fig. 5 B). When pollen absorbs water, it becomes hexagonal in polar view, because of the expansion of the apertural zones (Fig. 5 A).

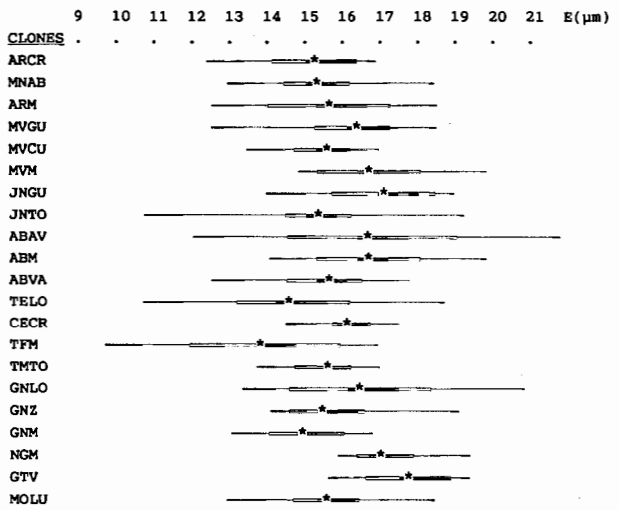

Fig. 2: Simpson & Roe test for E (equatorial axes).

Table 2

Pollen descriptions. Minimum, maximum and mean values and confidence interval (95 %) are indicated

CLONE	ORNAMENTATION APOCOLPIUM	SHAPE	SIZE	POLAR AXIS (P)	EQUATORIAL AXIS (E)	RATIO (P/E)
ARCR	Perforated-Rugulated	Prolate	Medium	25,37 30,29	13,87 16,99	1,67 2,05
				(28,10±1,15)	(15,50±0,75)	(1,82±0,08)
MNAB	Perforated-Rugulated	Prolate	Medium	25,39 31,68	14,68 18,52	1,68 2,02
				(29,46±0,83)	(16,11±0,47)	(1,83±0,05)
ARM	Perforated-Rugulated	Prolate	Medium	23,02 31,89	12,18 17,87	1,62 1,94
				(27,02±2,26)	(15,22±1,19)	(1,78±0,07)
MVGU	Perforated-Rugulated	Prolate	Medium	23,46 30,43	12,32 18,00	1,50 2,07
				(26,88±0,83)	(15,62±0,69)	(1,73±0,06)
MVCU	Rugulated	Prolate	Medium	26,82 32,67	13,42 16,95	1,72 2,09
				(28,64±0,76)	(15,45±0,47)	(1,86±0,05)
MVM	Rugulated	Prolate	Medium	27,19 30,92	14,51 19,40	1,54 2,01
				(28,91±0,80)	(16,02±1,08)	(1.82±0,13)
JNGU	Perforated-Rugulated	Prolate	Medium	28,23 33,80	13,49 18,79	1,59 2,26
				(30,20±1,31)	(16,70±1,10)	(1,82±0,13)
JNTO	Perforated	Prolate	Small	16,37 32,13	10,72 18,51	1,03 1,93
				(21,12±2,46)	(15,18±1,25)	(1,41±0,18)
ABAV	Perforated-Rugulated	Prolate	Medium	19,36 34,28	11,76 21,38	1,40 1,86
				(25,88±3,50)	(15,78±1,99)	(1,64±0,11)
ABM	Perforated-Rugulated	Prolate	Medium	25,97 29,52	13,68 19,19	1,52 2,00
				(28,00±0,80)	(15,94±1,11)	(1,77±0,10)
ABVA	Perforated-Rugulated	Prolate	Medium	21,66 31,20	12,36 17,81	1,48 2,20
				(26,56±1,39)	(14,54±0,73)	(1,84±0,10)
TELO	Perforated-Rugulated	Perprolate	Medium	22,31 34,12	10,14 18,86	1,58 2,59
				(28,78±1,42)	(14,12±1,19)	(2,08±0,15)
CECR	Perforated-Rugulated	Prolate	Medium	22,73 30,98	14,50 17,20	1,39 2,00
				(26,76±1,65)	(15,87±0,54)	(1,69±0,12)
TFM	Perforated	Prolate	Small	16,26 29,01	9,80 16,77	1,36 2,09
				(23,88±3,16)	(13,66±1,55)	(1,75±0,15)
TMTO	Perforated-Rugulated	Prolate	Medium	25,46 30,01	13,85 16,71	1,59 1,92
				(27,58±0,80)	(15,48±0,49)	(1,79±0,05)
GNLO	Perforated-Rugulated	Prolate	Medium	26,30 35,63	13,25 19,59	1,46 2,19
				(29,44±2,00)	(16,03±1,67)	(1,86±0,20)
GNZ	Perforated-Rugulated	Prolate	Medium	22,51 28,24	13,09 17,82	1,36 1,97
				(25,83±1,65)	(14,73±1,09)	(1,77±0,15)
GNM	Perforated-Rugulated	Prolate	Medium	26,82 30,75	13,04 16,55	1,64 2,22
				(28,80±0,66)	(14,78±0,56)	(1,96±0,07)
NGM	Perforated-Rugulated	Prolate	Medium	28,17 32,54	15,81 19,18	1,48 1,93
				(29,73±0,77)	(16,94±0,70)	(1,76±0,10)
GTV	Perforated-Rugulated	Prolate	Medium	26,33 33,56	15,46 18,90	1,64 1,98
				(30,55±1,50)	(17,41±1,00)	(1,76±0,08)
MOLU	Perforated-Rugulated	Prolate	Medium	26,11 31,50	12,98 18,29	1,62 2,37
				(29,29±0,80)	(15,27±0,65)	(1,93±0,09)

The average P/E relationship is around 1.71, which defines the shape of the pollen grain as prolate, according to ERDTMAN (1969). The clone Jaén from Toledo (JNTO) has the min. P/E value, and the maximum corresponds to Tempranillo from Logroño (TELO), which would define the pollen grains as perprolate ERDTMAN (1969) (Tab. 2).

Pollen size is mediumm, with average values of approximately 27.68 µm for the polar (P) axes. The maximum and minimum average mean values are 35.63 and 16.26 µm respectively for the polar axes (P), that correspond to GNLO and TMF; there are two clones, which have small pollen grains: JNTO and TFM.

The apertures are compound, with ecto- and endoapertures. Exine is perforate-rugulate, with elongated perforations intermixed with the rugulae, giving a reticulate aspect chose to the apocolpium (Fig. 6). In the mesocolpium

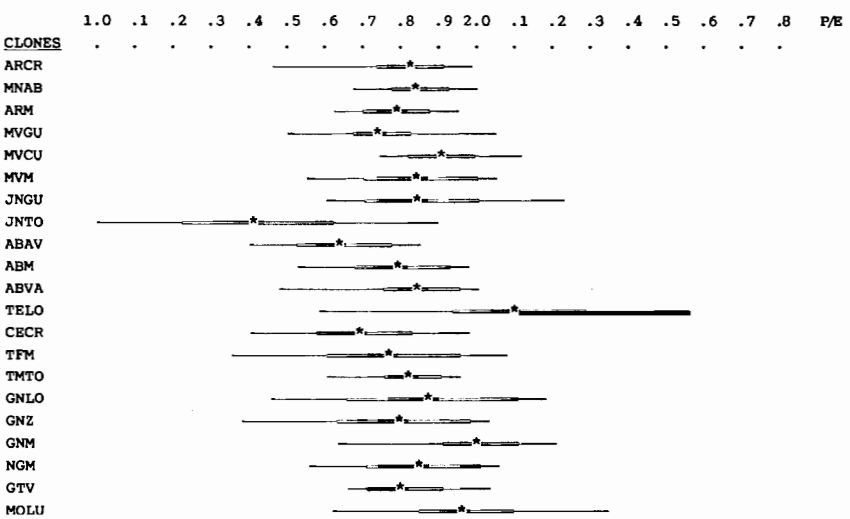

Fig. 3: Simpson & Roe test for P/E (polar/equatorial axes).

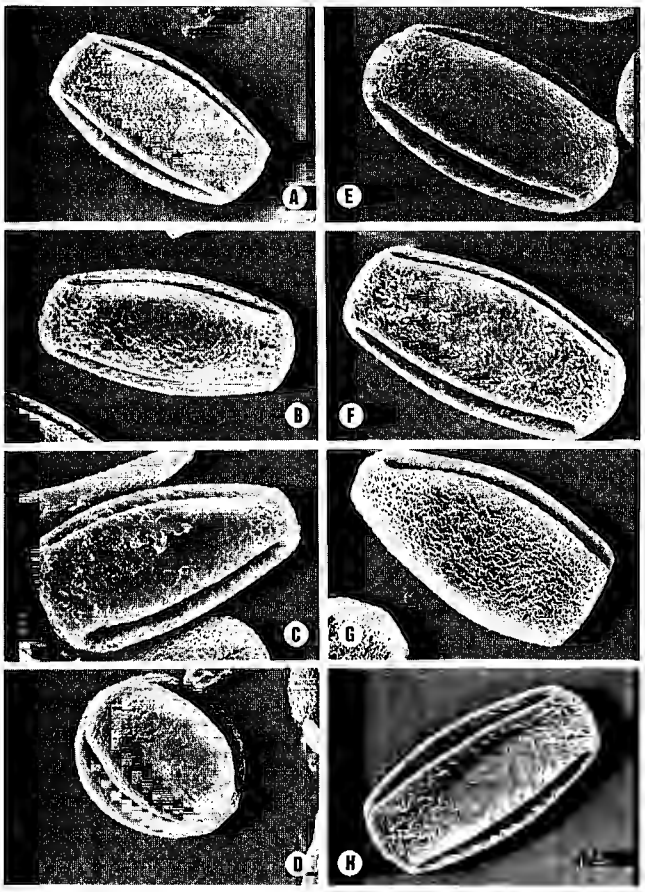

Fig. 4: Pollen grains of *Vitis vinifera* L. in equatorial view. A: Tinto Fino from Madrid (TFM), B: Garnacha from Madrid (GNM), C: Negral from Madrid (NGM), D: Jaén from Toledo (JNTO), E: Albillo from Valladolid (ABVA), F: Malvar from Cuenca (MVCU), G: Airén from Ciudad Real (ARCR), H: Jaén from Guadalajara (JNGU).

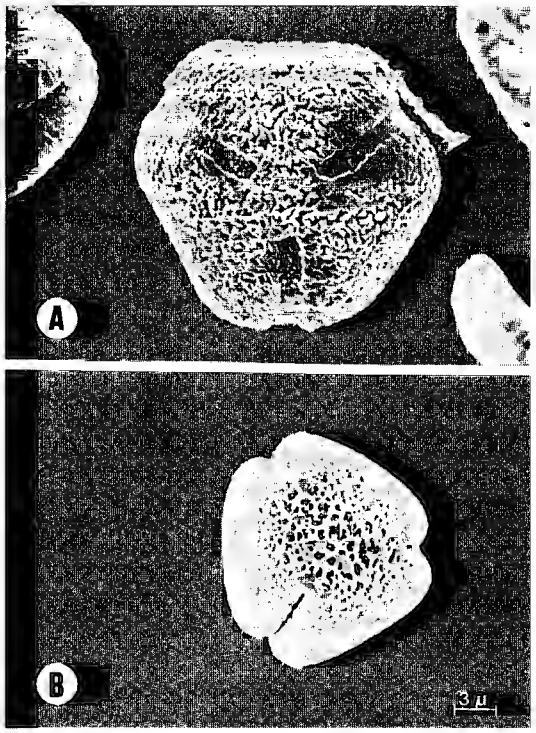

Fig. 5: Pollen grains of *Vitis vinifera* L. in polar view. A: Pollen grain inflates by absorption of water in hexagonal shape. B: Pollen grain not inflated in triangular shape.

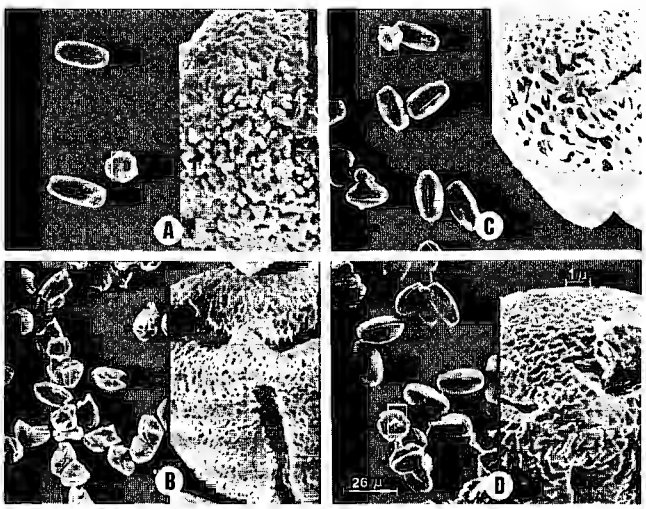

Fig. 6: Pollen grains of *Vitis vinifera* L. in polar view showing apocolpi. A: Garnacha from Zaragoza (GNZ) with perforated-rugulated type, B: Tinto Fino from Madrid (TMF) with perforated type, C: Jaén from Guadalajara (JNGU) with perforated-rugulated type, and D: Malvar from Madrid (MVM) with rugulated type.

the perforations disappear and the rugulae are denser (Fig. 4). Three groups can be recognized according the ornamentation of the apocolpium: perforate, rugulate and perforate-rugulate.

The clones Malvar from Madrid (MVM) (Fig. 6 D) and from Cuenca (MCV) belong to the rugulate type, Tinto Fino from Madrid (TFM) (Fig. 6 C) as well as Jaén from Toledo (JNTO) belongs to the perforate type and the rest of the cultivars to the perforate-rugulate type (Tab. 2).

Discussion

In a general overview, no marked differences among the studied pollen grains were observed, except for the clones with heteropolar grains, as well as Jaén from Toledo (JNTO) which has the rounded, small type described above; Tinto Fino from Madrid (TFM) with the small type and Tempranillo from Logroño (TELO), which has a perprolate shape.

Comparison of our results with some of the former studies on *Vitis vinifera* L. pollen shows, some differences exist in the description of the polar view by Bueno (1989) which depicts them as hexagonal. Our results agree with those of Fernández (1987) who describes them as triangular as shown in Fig. 5 B. As mentioned before, the hexagonal aspect is probably only the result of hydration.

Although in the revised literature the pollen grain is described as isopolar, a certain amount of heteropolar grains were found , especially in the two synonymous cultivars Negral (NM) and Garnacha Tintorera (GTV) as well as in one of the clones of Airén (ARCR). The relatively frequent occurrence of this characteristic in the clones is an

indication of the probability of its genetically fixed situation.

The ornamentation of the apocolpi, with the two defined groups, rugulate and perforate, with predominance of the last one, is not typical of an anemophilous species. According in MARTINEZ DE TODA (1990), the grapevine has three types of pollination: anemophilous, entomophilous and self-pollination. According to VORWOHL (1977) nectar secretion in the grapevine flowers seems to be quite restricted, since only sporadic visits of insects occur. The same author observed a maximum of 28 bees per vinestock per hour during the period of maximum bloom. However, by using pollen traps in the hives close to a vineyard during the blooming period, he observed that in some cases most of the collected pollen came from the vines. From this fact, he concluded that this species has a certain interest for honeybees due to the supply of pollen produced.

In our opinion, based on the observed ornamentation of the pollen grains, and on the authors cited, very likely it seems that a high percentage of the grapevines are autogamous and the rest correspond to an entomophilous allogamy and a few are wind-pollination.

Our results indicate that the pollen grains are of subsidiary use for characterising of vine clones and cultivars, which agrees with the conclusions of CASTELLI *et al.* (1985). These measurements and observations should be taken together with other morphological and biochemical features, since the restricted use of pollen grains is not enough to differentiate cultivars.

The present study confirms the triangular polar shape of the pollen grains as well as the predominant isopolar form, as well as the presence of some heteropolar types. Some differences in size, polarity and ornamentation were observed among some of the studied cultivars even in some cases among clones of the same cultivar. Palynology of *Vitis vinifera* L., although limited for characterization of cultivars, is an adequate and complementary observation for identification, when added in other types of measurements.

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