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KARYOTYPE ANALYSIS IN GRAPEVINES[1]

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ABSTRACT – Seven species of *Vitis* L., *V. champinii* Planchon*, V. cinerea* (Engelm in Gray) Engelm*, V. girdiana* Munson*, V. labrusca* L., *V. rotundifolia* Michaux, *V. rupestres* Scheels and *V. vinifera* L. were studied with the purpose of complementing the karyomorphometric information for further comparative analyses. Based on ideograms and on chromosome measures obtained it was possible to see several differences among the species, which were enough to distinguish at least *V. champinii* and *V. girdiana* from the others as well as *V. labrusca* for the lowest measures and *V. rotundifolia* for the highest mean value of arm ratio. It seems that during the species diversification process the most crucial differences among them did not involve drastic changes in chromosome morphometry.
Index terms: *Vitis*, ideogram, Vitaceae, mitosis, satellite segment.

ANÁLISE DE CARIÓTIPOS EM VIDEIRAS

RESUMO – Sete espécies de *Vitis* L., *V. champinii* Planchon, *V. cinerea* (Engelm in Gray) Engelm, *V. girdiana* Munson, *V. labrusca* L., *V. rotundifolia* Michaux, *V. rupestres* Scheels e *V. vinifera* L. foram estudadas com o propósito de complementar as informações cariomorfométricas para posteriores análises comparativas. Baseado nos ideogramas e nas medidas cromossômicas obtidas foi possível distinguir pelo menos *V. champinii* e *V. girdiana* das demais espécies pelas maiores medidas, bem como *V. labrusca* pelas menores medidas e *V. rotundifolia* pelo maior valor médio da razão de braços. Parece que durante o processo de diversificação das espécies as diferenças mais cruciais entre elas não envolveram mudanças drásticas na morfometria cromossômica.
Termos para indexação: *Vitis*, ideograma, Vitaceae, mitose, segmento satélite.

In the Vitaceae family the genus *Vitis* L. excels for its agronomic and economic importance. Domesticated and cultivated since the Neolithic period, at first in the Anatolia region, the vinifera grape spread and generated thousands of varieties and cultivars through improvements carried out by different civilizations (THIS et al., 2006). Due to its remarkable capacity for adapting to growth in subtropical regions, this grape culture arrived in Brazil in 1532 when the Portuguese brought the first plants, shoots and twigs directly from Europe. Since then, the grape culture has spread to some favorable Brazilian regions such as the South and Southeast (SOUSA, 1996).

In the state of São Paulo, a breeding program for grape improvement began around the end of the 19th Century at the 'Instituto Agronômico de Campinas (IAC)' by Franz W. Dafert who introduced about 300 varieties belonging to American species as well as to *V. vinifera* (cited by MARTINS et al., 2010). Some of these American grapes such as *V. champinii*, *V. riparia* and *V. rupestris*, for instance, along with some other Asian ones, have been used either in single or double crossing combinations, giving rise to resistant rootstocks for *V. vinifera* due to the compatibility between vinifera grafts (SILVA et al., 2010). Wild grape species have also been recognized as a valuable source of resistant genes (*R*-genes) for downy mildew (*Rpv* genes) and powdery mildew (*Re* genes) (BORNEMAN et al., 2013) building up tolerance to abiotic stressors such as cold, which is found in *V. labrusca* and *V. riparia*, or drought as observed in *V. champini* and *V. rupestris* (REISCH and PRATT, 1996). This fact has led to a continuously increasing interest in the study of wild

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grapes using different approaches (CHAVARRIA et al., 2012; SATO et al., 2011).

Chromosome research in grapes began at the end of the 1920s. The first phase that predominantly focused on the determination of chromosome number started with the finding of two *Vitis* species (OLMO, 1937) and has continued until now mainly regarding the detection of chromosome duplication, which either may appear spontaneously, or might be induced by chemical agents or may also appear in plants regenerated through tissue culture (KUKSOVA et al., 1997). This phase also comprised studies that focused on microsporogenesis of interspecific hybrids predominantly those between *V. vinifera* and *V. rotundifolia* aiming at generating knowledge on chromosome behavior and genome affinities including the relationship among the parental species. The second phase encompassed some attempts toward mitotic chromosome individualization and characterization, with further recognition that grapes have chromosomes of small size, which have challenged researchers. Chromosome banding with specific fluorescent dyes in some *Vitis* species was first reported by Pinto-Maglio et al. (2010) who observed a predominance of two pairs of chromosomes displaying positive CMA-bands. NOR banding was later applied to some *Vitis* species aimed at the obtainment of more detailed chromosome information (PIEROZZI, 2011). However, a media ideogram for each of the species studies with each chromosome individualized in the genome has yet been achieved or displayed. Therefore, additional studies in grapes regarding chromosome analyses were carried out with the purpose of drawing an individualized ideogram for some grapes species as an attempt towards germplasm characterization for further comparative studies among species at chromosomal level, adding to grape breeding program developed at '*Instituto Agronômico de Campinas – IAC'*.

The seven *Vitis* species analyzed, *V. champinii* Planchon, *V. cinerea* (Engelm in Gray) Engelm, *V. girdiana* Munson, *V. labrusca* L., *V. rotundifolia* Michaux var. Regale, *V. rupestris* Scheels, and *V. vinifera* L. var. Italia, belong to the grapevine collection of *Centro de Frutas* (IAC) in Jundiaí, SP. Hardwood cuttings from at least five different germplasm of each species were rooted and later transferred to the Plant Genetic Resources Center, at the Agronomic Institute in Campinas, SP – IAC (*CPD Recursos Genéticos Vegetais – IAC, Campinas*) where they have been growing. Roots were collected, pre-treated with a saturated solution of *p*-DB at 16° C for 2h and 30min, fixed in fresh 3:1 solution (ethanol:acetic acid) and stored at -20°C. Fixed roots were briefly washed in distilled water, transferred to an enzymatic mixture of 20% pectinase and 2% cellulase at 37° C for 1 hour and then squashed in 45% acetic acid solution and the cover slips were removed. Slides were stained with a fresh 2% Giemsa solution from 2 to 5 minutes at room temperature, dried and mounted with Permount media. Ten metaphase cells of each species were chosen for morphometric data. Since only length mean values for the longest and for the shortest chromosome of each genome were reported by Pierozzi (2011) the remaining were measured and individualized by absolute (μm) and relative (%) length mean values. Arm ratio was also calculated (the long arm divided by the short arm) for each chromosome of each species and also (a) arm ratio mean values with standard deviation (R_{mean}); and (b) differences between the longest and the shortest chromosome relative length (L-S%) for each species analyzed. Differences observed were analyzed by Tukey test at 1%. An individual ideogram was displayed for each species. Some slides were photomicrographed under an Olympus Vanox photomicroscope with Kodak Ultra 400 film.

Chromosome morphometric data along with median ideograms have fostered plant research on the identification and characterization of wild species and populations as well as improving the knowledge of a genus. In South American races of maize, for instance, McClintock et al. (1981) was able to individualize and characterize Andean maize and several autochthone races through chromosome morphometry differences observed among them. In regards to grapevines, it is the first time that chromosome measures of an entire genome has been obtained along with comprehensive ideograms for *V. vinifera* var. Italia, *V. rotundifolia* var. Regale and also for the five other American grape species studied (Figures 1, 2 and 3; Table 1). In the seven species studied chromosome 3 was the only one carrying a secondary constriction associated with a satellite segment on the long arm (Figures 2 and 3). Pierozzi (2011) observed only one pair of chromosomes with a terminal NOR band after silver impregnation in some grapes. However, it was not possible for the authors to discriminate which chromosome pair it was. Despite knowing that silver impregnation (NOR band) unveils the 45S ribosomal DNA sites (45S rDNA) anchored in the secondary constriction, which were active in the last mitotic interphase, it was concluded that the chromosome 3 is the same firstly targeted by silver impregnation. Both satellite segment and the secondary constriction

sizes varied among the species analyzed (Figures 2 and 3). Satellite segment size varied from 0.15μm in *V. labrusca* to 0.27μm in *V. champinii* while the secondary constriction varied from 0.14μm in *V. labrusca* to 0.26 μm in *V. champinii* (Table 2). Although there were no significant differences at 1% after Tukey test, the size of the satellite and secondary constriction turned out to be remarkable features to distinguish and separate *V. labrusca* and *V. champinii* from the remaining species studied (Figure 4). In fact, the use of secondary constriction and satellite segment measures have been strong enough for distinguishing some morphologically similar species of *Lathyrus*, for instance, mainly those with an uncertain classification (GÜNES, 2011).

V. labrusca displayed the lowest values for absolute chromosome length for almost all the chromosomes of the genome, while *V. champinii* along with *V. girdiana* displayed the highest values for absolute chromosome length and secondary constriction (Tables 1 and 2). The few and discrete chromosome differences observed among the species studied added up to those differences reported by some authors (ZECCA et al., 2012, for instance) in regards to phenological cycle associated to DNA specific sequences, may probably have contributed to assure the species identity. Arandhya et al. (2013) emphasized that the geographic isolation of some species of *Vitis* was not the only process involved in reproductive isolation, but the preferences for markedly different habitats or shift in flowering time that likely played an important role in promoting and maintaining isolation among sympatric species.

In addition to the well-known different chromosome number (2n=40) *V. rotundifolia* could be characterized by the lowest mean values for chromosome relative length in almost all of the chromosomes of the genome; also, by the highest mean value for arm ratio (R), which also distinguished this species from the others that showed almost the same arm ratio mean values (Table1). Peruzzi and Eroglu (2013) stated that the arm ratio is one of the most popular, cheapest and widely used approaches, employed to estimate the intrachromosomal asymmetry of a genome, which may very well assist the species characterization. Therefore, *V. rotundifolia* 'Regale' was the species, which displayed the most intrachromosomal asymmetry among the grapevines studied.

V. vinifera is a species that comprises thousands of varieties almost all raised from successive crossings among different varieties, which enabled the enhancement of the grapevine culture boundaries allowing for crop occupation of new environments. Although native to the European continent, *V. vinifera* presented some similarities with some American wild grapes studied by means of chromosome data and ideograms, mainly regarding *V. labrusca* with which it displayed a narrow affinity. Indeed, crossings between these two species have given rise to many vigorous hybrids, which have been very much appreciated as either table grapes or fruit juice in Brazil (MAIA, 2012). It is interesting to notice that these chromosome similarities observed among species, strengthen the hypothesis about *Vitis* origin as an ancient secondary polyploid (paleo-allohexaploid) firstly proposed by Patel and Olmo (1955). Recent comparative studies focusing on nuclear and chloroplast DNA sequences (ZECCA et al., 2012; ARADHYA et al., 2013) supported a monophyletic origin of *Vitis*, probably in the Eurasian lands, despite two different chromosome numbers, i.e., 2n=38 and 2n=40.

Although the supernumerary B chromosome has been widely reported in many plants, it has not yet been seen in the grape species studied. In some way it was expected, since almost all the cases studied and reported, dealt with plants characterized by a large or median chromosome size as perceived through the revision done by Houben et al. (2014), in contrast to the small chromosomes of grape. However, in *Aegilops mutica* Bs were remarkably seen only within aerial tissues and not in roots, leading to the questioning if B chromosomes could also occur in aerial parts of tissues of grapes.

Chromosome information recorded for the seven *Vitis* species analyzed, suggest that during the species diversification process the most crucial differences among them did not involve drastic changes in chromosome morphometry. Loss of DNA segments in some species or gain of repeated DNA segments in others, as well as small structural rearrangements may also have occurred, as suggested by Pierozzi (2011). However, this remains an open question requiring further studies which may open possibilities and furbish some clues through a comparative analysis of the genome organization of the species. The chromosomal information including the satellite and secondary constriction data obtained in the present research allowed for grapes characterization. Chromosome data may be used along with other approaches toward grape for the betterment of the comprehension of evolutionary tendencies into the genus.

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TABLE 1- Chromosome absolute (µm) and relative (%) lengths and the arm ratio (R) for *V. champinii*, *V. cinerea*, *V. girdiana*, *V. labrusca*, *V. rotundifolia* 'Regale' (Rotundifolia), *V. rupestris* and *V. vinifera* 'Italia'(Vinifera). N°= chromosome pair. R_{mean} = R mean values with standard deviation for each species; L-S% = difference of longest and shortest chromosome relative length.

	Champinii			**Cinerea**			**Girdiana**			**Labrusca**			**Rodundifolia**			**Rupestris**			**Vinifera**		
N°	**µm**	**%**	**R**	**µm**	**%**	**R**	**µm**	**%**	**R**	**µm**	**%**	**R**	**µm**	**%**	**R**	**µm**	**%**	**R**	**µm**	**%**	**R**
01	1,93*	6,91*	2,02	1,70*	7,31*	1,83	2,09*	7,40*	1,90	1,57*	7,63*	1,96	1,61*	7,02*	2,22	1,61*	7,19*	2,16	1,68*	7,40*	2,11
02	1,83	6,53	1,95	1,52	6,53	1,92	1,90	6,73	2,06	1,40	6,80	1,74	1,45	6,39	2,02	1,45	6,59	1,96	1,56	6,85	2,00
03	1,79	6,39	2,03	1,50	6,52	2,25	1,82	6,44	1,76	1,36	6,62	2,16	1,34	6,34	1,85	1,37	6,41	1,85	1,49	6,51	2,04
04	1,74	6,24	1,95	1,43	6,15	1,92	1,74	6,14	2,11	1,28	6,21	1,78	1,28	5,80	1,91	1,34	6,22	1,85	1,36	5,95	1,89
05	1,68	5,99	1,37	1,36	5,84	1,52	1,70	6,00	1,43	1,24	6,04	1,43	1,25	5,71	1,50	1,27	6,04	1,40	1,33	5,82	1,46
06	1,64	5,87	1,78	1,33	5,70	1,77	1,64	5,79	1,93	1,18	5,72	1,88	1,18	5,92	1,95	1,22	5,81	1,77	1,28	5,61	1,91
07	1,59	5,70	1,89	1,29	5,56	1,80	1,57	5,56	1,85	1,14	5,56	1,85	1,15	5,21	1,86	1,17	5,58	1,79	1,27	5,58	1,82
08	1,55	5,55	1,31	1,27	5,45	1,31	1,51	5,34	1,32	1,09	5,27	1,27	1,13	5,17	1,35	1,16	5,54	1,32	1,25	5,51	1,36
09	1,49	5,33	1,76	1,23	5,31	1,67	1,48	5,23	1,79	1,08	5,23	1,92	1,12	5,03	1,87	1,13	5,40	1,69	1,18	5,18	1,74
10	1,46	5,21	1,81	1,19	5,13	1,70	1,46	5,17	1,75	1,06	5,15	1,94	1,09	4,94	2,02	1,11	5,17	1,77	1,17	5,11	1,72
11	1,42	5,07	1,78	1,16	5,01	1,76	1,44	5,09	1,77	1,04	5,08	1,97	1,04	4,85	1,97	1,08	5,08	1,84	1,15	5,04	1,74
12	1,40	4,99	1,33	1,15	4,93	1,35	1,42	5,01	1,41	0,98	4,78	1,28	1,01	4,80	1,24	1,05	4,94	1,44	1,11	4,86	1,41
13	1,37	4,89	1,85	1,13	4,79	1,51	1,38	4,90	1,76	0,96	4,70	1,91	0,99	4,58	1,91	1,02	4,76	1,91	1,09	4,79	1,79
14	1,32	4,74	1,81	1,11	4,74	1,78	1,34	4,75	1,68	0,93	4,53	1,91	0,98	4,44	1,97	1,00	4,62	1,94	1,06	4,65	1,94
15	1,28	4,59	1,84	1,07	4,60	1,74	1,30	4,60	1,55	0,92	4,48	1,87	0,97	4,35	2,03	0,99	4,44	1,20	1,04	4,58	1,31
16	1,18	4,23	1,36	1,01	4,35	1,30	1,21	4,30	1,37	0,88	4,29	1,44	0,95	4,21	1,38	0,98	4,35	1,33	1,01	4,41	1,24
17	1,15	4,14	1,30	0,97	4,20	1,36	1,15	4,07	1,30	0,85	4,13	1,30	0,93	4,17	1,33	0,97	4,30	1,31	0,93	4,35	1,27
18	1,11	3,94	1,31	0,95	4,08	1,26	1,08	3,84	1,20	0,82	3,99	1,28	0,85	3,99	1,30	0,90	3,84	1,25	0,86	4,06	1,26
19	1,03*	3,69*	1,24	0,89*	3,80*	1,28	1,03*	3,64*	1,10	0,78*	3,79*	1,29	0,83	3,81	1,31	0,86*	3,71*	1,26	0,85*	3,75*	1,36
20													0,75*	3,57*	1,32						
R_{mea}	1,65±0,06[a]			1,65±0,06[a]			1,73±0,10[a]		[b]	1,69±0,07[a]			1,81±0,03[b]			1,68±0,03[a]			1,66±0,04[a]		
L-S%	3,22±0,57[a]			3,51±0,70[a]			3,75±0,19[a]			3,84±0,55[a]			3,97±0,47[a]			3,47±0,27[a]			3,64±0,63[a]		

* data from Pierozzi (2011) obtained only for chromosome pair 1 and 19 (20) and employed for comparison.
Mean values followed by the same letter = not significant at 1%
Mean values followed by different letters = significant by Tukey test

TABLE 2- Mean values in micrometer (μm) of secondary constriction (SC) and satellite segment (SAT) for *V. champinii*, *V. cinerea*, *V. girdiana*, *V. labrusca*, *V. rotundifolia* 'Regale', *V. rupestris* and *V. vinifera* 'Italia'

Species	SAT (μm)	SC (μm)
V. champinii	0,27 ± 0,06[a]	0,26 ± 0,09[a]
V. cinerea	0,20 ± 0,08[a]	0,17 ± 0,08[a]
V. girdiana	0,20 ± 0,06[a]	0,17 ± 0,06[a]
V. labrusca	0,15 ± 0,08[a]	0,14 ± 0,06[a]
V. rotundifolia	0,20 ± 0,07[a]	0,16 ± 0,03[a]
V. rupestris	0,17 ± 0,07[a]	0,16 ± 0,06[a]
V. vinifera	0,21 ± 0,08[a]	0,21 ± 0,06[a]

Mean values followed by the same letter = not significant at 1%
Mean values followed by different letters = significant by Tukey test

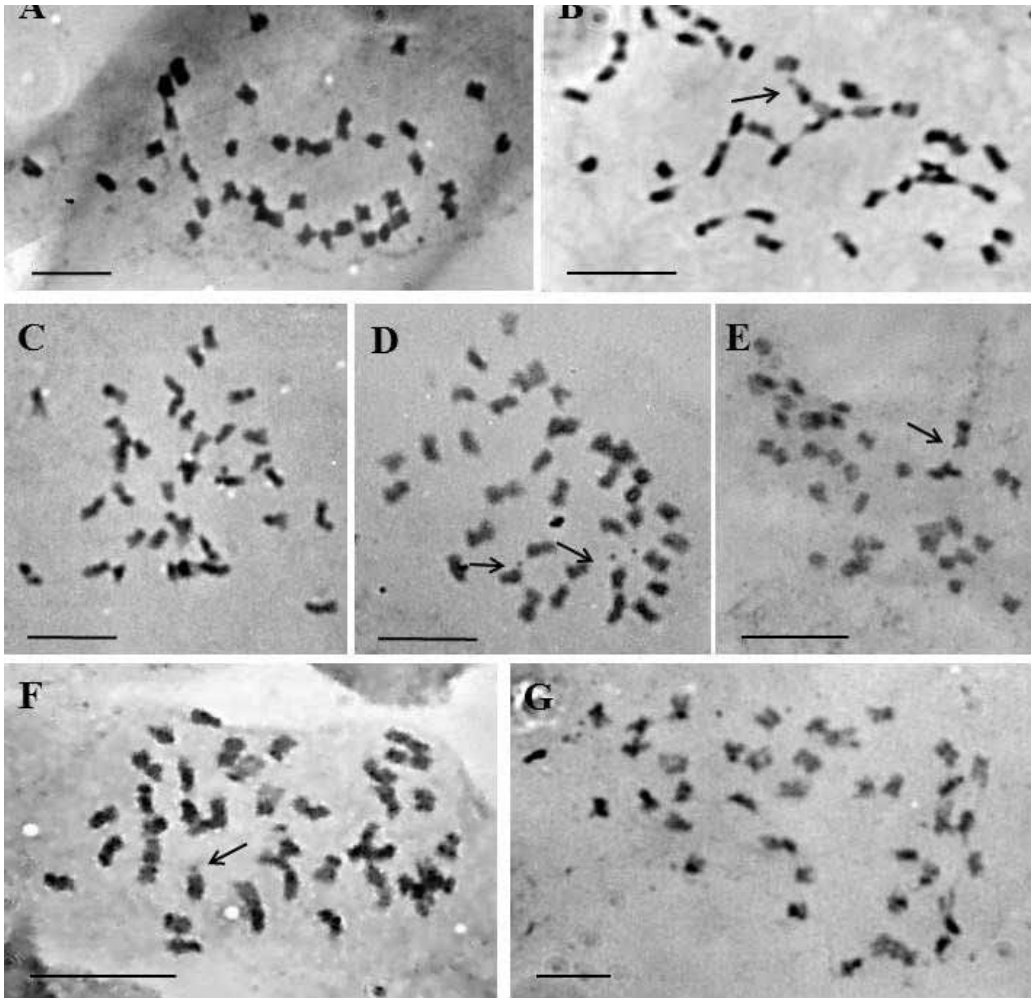

FIGURE 1 - Photomicrographies of mitotic chromosomes of **A-** *V. champinii*; **B-** *V. rotundifolia* 'Regale'; **C-** *V. girdiana*; **D-** *V. rupestris*; **E-** *V. vinifera* 'Italia'; **F-***V. labrusca*; **G-** *V. cinerea* after Giemsa staining. Bar = 5 μm. Arrows = chromosome with Satellite segment (SAT).

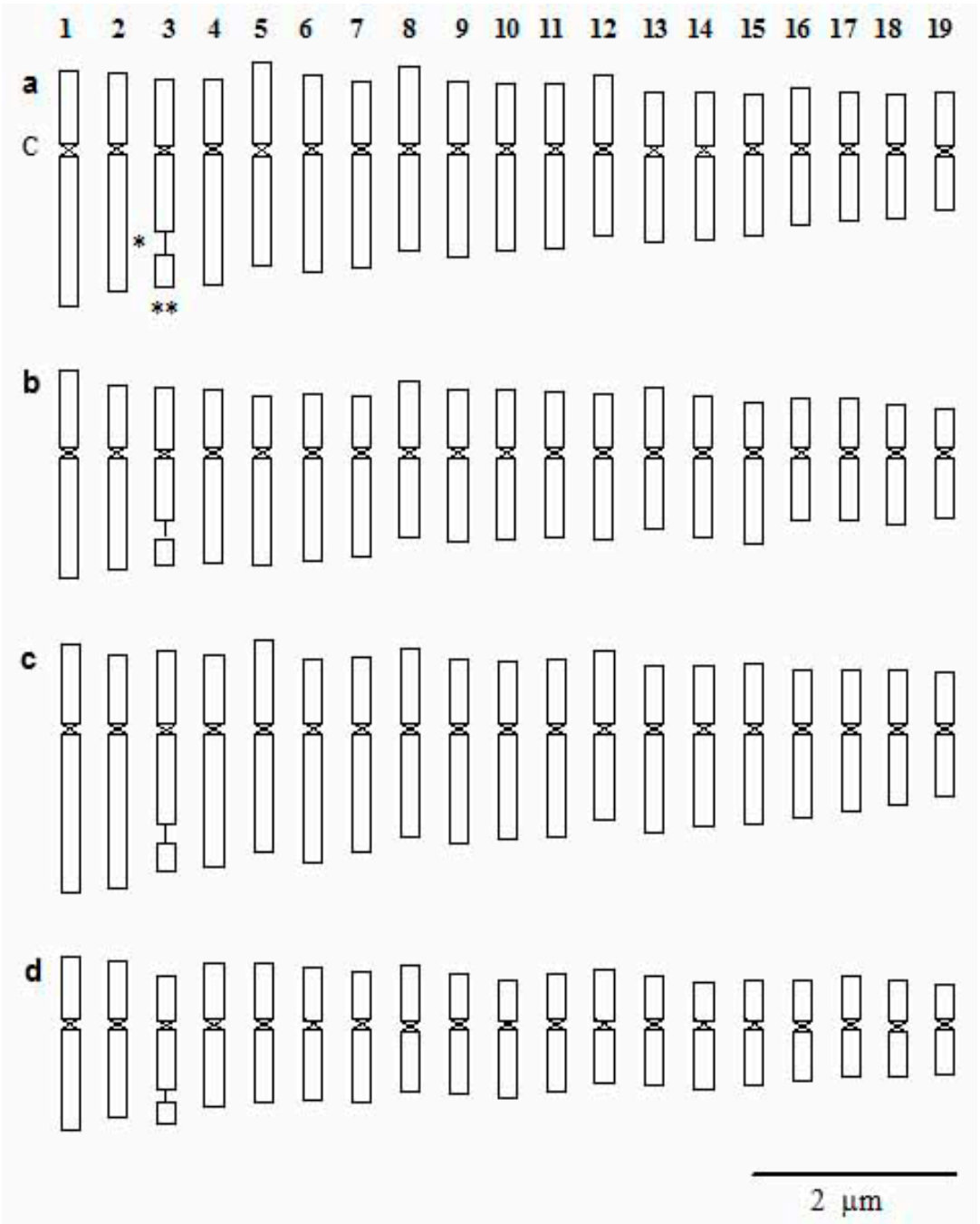

FIGURE 2- Ideograms obtained for **a**- *V. champinii*, **b**- *V. cinerea*, **c**-*V. girdiama*, and d- *V. labrusca*.C = centromere; * = secondary constriction (SC); ** = satellite segment (SAT).

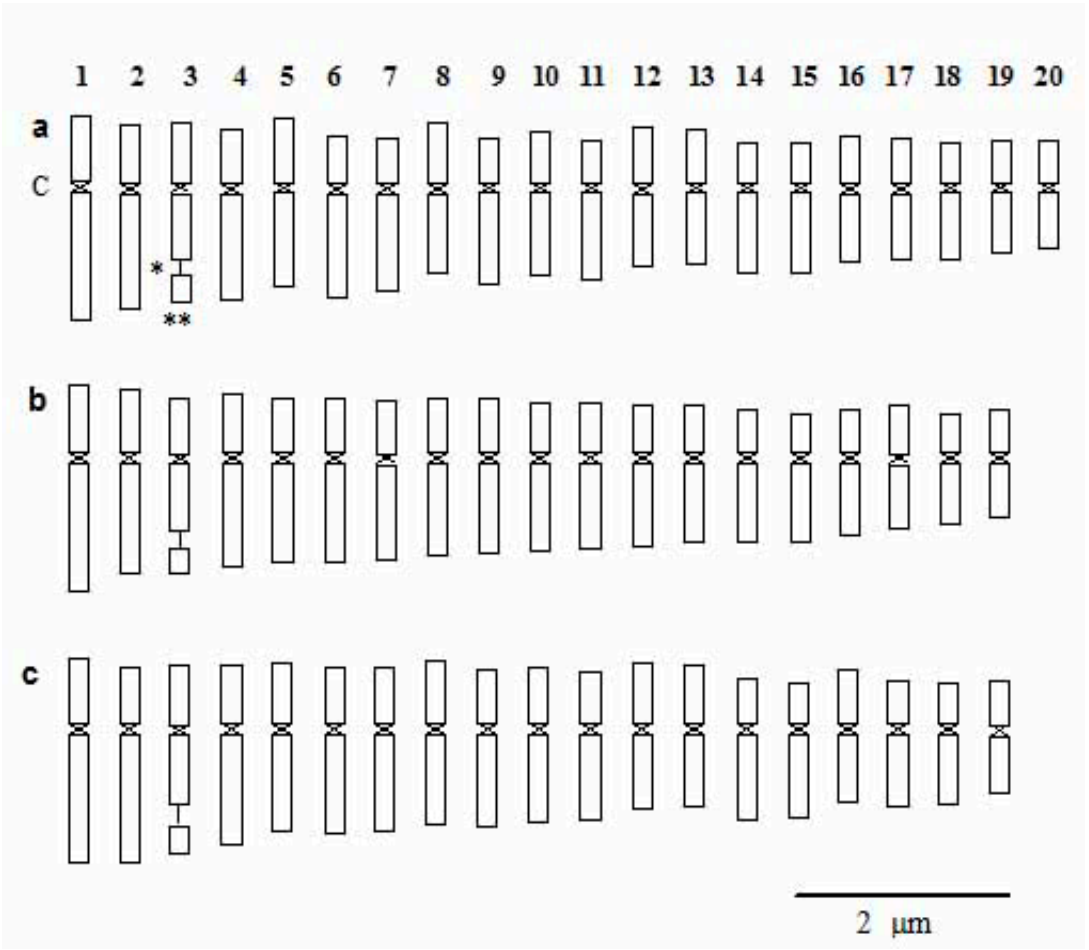

FIGURE 3- Ideograms obtained for **a**- *V. rotundifolia* 'Regale', **b**- *V. rupestris* and **c**- *V. vinifera* 'Italia'. C = centromere; * = secondary constriction (SC); ** = satellite segment (SAT).

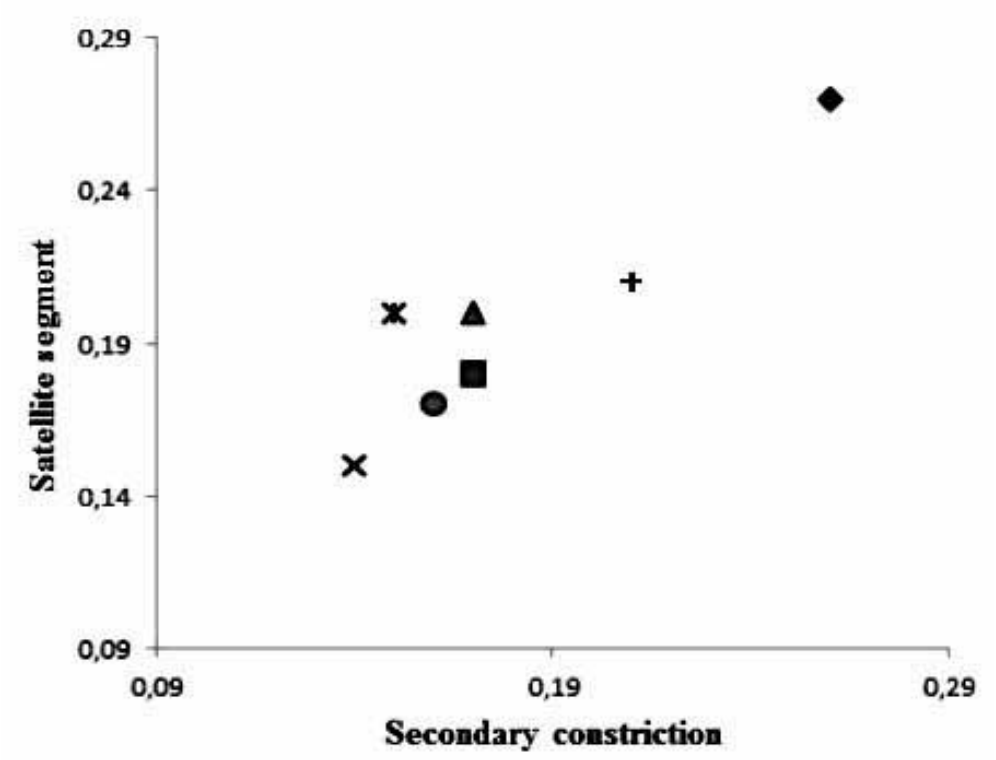

FIGURE 4- Scattered diagram for *V. champinii* (♦), *V. cinerea* (■),*V. girdiana* (■), *V. labrusca* (✖), *V. rotundifolia* (✱), *V. rupestris* (●) and *V. vinifera* (✚) based on satellite and secondary constriction measures.

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