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Seeds

In vitro germination to overcome dormancy in seeds of 'Red Globe', 'Italia' and 'Niagara Rosada' grapes

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Abstract- Dormancy in grape seeds slows the progress of many breeding programs of the crop, culminating in low uniformity and low germination percentages. Conventional methods used to overcome dormancy are time-consuming. Thus, *in vitro* seed germination emerges as a promising alternative to ensure the germination of grape seeds. In this study, we examined the *in vitro* germination and vigor of seedlings originating from seeds of 'Red Globe', 'Italia' and 'Niagara Rosada' grapes in growth media supplemented with five concentrations of gibberellic acid (GA_3) (0, 1.41, 2.83, 4.24 and 5.66 µmol L^{-1}) for 47 days. The use of GA_3 increased seed germination percentage and seedling vigor, in the three varieties. Therefore, for the *in vitro* germination of seeds of 'Red Globe' can be used between 1.41 to 4.24 µmol L^{-1} of GA_3. For 'Italia' grapes is indicated 1.41 µmol L^{-1} from GA_3 and for 'Niagara Rosada' grape is as between 1.41 to 5.66 µmol L^{-1} from GA_3 can be used.

Index terms: Gibberellic acid, plant tissue culture, grapes.

Germinação *in vitro* para a superação da dormência de sementes de videira 'Red Globe', 'Italia' e 'Niagara Rosada'

Resumo- A dormência em sementes de videira dificulta o avanço de muitos programas de melhoramento genético da cultura, ocasionando desuniformidade e baixa porcentagem de germinação. Os métodos convencionais utilizados para superar a dormência são demorados. Assim, a germinação *in vitro* das sementes torna-se uma alternativa promissora para garantir a germinação das sementes de videira. Buscou-se avaliar a germinação *in vitro* e o vigor das plântulas, oriundas de sementes das cultivares de videira 'Red Globe', 'Italia' e 'Niagara Rosada' em meios de cultivo suplementados com cinco concentrações de ácido giberélico (GA_3) (0; 1.41; 2.83; 4.24 e 5.66 µmol L^{-1}), por 47 dias. O uso do GA_3 promoveu um aumento na porcentagem de germinação das sementes e no vigor das plântulas das três variedades. Portanto, para a germinação *in vitro* de sementes de variedades comerciais de 'Red Globe' pode ser utilizado entre 1,41 e 4,24 µmol L^{-1} de GA_3. Para as uvas 'Italia', é indicado 1,41 µmol L^{-1} de GA_3, e para a uva 'Niagara Rosada' pode ser usado entre 1,41 e 5,66 µmol L^{-1} de GA_3.

Termos para Indexação: Ácido giberélico, cultura de tecidos vegetais, uvas.

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In grape breeding programs, seeds obtained from controlled crosses are very important materials of propagation (GISBERT et al., 2018). However, the low germination percentage of grape seeds constitutes a major obstacle to breeders (WANG et al., 2009; VAL et al., 2010). In grape seeds, dormancy is the main cause of low germination percentages (CELIK, 2001; WANG et al., 2009). In the search for methods to overcome dormancy in grape seeds, several authors have suggested cold stratification (BRASIL, 2009; WANG et al., 2009) along with exogenous application of gibberellic acid (GA_3) (ELLIS et al., 1983; ERGENOGLU et al., 1997; CELIK, 2001). However, in addition to not being effective in inducing germination in many seeds, these treatments require more than 120 days to produce normal seedlings.

In this scenario, the use of plant tissue culture with *in vitro* germination of grape seeds is a viable alternative to break dormancy, ensure seed germination and provide new genotypes for grape breeding programs. The present study proposes to examine *in vitro* germination and vigor of seedlings originating from seeds of table grapes in growth media supplemented with five concentrations of GA_3 (0, 1.41, 2.83, 4.24 and 5.66 µmol L^{-1}).

The plant material consisted of seeds of three commercial genotypes of table grape, namely, 'Red Globe', 'Italia' and 'Niagara Rosada'. Seeds were removed from the pericarp, washed in running water, disinfected with neutral detergent, left to dry at room temperature for 48 h and then stored in a refrigerator (±4°C) for seven days, until the *in vitro* germination test was assembled. The experiment was set up as a completely randomized design with a 3 × 5 factorial arrangement in which the *in vitro* germination of three table grape genotypes was tested in growth media with five concentrations of GA_3 (0, 1.41, 2.83, 4.24 and 5.66 µmol L^{-1}). Seven replicates were used, each of which was composed of a culture bottle containing 40 mL of growth medium and five seeds.

Seeds were cut in the micropyle region and in the upper region using cuticle pliers, in accordance with the methodology of Val et al. (2010). In a laminar flow hood, the seeds were then disinfected in 70% alcohol for 30 s, then in for 20 min a 1.0% sodium hypochlorite (NaClO) solution with two drops of Tween® 20 deposited for every 100 mL and lastly washed three times in autoclaved deionized water. The seeds were inoculated in growth medium constituted by half of the concentrations of MS medium mineral salts (MURASHIGE and SKOOG, 1962), White vitamins, 100 mg L^{-1} myo-inositol, 30 g L^{-1} sucrose, 200 mg L^{-1} polyvinylpyrrolidone (PVP) and five concentrations of GA_3 (Vetec®) (0, 1.41, 2.83, 4.24 and 5.66 µmol L^{-1}), with the pH adjusted to 5.7, and solidified with 7 g L^{-1} pure bacteriological agar (Vetec®). The growth medium was autoclaved for 20 min at 121 °C and 1.1 atm and 40 mL were distributed per culture bottle. The growth regulator GA_3 was added to the growth medium after autoclaving.

The bottles with the seeds were kept in the growth room at a temperature of 27±2 °C, photoperiod of 16:8 (light:dark) and luminosity supplied by Osram® daylight lamps with a luminous intensity of 54 µmol m^{-2} s^{-1}. After 47 days of growth, the seeds were examined for the germination percentage of normal seedlings, abnormal seedlings, seeds with primary root emission and ungerminated seeds (seedlings were considered normal only when showing expanded shoots and roots) and the vigor variables of number of leaves, shoot length, root length, total dry matter and germination speed index (GSI), which was evaluated at a two-day interval, during the 47 days of germination, by observing the number of seeds producing primary roots (MAGUIRE, 1962). The variables were subjected to an initial normality test (Shapiro-Wilk). Subsequently, an analysis of variance was performed and means were separated by the t test (LSD) at $P<0.05$, using Sisvar statistical software (FERREIRA, 2011).

Dormancy is characterized by a condition in which some seeds do not germinate even when exposed to favorable environmental conditions (BASKIN and BASKIN, 2004). In grape seeds, one of the reported types of dormancy is physical, caused by the thickness and hardness of the seed integument, which preclude the entry of water into the embryo (ELLIS et al., 1983). In *in vitro* conditions, Val et al. (2010) suggested making cuts on the seed integument to facilitate water imbibing by the embryo. Thus, the cuts made in the micropyle region and in the upper region, in our study, managed to overcome physical dormancy and hydrate the embryo. However, differences were observed for the germination and percentage results across the studied varieties. 'Itlia' grape exhibited the highest percentage of germination into normal seedlings (58.5%), differing statistically from 'Red Globe' (43.9%) and 'Niagara Rosada' (53.6%) (Figure 1a). At 47 days of germination *in vitro*, several 'Italia' seeds (16.6%) inoculated in growth medium with 1.41 µmol L^{-1} GA_3 were still producing primary root (Table 1). Additionally, the highest means observed for the vigor-related variables were obtained by varieties 'Red Globe' (number of leaves, shoot length and dry matter) and 'Italia' (GSI and number of leaves) (Table 2). For root length, however, no difference was detected between the three varieties (Figure 1e).

The use of GA_3 as a plant growth regulator provided a significant increase in seed germination percentage across the grape varieties, which rose from 13% (treatment without GA_3) to more than 56%, irrespective of the concentration (Figure 1b). Lack of GA_3 in the growth medium resulted in the highest percentages of ungerminated seeds for all studied varieties (Table 1). The use of GA_3 provided an increase in seedling vigor through GSI for 'Italia' grape and in shoot length for varieties

'Red Globe' and 'Italia'. Additionally, GA_3 considerably increased germination rate and speed in the three grape varieties (Figure 2). However, addition of 2.83 µmol L^{-1} of GA_3 to the growth medium led to the emergence of abnormal seedlings (10.4%) (Figure 1d).

A positive effect of GA_3 on the *in vitro* germination percentage of 'Niagara Rosada' seeds was reported by Val et al. (2010) and in 'Isabella' grape by Celik (2001), in different substrates. In seeds, GA_3 acts on the synthesis and activation of the hydrolytic enzymes present in the endosperm tissue (MOSHKOV et al., 2008).

The conventional method provided by the Rules for Seed Testing (RST) for dormancy breaking in seeds of *Vitis vulpina* indicates pre-chilling in wet substrate at a temperature of 3-5ºC for a period of three months, to only then set them to germinate (BRASIL, 2009). The entire process may exceed 120 days. By contrast, *in vitro* germination of grape seeds with GA_3 supplementation is a promising strategy in grape breeding programs, as it allows for breaking seed dormancy, accelerating the germination period and obtaining vigorous seedlings (Figure 3) in 47 days. The significant variability observed among the three varieties was due to the genotypic difference between species. Therefore, for the *in vitro* germination of seeds of 'Red Globe' can be used between 1.41 to 4.24 µmol L^{-1} of GA_3. For 'Italia' grapes is indicated 1.41 µmol L^{-1} from GA_3 and for 'Niagara Rosada' grape is as between 1.41 to 5.66 µmol L^{-1} from GA_3 can be used..

Table 1. The interaction between three grape seeds varieties and five concentrations of GA_3 after 47 days of *in vitro* germination, for the variables: seeds with primary root emission and ungerminated seeds.

Primary root emission (%)					
Varieties	GA_3 (µmol L^{-1})				
	0	1.41	2.83	4.24	5.66
Red Globe	8.4 Aa	2.8 Ab	5.6 Aa	2.8 Aa	0.0 Aa
Italia	0.0 Ba	16.6 Aa	2.8 Ba	0.0 Ba	2.8 Ba
Niagara Rosada	2.8 ABa	0.0 Bb	11.4 Aa	3.2 ABa	5.6 ABa
Ungerminated seeds (%)					
Varieties	GA_3 (µmol L^{-1})				
	0	1.41	2.83	4.24	5.66
Red Globe	77.0 Aa	57.0 Ba	34.2 Ca	37.0 Ca	34.2 Ca
Italia	85.6 Aa	0.0 Cc	8.4 BCb	20.0 Ba	8.4 BCb
Niagara Rosada	82.8 Aa	28.4 Bb	20.0 Bab	23.2 Ba	25.6 Bab

Note. Different letters indicate significant differences by t test (LSD) ($p \leq 0.05$). Uppercase letter comparisons in the row and lowercase letter comparisons in the column.

Table 2. The interaction between three grape seeds varieties and five concentrations of GA_3 after 47 days of *in vitro* germination, for the variables: GSI, number of leaves, shoot length and dry matter. .

GSI					
Varieties	GA_3 (µmol L^{-1})				
	0	1.41	2.83	4.24	5.66
Red Globe	0.88 Da	1.51 Cb	2.21 ABa	2.53 Aa	1.73 BCb
Italia	0.59 Ba	2.25 Aa	2.43 Aa	2.79 Aa	2.53 Aa
Niagara Rosada	0.61 Ca	1.43 Bb	2.00 ABa	1.57 ABb	2.08 Aab
Number of leaves					
Varieties	GA_3 (µmol L^{-1})				
	0	1.41	2.83	4.24	5.66
Red Globe	5.9 ABa	7.5 Aa	5.3 Ba	5.9 ABa	6.0 ABa
Italia	4.2 Ba	7.3 Aa	5.7 ABa	5.1 Ba	4.7 Ba
Niagara Rosada	6.0 Aa	4.2 Ab	5.3 Aa	5.4 Aa	4.8 Aa
Shoot length (mm)					
Varieties	GA_3 (µmol L^{-1})				
	0	1.41	2.83	4.24	5.66
Red Globe	73.8 Ca	118.9 Aa	89.2 BCa	107.4 ABa	122.9 Aa
Italia	34.3 Bb	77.3 Ab	68.6 Ab	58.2 Ab	68.1 Ab
Niagara Rosada	36.6 Ab	41.3 Ac	49.1 Ab	42.2 Ab	50.6 Ab
Dry matter (g)					
Varieties	GA_3 (µmol L^{-1})				
	0	1.41	2.83	4.24	5.66
Red Globe	0.0323 Ba	0.0792 Aa	0.0277 Ba	0.0481 Ba	0.0421 Ba
Italia	0.0168 Aa	0.0456 Ab	0.0437 Aa	0.0412 Aa	0.0421 Aa
Niagara Rosada	0.0224 Aa	0.0279 Ab	0.0342 Aa	0.0379 Aa	0.0457 Aa

Note. Different letters indicate significant differences by t test (LSD) ($p \leq 0.05$). Uppercase letter comparisons in the row and lowercase letter comparisons in the column.

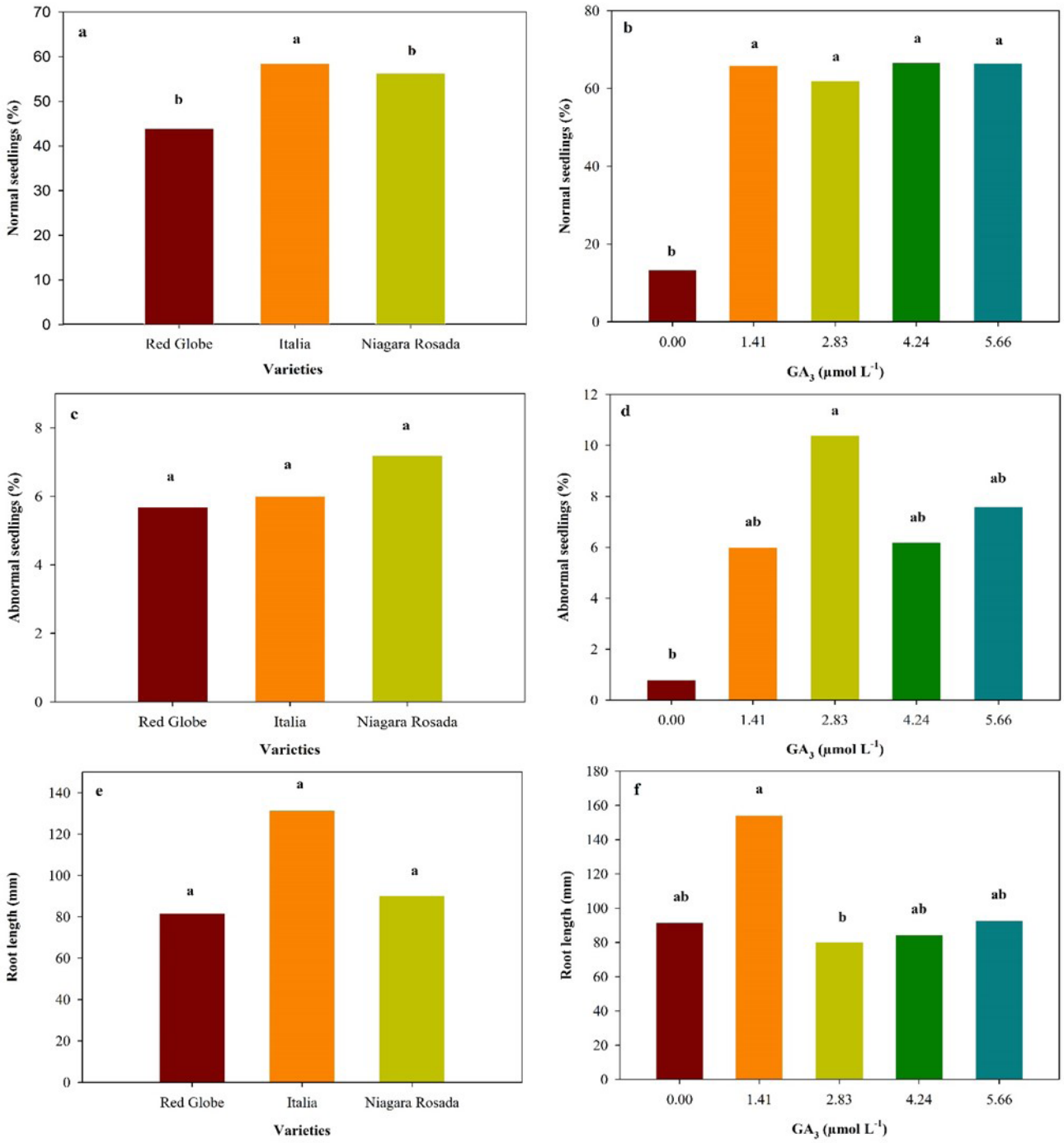

Figure 1. *In vitro* germination means of normal seedlings of three grape varieties (a) in culture medium containing five concentrations of GA_3 (b) after 47 days of cultivation. *In vitro* germination means of abnormal seedlings of three grape varieties (c) in culture medium containing five concentrations of GA_3 (d) after 47 days of cultivation. Roots length of three grape varieties seedling (e) placed to germinate *in vitro* in medium containing five concentrations of GA_3 (f) for 47 days. Means followed by the same lowercase letter do not differ by t test (LSD) at $p < 0.05$.

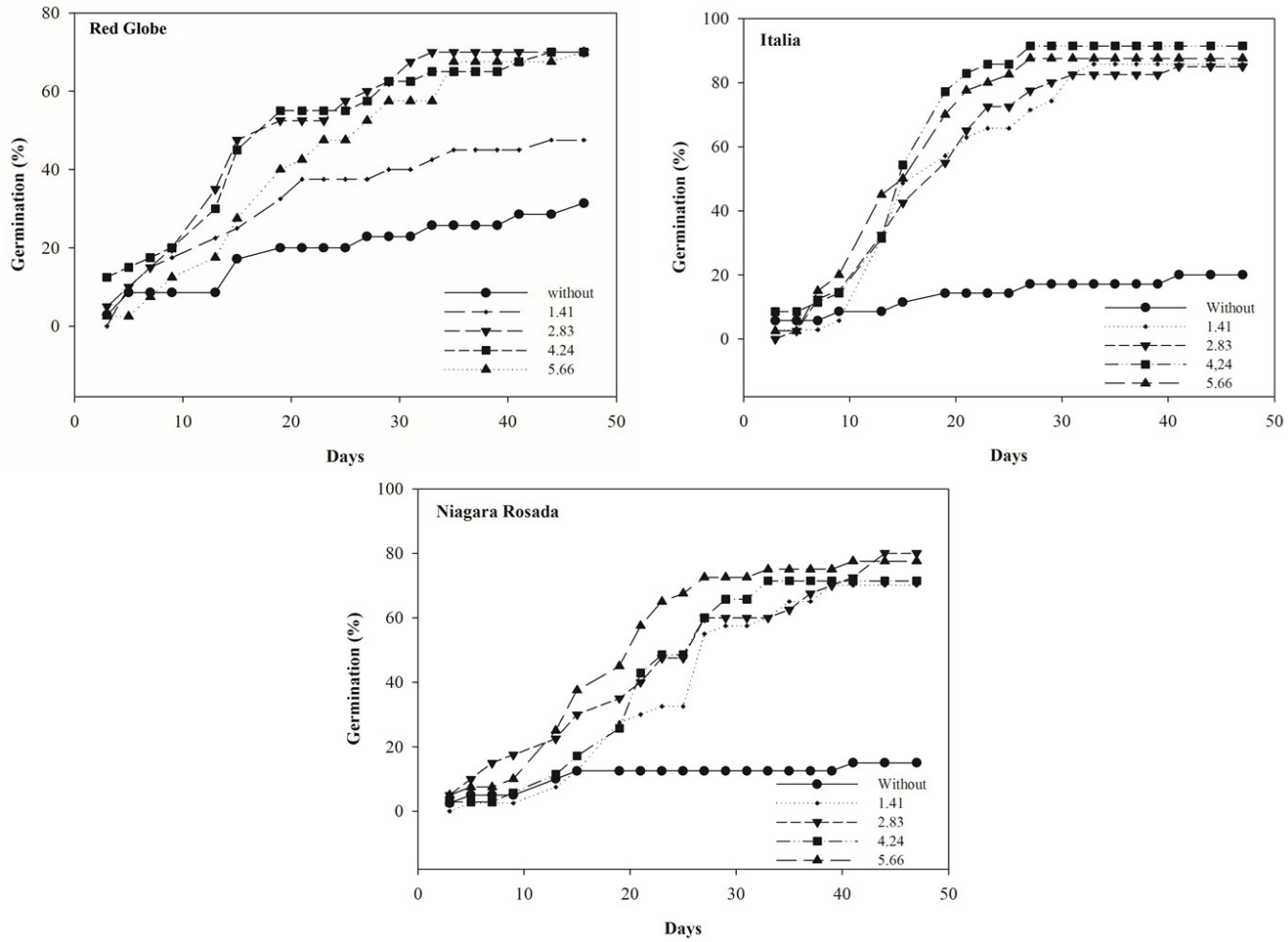

Figure 2. *In vitro* germination speed of the grape varieties Red Globe, Italia and Niagara Rosada during a period of 47 days of *in vitro* germination in culture medium containing five concentrations of GA_3 (without, 1,41, 2,83, 4,24 e 5,66 µmol L^{-1}).

Figure 3. Normal seedlings of Red Globe grape after 47 days of *in vitro* germination in mediun culture: without GA_3 (a), with 1.41 µmol L^{-1} of GA_3 (b), 2.83 µmol L^{-1} of GA_3 (c), 4.24 µmol L^{-1} of GA_3 (d) and 5.66 µmol L^{-1} of GA_3 (e).

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