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Effect of different treatments on germination of Romi red grape seeds

by

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Der Einfluß verschiedener Behandlungsbedingungen auf die Samenkeimung bei der Rebsorte Romi red

Zusammenfassung. — Die Samen der Rebsorte Romi red keimten nur nach Stratifikation, entweder unter Kälte- oder unter Wärmebedingungen (5 ± 1 °C bzw. 18 ± 1 °C). Normale Sämlinge, d. h. solche mit Plumula, entwickelten sich nur bei einer Stratifikationsdauer von mindestens 60 d. Durch Kaltstratifikation wurde der Anteil keimender Samen und normaler Sämlinge bedeutend erhöht, wenn die Samen zuvor warmstratifiziert worden waren. Das Einweichen der Samen vor der Aussaat in unterschiedlichen Konzentrationen von IAA, IBA oder Ethrel förderte die Bildung normaler Sämlinge in keiner Weise. Umgekehrt wurden im Hinblick auf die Samenkeimung und die Bildung normaler Sämlinge die besten Ergebnisse mit GA_3, vor allem in der Konzentration von 5000 ppm erzielt; auch Thioharnstoff und Kinetin zeigten einen positiven Einfluß. GA_3-Behandlung der Samen unmittelbar vor der Stratifikation war wirksamer als danach.

Introduction

As already known, grape seeds are dormant when freshly extracted from fruits (Scott and Ink 1950). Various plant regulators and chemicals, such as gibberellic acid, thiourea, indoleacetic acid, kinetin and ethephon, were found to substitute completely or partially the conditions needed for improving seed germination of different plants (Chadha and Manon 1969, Pal *et al.* 1976, Sinha *et al.* 1977, Forlani and Coppola 1978).

The grapevine Romi red being an important table grape cultivar, more tolerant to salinity than Thompson Seedless (Taha *et al.* 1972), was chosen for this study; to our knowledge, the dormancy requirement of seeds of this cultivar has not been studied up to now. So trials were conducted to investigate the germination conditions and to elaborate an effective method for producing seedlings of Romi red.

Material and methods

The required seeds of Romi red grape cultivar were obtained from vines grown at the north western coastal belt of Egypt in the years 1975—1977. Immediately before use, the seeds were immersed in a 2 % solution of sodium hypochlorite for 5

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min to avoid microorganism infection, then washed with sterile distilled water for several times. Seeds were subjected to either of the following treatments:

1. Cold (5 ± 1 °C) or warm (18 ± 1 °C) stratification in sterilized moist sand for 0, 30, 60, 90, 120 and 150 d.
2. Alternate stratification temperatures keeping the seeds at 18 ± 1 °C for 30 or 60 d and then at 5 ± 1 °C for 15 or 30 d.
3. Soaking them in one of the following aqueous solutions of growth regulators for 24 h before sowing: GA_3, thiourea, kinetin, IAA, IBA, ethephon, or distilled water (control). The concentrations used for experiment are mentioned in Tables 3 and 4.
4. Combination of cold stratification for 60 d and growth regulator treatment mentioned under 3.
5. Applying GA_3 at 50 or 5000 ppm before or after cold stratification for 30 d.

After each treatment, seeds were washed with sterile distilled water. They were sown in plastic pots filled with sterilized clean sand, each pot containing 30 seeds. Three pots representing three replicates were utilized for each treatment. The pots were placed in an incubator at 30 °C with relative humidity of 60—65 % and a light period of 12 h/d (Yeou-Der *et al.* 1968).

The percentages of germination and normal seedlings (seedlings with plumule) were counted 60 d after sowing.

Table 1

Effect of cold and warm stratification on percentages of germination and normal seedlings during 1975—1976 and 1976—1977

Einfluß der Kalt- und Warmstratifikation auf den Anteil gekeimter Samen und normaler Sämlinge (1975—1976 and 1976—1977)

		1975—1976		1976—1977	
	Length of stratification d	Germination %	Normal seedlings %	Germination %	Normal seedlings %
Cold at 5 ± 1 °C	0	0.0	0.0	0.0	0.0
	30	10.0	0.0	12.0	0.0
	60	30.7	6.8	29.3	7.9
	90	45.3	16.5	32.0	12.0
	120	52.0	29.3	53.4	28.5
	150	29.3	8.3	28.0	8.2
Warm at 18 ± 1 °C	0	0.0	0.0	0.0	0.0
	30	16.0	0.0	15.0	0.0
	60	36.0	7.3	34.7	8.7
	90	50.0	15.5	37.3	12.9
	120	58.7	32.1	50.0	25.2
	150	0.0	0.0	0.0	0.0
	New L.S.D.				
	0.05	0.9	0.2	1.0	0.2
	0.01	1.3	0.3	1.3	0.3

Results and discussion

It can be seen from the results as shown in Table 1 that germination of Romi red grape seeds required a stratification period either at cold or warm conditions. Germination percentage was gradually increased with the progress of stratification period up to 120 d. These findings are in agreement with those reviewed by HARMAN and WEINBERGER (1959) according to which stratification of grape seeds in damp sand at low temperature markedly improved germination. CHOHAN and DHILLON (1976) also showed that long stratification periods were generally better for seed germination of different grape cultivars than shorter periods. Production of normal seedlings (with plumule) required at least 60 d of stratification. After that period, there was a gradual raise in the percentage of normal seedlings up to 120 d. These findings ascertained the earlier results of SCOTT and INK (1950) that direct sowing of grape seeds obtained from freshly harvested fruits gave very low germination capacity and that plants which had grown were dwarfs.

Treatments of alternate stratification temperatures (Table 2) indicated that seed germination and production of normal seedlings increased pronouncedly when seeds were first subjected to warm stratification conditions and then cold stratified. These findings supported the results of SUSZKA (1976) on different species of prunus.

Regarding the effect of growth regulators, the data shown in Table 3 proved that soaking grape seeds in indolebutyric acid, indoleacetic acid or Ethrel for 24 h before sowing resulted in a limited effect on seed germination, but failed completely to produce normal seedlings. In this respect, RANDHAWA and PAL (1968) proved that IAA or IBA did not affect subsequent seedling growth of Bhokri grape cultivar. FORLANI and COPPOLA (1978) also showed that soaking grapevine seeds in solutions of IAA or ethephon for 24 h had little effect on germination percentage. Conversely, gibberellic acid, especially at 5000 ppm, gave the best results with respect to germination percentage and seedlings with plumule percentage. Similarly, it was found that gibberellic acid treatments promoted germination of grape seeds (PAL *et*

Table 2

Effect of alternate stratification temperatures on germination parameters (1976—1977)

Einfluß alternierender Stratifikationstemperaturen auf die Keimungsparameter (1976—1977)

Warm stratification at 18 ± 1 °C d	Cold stratification at 5 ± 1 °C for 15 d		30 d	
	Germination %	Normal seedlings %	Germination %	Normal seedlings %
0	8.2	0.0	12.0	0.0
30	60.0	26.3	76.0	48.8
60	67.9	35.7	75.0	49.6
Average	45.3	20.7	54.3	32.9
New L.S.D.				
0.05	0.05	0.03	0.07	0.05
0.01	0.07	0.04	0.11	0.07

al. 1976). Thiourea at 2.5 % or 5.0 % ranked second with respect to germination percentage. Accordingly, thiourea treatments increased the seed germination of Rosem-T-Lahore grape cultivar (Randhawa and Negi 1964). On the other hand, seeds treated with kinetin led to a higher percentage of normal seedlings as compared with seeds treated with thiourea. Kinetin also increased the percentage of germination of the grapevine cultivar Rabose Piave (Forlani and Coppola 1978).

Concerning the effect of the combined treatments of stratification and growth regulators, in general all combinations increased significantly the percentage of germinated seeds as compared with stratification (Table 4). Gibberellic acid, especially at 5000 ppm, was the most active growth regulator for raising the germination

Table 3

Effect of growth regulators on percentages of germination and normal seedlings during 1975—1976 and 1976—1977

Einfluß von Wachstumsregulatoren auf den Anteil gekeimter Samen und normaler Sämlinge (1975—1976 und 1976—1977)

1975—1976				1976—1977			
Treatments		Germination %	Normal seedlings %	Treatments		Germination %	Normal seedlings %
Control[1])	A	0.0	0.0	Control[1])	A	0.0	0.0
"	B	4.2	0.0	"	B	4.0	0.0
GA_3	50 ppm	15.0	4.1	GA_3	50 ppm	14.6	4.0
"	100 "	14.7	4.9	"	500 "	20.0	8.0
"	500 "	26.6	9.3	"	5000 "	42.7	20.1
"	1000 "	35.3	17.3	Kinetin	5 ppm	12.0	3.3
"	5000 "	56.7	29.4	"	10 "	13.3	4.1
"	10000 "	41.4	16.6	"	20 "	14.6	4.8
Thiourea	0.25 %	6.6	0.0	Thiourea	0.25 %	8.0	0.0
"	0.5 "	6.3	0.0	"	1,0 "	13.3	2.2
"	1,0 "	11.6	2.2	"	2.5 "	25.0	3.7
"	2.0 "	14.0	2.9	IBA	50 ppm	6.0	0.0
"	2.5 "	32.1	3.7	"	100 "	10.6	0.0
"	5.0 "	30.7	0.0	"	500 "	12.0	0.0
IBA	50 ppm	6.0	0.0	IAA	50 ppm	10.6	0.0
"	100 "	8.7	0.0	"	100 "	12.0	0.0
"	250 "	8.7	0.0	"	500 "	13.3	0.0
"	500 "	10.0	0.0	Ethrel	50 ppm	10.6	0.0
				"	100 "	12.0	0.0
				"	200 "	6.5	0.0
New L.S.D.	0.05	1.4	0.16	New L.S.D.	0.05	1.4	0.1
	0.01	1.8	0.2		0.01	1.8	0.2

[1]) Control A = Without soaking in water.
" B = Soaked in water for 24 h.

percentage. It was followed by Kinetin, thiourea, IAA and IBA, while ethephon showed the least effect. Similarly, RHANDHAWA and PAL (1968) obtained the highest germination percentage when the grape seeds were cold stratified and then soaked in GA_3 solution before sowing. The same investigators also recommended the use of kinetin or thiourea for improving germination of stratified grape seeds. In contrast to our findings, SINHA *et al.* (1977) obtained the maximum percentage of germination of apple seeds by stratification for 60 d followed by treatment with Ethrel at 250 ppm. Generally GA_3 and then kinetin, at all tested concentrations, enhanced the production of normal seedlings from cold stratified seeds. FRANKLAND (1961) also suggested that gibberellins would overcome the dwarf growth of unstratified Rosaceous seedlings.

Table 4

Effect of cold stratification at 5 ± 1 °C for 60 d in combination with growth regulators on percentages of germination and normal seedlings during 1975—1976 and 1976—1977

Einfluß der Kaltstratifikation (60 d bei 5 ± 1 °C) in Verbindung mit Wachstumsregulatoren auf den Anteil gekeimter Samen und normaler Sämlinge (1975—1976 und 1976—1977)

1975—1976				1976—1977			
Treatments		Germination %	Normal seedlings %	Treatments		Germination %	Normal seedlings %
Control[1])	A	30.7	6.8	Control[1])	A	29.3	7.9
"	B	35.0	11.3	"	B	34.7	12.4
GA_3	50 ppm	59.7	31.3	GA_3	50 ppm	58.2	31.3
"	100 "	61.0	34.2	"	500 "	62.7	36.2
"	500 "	64.7	38.0	"	5000 "	70.7	48.9
"	1000 "	65.3	38.7	Kinetin	5 ppm	45.3	21.2
"	5000 "	81.4	57.2	"	10 "	50.7	26.8
"	10000 "	78.7	54.5	"	20 "	53.4	29.3
Thiourea	0.25 %	48.0	15.9	Thiourea	0.25 %	39.0	14.1
"	0.5 "	50.7	20.1	"	1.0 "	48.0	24.0
"	1.0 "	62.0	32.2	"	2.5 "	42.0	19.1
"	2.0 "	60.0	26.9	IBA	50 ppm	41.4	16.1
"	2.5 "	50.7	20.2	"	100 "	50.0	21.1
"	5.0 "	42.6	4.0	"	500 "	40.0	13.3
IBA	50 ppm	53.4	17.3	IAA	50 ppm	46.6	19.4
"	100 "	58.7	22.7	"	100 "	48.0	23.8
"	250 "	48.0	16.1	"	500 "	41.3	16.2
"	500 "	45.3	11.6	Ethrel	50 ppm	38.7	14.9
				"	100 "	42.7	18.6
				"	200 "	34.7	12.1
New L.S.D.				New L.S.D.			
	0.05	1.6	0.16		0.05	1.5	0.1
	0.01	1.9	0.2		0.01	1.9	0.16

[1]) Control A = Without soaking in water.
" B = Soaked in water for 24 h.

Table 5

Effect of GA_3 treatment before and after stratification at 5 ± 1 °C for 30 d on percentages of germination and normal seedlings during 1977—1978

Einfluß der Behandlung mit GA_3 vor und nach Kaltstratifikation (30 d bei 5 ± 1 °C) auf den Anteil gekeimter Samen und normaler Sämlinge (1977—1978)

	Treatments	Germination %		Normal seedlings %	
Control[1]) A		12.0		0.0	
" B		21.3		0.0	
GA_3 before stratification	50 ppm	66.7		32.1	
	5000 ppm	93.3		65.7	
Average		48.3		24.5	
Control[1]) A		12.0		0.0	
" B_1		14.6		0.0	
GA_3 after stratification	50 ppm	44.0		12.0	
	5000 ppm	60.0		31.9	
Average		32.7			
New L.S.D.		0.05	0.01	0.05	0.01
Treatment (T)		1.3	1.7	0.1	0.2
Stratification (S)		0.5	0.7	0.04	0.05
T × S		1.1	1.5	0.08	0.11

[1]) Control A = Without soaking in water.
" B = Soaked in water for 24 h before stratification.
" B_1 = Soaked in water for 24 h after stratification.

Applying GA_3, especially at 5000 ppm, to seeds immediately before cold stratification was more effective in raising germination capacity and percentage of normal seedlings than treatment after stratification (Table 5). However, both types of combined treatments of GA_3 and stratification increased the germination percentage of seeds considerably, compared with only stratified seeds. In this respect, best germination of Bangalore Blue grape seeds (Randhawa and Negi 1964), Bhokri, Black Hamburg and Hussaini grape cultivars (Randhawa and Pal 1968) was obtained when the seeds were first stratified and then soaked in GA_3, the reverse trend being more favourable for Black Muscat grape seeds (Kachru *et al.* 1972).

Summary

Germination of seeds required a stratification period either at cold (5 ± 1 °C) or warm (18 ± 1 °C) conditions. Besides, production of normal seedlings (seedlings with plumule) needed stratification of at least 60 d. Cold stratification treatments increased pronouncedly the percentages of seed germination and normal seedlings when seeds were first subjected to warm stratification conditions. Soaking the

seeds in IAA, IBA or Ethrel at different concentrations before sowing failed completely to produce normal seedlings. Conversely, the best results with respect to germination percentage and normal seedlings were obtained with GA$_3$, especially at 5000 ppm, followed by thiourea and kinetin. Applying GA$_3$ to seeds immediately before stratification was more effective than adding it after stratification.

Literature cited

CHADHA, K. L. and MANON, V. N., 1969: Studies on the germination of grape seeds. II. Germination of freshly extracted and after-ripened grape seed, as influenced by gibberellic acid and thiourea treatments. J. Res. Punjab Agricult. Univ., Ludhiana 6, 821—828.

CHOHAN, G. S. and DHILLON, B. S., 1976: Seed dormancy and endogenous growth substances in Anab-e-Shahi grapes. Vitis 15, 5—10.

FORLANI, M. and COPPOLA, V., 1978: The effect of various growth regulators and c-AMP on the germination of seeds of the grapevine cv. Raboso Piave (It.). Riv. Viticolt. Enol. 31, 99—104. [Abstr.: Hort. Abstr. 48, 8958.]

FRANKLAND, B., 1961: Effect of gibberellic acid, kinetin and other substances on seed dormancy. Nature 192, 678—679.

HARMAN, F. N. and WEINBERGER, J. H., 1959: Effect of storage and stratification on germination of *vinifera* grape seeds. Proc. Amer. Soc. Hort. Sci. 73, 47—50.

KACHRU, R. B., SINGH, R. N. and YADAV, I. S., 1972: Physiological studies on dormancy in grape seeds (*Vitis vinifera* var. Black Muscat). II. On the effect of exogenous applications of growth substances, low chilling temperature and subjection of the seeds to running water. Vitis 11, 289—295.

PAL, R. N., SINGH, R., VIJ, V. K. and SHARMA, J. N., 1976: Effect of gibberellins GA$_3$, GA$_{4+7}$ and GA$_{13}$ on seed germination and subsequent seedling growth in Early Muscat grape (*Vitis vinifera*). Vitis 14, 265—268.

RANDHAWA, G. S. and NEGI, S. S., 1964: Preliminary studies on seed germination and subsequent seedling growth in grape. Indian J. Hort. 21, 186—196.

— — and PAL, N. G., 1968: Further studies on seed germination and subsequent seedling growth in grape (*Vitis* spp.). Indian J. Hort. 25, 148—158.

SCOTT, D. H. and INK, D. P., 1950: Grape seed germination experiments. Proc. Amer. Soc. Hort. Sci. 56, 134—139

SINHA, M. M., PAL, R. S. and AWASTHI, D. N., 1977: Effect of stratification and plant growth regulating substances on seed germination and seedling growth in apples. Progr. Hort. 9, 27—30.

SUSZKA, B., 1976: Increase of germination capacity of mazzard cherry (*Prunus avium* L.) seed through the induction of secondary dormancy. Arboretum Kornickie 21, 257—270.

TAHA, M. W., EL-SEWEY, A. and FADLIAH, Z. G., 1972: Salt tolerance of grape, guava and olive plants. Alexandria J. Agricult. Res. 1, 123—135

YEOU-DER, K., WEAVER, R. J. and POOL, R. M., 1968: Effect of low temperature and growth regulators on germination of "Tokay" grapes. Proc. Amer. Soc. Hort. Sci. 92, 323—330.

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