Vitis 11, 289—295 (1972)

Division of Horticulture and Fruit Technology, Indian Agricultural Research Institute, New Delhi 12, India

Physiological studies on dormancy in grape seeds (Vitis vinifera var. Black Muscat)

II. On the effect of exogenous application of growth substances, low chilling temperature and subjection of the seeds to running water

by

Raj B. Kachru, R. N. Singh and I. S. Yadav

Physiologische Untersuchungen zur Dormanz von Rebensamen (Vitis vinifera var. Black Muscat). — II. Über den Einfluß äußerlich angewandter Wuchsstoffe, niedriger Temperaturen und des Auswaschens der Samen in fließendem Wasser

Zusammenfassung. — Um die Dormanz von Rebensamen in der kürzestmöglichen Zeit zu brechen, wurden sie stratifiziert, mit Wuchsstoffen behandelt und in fließendem Wasser ausgewaschen. Es wurde beobachtet, daß Rebensamen, die in einem Musselinsäckchen 8 Tage lang ausgewaschen wurden, schon in dem Säckchen keimten (34,00%). Der höchste Keimungsprozentsatz (73,00%) wurde erzielt, wenn die Samen 16 Tage lang mit fließendem Wasser behandelt wurden. Bei einer 12tägigen Behandlung (72,11% gekeimter Samen) war die Standfestigkeit der Sämlinge jedoch am besten. Das zweitbeste Resultat wurde mit GS_3-Behandlung (2000 ppm) vor der Stratifikation erzielt, wobei die Rebensamen nach 8tägiger Stratifikation keimten; der höchste Anteil gekeimter Samen betrug — nach 30tägiger Stratifikation — 81,05%. 1monatige Stratifikation mit anschließender GS_3-Behandlung lieferte das drittbeste Ergebnis. Ferner gaben Rebensamen, die 96 Std. lang in Wasser gequollen waren, einen wasserlöslichen Inhibitor ab. Dieser konnte die Keimung GS_3-behandelter Samen hemmen. Mit Hilfe des Kressewurzeltests ließ sich im Einweichwasser ein Hemmstoff mit einem R_f von 0,70—0,85 nachweisen. Möglicherweise stört der wasserlösliche Inhibitor den Stoffwechsel der Keimung, so daß die Wirkung der Wuchsstoffe in den Samen verdeckt wird.

Introduction

Grape seeds will not germinate until, after ripening, they have been kept under moist conditions and controlled temperature for about three months. The present work is therefore, focussed to break the dormancy period of grape seeds in the minimum possibe time, particularly with a view to raising grape hybrid seedlings earlier under North Indian conditions so that these seedlings may get established before the commencement of winter.

Materials and Methods

Grape seeds were collected from the variety Black Muscat grown at the experimental orchard of IARI, New Delhi. They were washed and divided into four lots. The treatments were:

a. Seeds stratified and treated with growth substances.
b. Seeds first treated with GA_3 and then stratified.
c. Seeds subjected to running water.
d. Dried seeds kept at room temperature (control).

a. Grape seeds stratified and treated with growth substances

Seeds under this lot were stratified in moist sand at a temperature of 5 ± 2^0 C. Each of the petri dishes was removed after a 15 day interval and the seeds were

treated with growth substances for 48 hours. The following growth substances and their combinations were used:

1. GA_3(500, 1000, 2000 and 2500 ppm),
2. Kinetin (500, 1000 and 2000 ppm),
3. Thiourea (1.0%, 2.0% and 2.5%),
4. GA_3 + Kinetin (i) 1000 ppm GA_3 + 1000 ppm Kinetin)
   (ii) 2000 ppm GA_3 + 2000 ppm Kinetin),
5. GA_3 + Thiourea (i) 1000 ppm GA_3 + 1.0% Thiourea)
   (ii) 2000 ppm GA_3 + 2.0% Thiourea).

After 48 hours the seeds were thoroughly washed and sown in the germinating pans containing a sterilized sand + soil medium (50% each). These pans were kept in the net house. The final germination count was done after five weeks of sowing and is expressed in percentages.

b. Seeds treated with GA_3 and then stratified

Under this lot, seeds were first soaked in aqueous solutions of different concentrations (1000, 2000 and 2500 ppm) of GA_3 for 48 hours. These seeds were dried on blotting paper and stratified as in experiment one. After the fixed interval of stratification storage period, the seeds were taken out of the petri dishes and sown in the germinating pans so as to conduct the germination percentage test.

c. Seeds subjected to running water

The third lot of seeds was placed in muslin cloth and tied to the water tap. Water was allowed to drip through these seeds at a very slow speed for the period of 4, 8, 12 and 16 days. After this, the seeds were sown separately in the germinating pans and observations on germination were recorded.

d. Extraction of the inhibitor

One hundred fresh seeds were placed in each of four flasks and covered with 20 ml of water. The extraction flasks were then placed at a temperature of $5^0 \pm 2^0$ C. The water extract was poured into another set of beakers after 24, 48, 72 and 96 hours and replaced with a fresh lot of 20 ml water. The collected water extract of two flasks was then evaporated to dryness. The water extract of other two flasks was kept separate for further use. The dried extracts of the two flasks were separately taken up in 5 ml of ethanol. The concentration of this extract was thus equivalent to 20 seeds/ml of ethanol.

e. Chromatography

Descending type of chromatography was followed using Whatman No. 1 filter paper. The solvent used was Isopropanol : ammonia : water (10 : 1 : 1). The alcoholic aliquot equivalent to 5 and 10 seeds sample was loaded on the chromatograms. These loaded chromatograms were used for the cress seed germination test.

f. Bioassy technique

Cress seed germination test: Chromatographic strips were placed in petri dishes of 5 cm diameter and moistened with 2 ml distilled water. In each of these petri dishes, 50 cress seeds were allowed to germinate in darkness at a temperature of $25^0 \pm 2^0$ C. The number of germinated seeds was counted after 48 hours and the percentage inhibition of germination was calculated from duplicate tests.

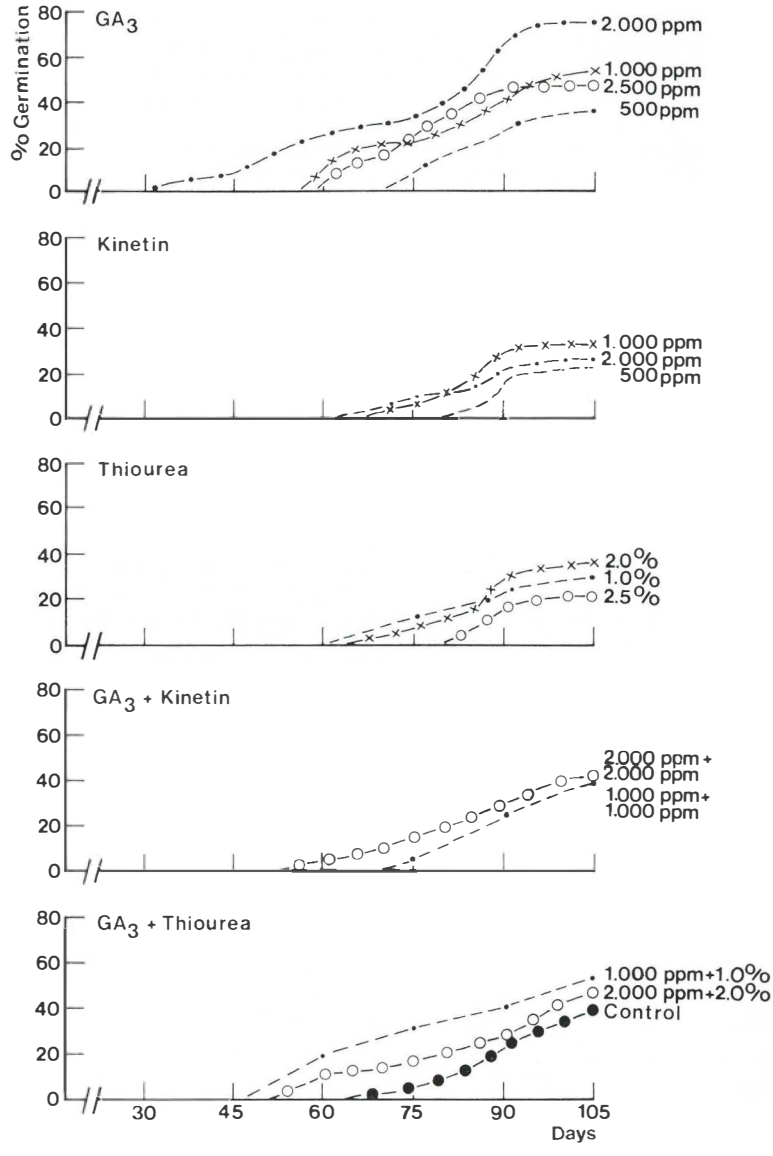

Fig. 1: Percentage germination of grape seeds stratified and treated with different chemicals.

g. Antagonism of GA_3 effect by water extract of fresh grape seeds

Fifty grape seeds treated with GA_3 (2000 ppm) and stratified for one month were taken and soaked in water extract collected from the fresh seeds. This was repeated twice. Both the repeated sets were kept at a temperature of $5^0 \pm 2^0$ C for 72 hours. Subsequently the seeds were taken out and sown in the germinating pan along with the control (GA_3 treated and stratified) so as to record the germination percentage.

Table 1

Percentage germination of grape seeds treated with GA_3 before stratification

Treatments	Concentrations	Period of stratification				
		4 days	8 days	12 days	16 days	30 days
GA	(i) 1000 ppm	0	15.00	42.10	69.38	78.12
	(ii) 2000 ppm	0	10.79	39.62	57.00	81.05
	(iii) 2500 ppm	0	4.03	12.90	28.17	45.00
Control	0	0	0	0	0	0

Remark: At 2500 ppm GA_3 grape seedling growth was very slender and long compared to other concentrations and control.

Results

The germination percentage of the seeds stratified for definite intervals and then treated with different growth substances at various concentrations are given in Fig. 1.

It is clear from Fig. 1 that GA_3 was much more effective compared to other growth substances used. GA_3 at 2000 ppm induced 2.84 per cent of the seeds to germinate after a one month storage period, which subsequently increased to 79.69 per cent after 105 days as against 37.00 per cent in the control. Kinetin and Thiourea were not significantly effective in breaking the dormancy of grape seeds (var. Black Muscat). After 75 days' storage period, 7 and 13 per cent germination was observed by Kinetin (2000 ppm) and Thiourea (1.0%) respectively. When Kinetin and Thiourea were used in combinations with GA_3 at optimum concentrations, there was comparatively better germination. Thiourea (1.0%) in combination with GA_3 (1000 ppm) gave a germination of 20 per cent after sixty days, followed by second combination of Thiourea (2.0%) and GA_3 (2000 ppm) where the germination percentage was 11.73. This showed that Thiourea in combination with GA_3 at a lower concentration could partially break the dormancy of grape seeds compared, after 60 days of storage period, to Thiourea alone and the control.

Grape seeds treated with GA_3 and then stratified

This treatment showed a very significant effect in breaking the dormancy of grape seeds within a month of storing. The results are shown in Table 1.

Table 2

Percentage germination of grape seeds subjected to running water

Treatments	Duration of running water			
	4 days	8 days	12 days	16 days
Running water (Muslin cloth bags tied up with water tap)	12.00	34.00	72.11	73.00
Control	0	0	0	0

Remark: After the 12th day the tips of all the exposed roots from the muslin cloth were dried by the atmospheric air and the germinated seeds did not grow further, only 5% seedlings grew successfully after 16 days' treatment.

It is evident from the results that grape seeds treated with GA_3 and stratified for one month showed very remarkable germination. After 8 days of stratification, the germination percentage was 15.00, 10.79 and 4.03 per cent with 1000, 2000 and 2500 ppm GA_3 respectively. These percentages subsequently increased after one month of stratification, when the highest grape seed germination percentage was 81.05 with 2000 ppm GA_3, followed by the concentration of 1000 ppm GA_3 (78.12% germination), whereas the control showed no germination.

A perusal of Table 2 shows that after 8 days of running water, 34 per cent of the seeds germinated and rootlets were seen growing through the muslin cloth. After 12 days, germination percentage increased to 72.11 per cent. When this treatment was prolonged for 16 days, germination percentage did increase but the exposed rootlets through the muslin cloth turned brown at the tips. Germinated seeds of different time intervals were sown in the germinating pans. It was observed that the seeds germinated till 12 days by running water treatment exhibited good stand, whereas germinated seeds of the 16 days lot, did not establish in soil and sand medium afterwards, and because of browning root-tips in the exposed atmosphere when still tied up to the water tap, only 5 per cent of such seedlings showed better growth.

Inhibitor estimation

Observations made suggested that the dormancy of grape seeds is perhaps largely due to the presence of a water soluble inhibitor. It was evident, from Fig. 2. that this inhibitor hindered the germination of cress seeds at R_f 0.7 to 0.85.

The presence of a water soluble inhibitor in grape seeds was further confirmed when the grape seeds treated with GA_3 and stratified for a month were soaked in the water extract of the fresh grape seeds for 72 hours and kept at a temperature of $5^0 \pm 2^0$ C. As a result of this treatment no germination of grape seeds could be observed, whereas in control (GA_3 treated + stratified for one month) 77 per cent of the seeds germinated. This indicated that the water extract of grape seeds could antagonize the germination of GA_3 treated + stratified seeds.

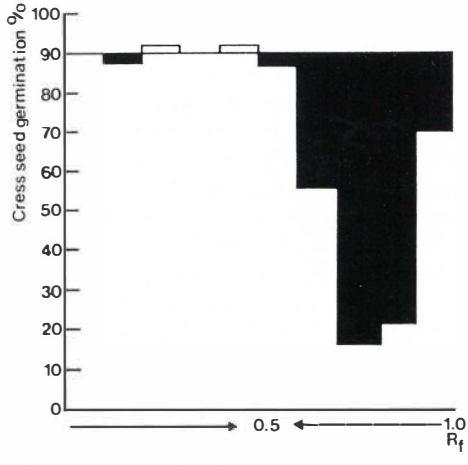

Fig. 2: R_f value of the water soluble inhibitor extracted from fresh grape seeds.

Discussion

Grape seeds subjected to various treatments showed that it is possible to reduce the after-ripening period to a minimum. From the present data, it is observed that GA_3 has partially substituted this after-ripening period, thereby hastening seed germination. This is also reported by RANDHAWA and NEGI (1964), CHADHA (1965) and by AMEN (1967). It was further noted that when Thiourea is combined with GA_3 at optimum concentrations, the results are better than when Thiourea is used alone. This could possibly be due to some interaction effect of these chemicals when mixed together.

Much better results were obtained when grape seeds were first treated with GA_3 and then stratified. Within 12 days of stratification after GA_3 treatment, 50 per cent of the seeds germinated and after 30 days the germination increased to 81.05 per cent. The increased grape seed germination within a month of stratification by pre-treatment with GA_3 might be either due to diffusion of endogenous auxin-like substances in the seeds, or GA_3 might have enhanced the auxin effectiveness (SASTRY and MUIR 1963, LEOPOLD 1964). Further, this reaction might have been accelerated by subsequent chilling.

Our present findings have also clearly indicated that there is some water soluble inhibitor present in grape seeds which does not allow these seeds to germinate. When grape seeds were subjected to running water, these seeds germinated in the muslin cloth itself within few days. When the water extract of the grape seeds was bioassayed for inhibitor estimation positive results were obtained. The inhibitor could hinder the germination of cress seeds at R_f 0.7 to 0.85. This water soluble inhibitor is also alcohol soluble, as the extract was taken finally in ethanol for chromatography. Further, the presence of this inhibitor in the water extract of grape seeds was confirmed by its antagonizing effect on GA_3 treated and stratified seeds. It is, therefore, obvious that once this inhibitor is leached out, the seeds will readily germinate. FERENCZY (1955) has concluded that most of the inhibitory material of *Fraxinus excelsior* seeds is present in a mucilaginous layer surrounding the seeds. He found decrease in the inhibitory material during moist storage at 5° C. Moreover, he observed that leaching is necessary to induce germination in unchilled seeds. LUCKWILL (1952) reported that the synthesis of growth promoting substances coupled with the leaching out of the inhibitor from the seeds during low temperature, resulted in the germination of apple seeds. Therefore, there is a possibility that this water soluble inhibitor of grape seeds is leached out during stratification or its action may be antagonized with the increased level of auxins by chilling (KACHRU *et al.* 1969). The inhibitor was not identified; however, LOTT (1968) could prove that in all probability abscisic acid was present in grape seeds.

It is well known that GA_3 can partially replace the after ripening period. KANG YEOU DER *et al.* (1968) have used 8000 ppm GA_3 which resulted in a better and early germination of grape seeds. However, present findings have shown that pretreatment of seeds with a much lower concentration of GA_3 followed by a short period of stratification could considerably minimize the after ripening period of seeds. Further, even without using GA_3, it is possible to induce germination in grape seeds by leaching the inhibitor with the help of running water. KANG YEOU DER *et al.* (1968) have also discussed that the response of exogenous GA_3 could be due to deficiency of gibberellins in the seeds. From the present findings, it is evident that apart from other possible factors, the presence of an inhibitor in the seeds plays an important role in inducing dormancy in grape seeds. Exogenous application of GA_3 might accelerate the metabolic activities of the auxin-like growth promoting substances

during the chilling period (KACHRU *et al.* 1969), which might be directly involved in germination or might antagonize the effect of the inhibitor present in grape seeds.

Thus it would be seen that grape seed dormancy is mainly caused by the presence of an inhibitor, and the dormancy breaking effect of chilling is due to accumulation of a germination promoter which enables seeds to overcome the effect of the inhibitor. The control of dormancy and germination in the grape seed appears to involve the interaction between an inhibitor and a promoter.

Summary

In order to break the dormancy of grape seeds in the minimum possible time, a number of treatments, including stratification, growth substances and subjecting the seeds to running water, were used. It was observed that grape seeds kept in running water for 8 days germinated in the muslin cloth itself (34.00%). The maximum percentage of germination obtained was 73.00% when the seeds were kept in running water for 16 days. However, the seedling stand was best under the 12 day treatment (72.11%). This was followed by GA_3 treatment (2000 ppm) before stratification, whereby grape seeds germinated within 8 days of stratification and maximum germination percentage was 81.05 after one month. Third best result was obtained under stratification for one month and then a treatment with GA_3. Further, grape seeds, when soaked in water for 96 hours, leached some water soluble inhibitor. This could antagonize the germination of GA_3 treated grape seeds. When this leached water was bioassayed by the cress seed germination test, it showed the presence of inhibitor at R_f 0.7 to 0.85. It appears that the water soluble inhibitor might be responsible for masking the effect of growth substances present in the seeds and thereby disturbing the metabolism of germination.

Literature Cited

1. AMEN, R. D., 1967: The effect of gibberellic acid and scarification on the seed dormancy and germination of *Lazula spicata*. Physiol. Plant. (Kopenhagen) **20**, 6—12.
2. CHADHA, K. L., 1965: Studies on phenotypic variability and seed germination in some grape cultivars. Unpubl. Thesis, Indian Agricult. Res. Inst., New Delhi.
3. FERENCZY, L., 1955: The dormancy and germination of seeds of *Fraxinus excelsior* L. Acta Biol. Szegediensis N. S. **1**, 17—24.
4. KACHRU, R. B., CHACKO, E. K. and SINGH, R. N., 1969: Physiological studies on dormancy in grape seeds *(Vitis vinifera)*. Vitis **8**, 12—18.
5. KANG YEOU DER, WEAVER, R. J. and POOL, R. M., 1968: Effect of low temperature and growth regulators on germination of seeds of 'Tokey' grapes. Proc. Amer. Soc. Hort. Sci. **92**, 323—330.
6. LEOPOLD, A. C., 1964: Plant growth and development. McGraw-Hill Book Comp., USA.
7. LOTT, H., 1968: Über den Nachweis von Abscisinsäure im Samen von Reben. Vitis **7**, 221—222.
8. LUCKWILL, L. C., 1952: Growth inhibiting and growth promoting substances in relation to dormancy in apple seeds. J. Hort. Sci. **27**, 53—67.
9. RANDHAWA, G. S. and NEGI, S. S., 1964: Preliminary studies on seed germination and subsequent seedling growth on grapes. Indian J. Hort. **21**, 186—196.
10. SASTRI, A. A. and MUIR, R. M., 1963: Gibberellin effect on diffusible auxin in fruit development. Science **140**, 494—495.

Eingegangen am 21. 3. 1972

Dr. R. N. SINGH
Div. of Horticult. and
Fruit Technology
New Delhi 12
India