Vitis **15**, 5—10 (1976)

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Seed dormancy and endogenous growth substances in Anab-e-Shahi grapes

by

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Samenruhe und endogene Wachstumsregulatoren bei der Rebensorte Anab-e-Shahi

Zusammenfassung. — Der Gehalt an Auxin, Gibberellin und Hemmstoffen in frisch isolierten Samen von Anab-e-Shahi sowie in Samen, die 30, 45, 60, 75 und 90 Tage lang feucht stratifiziert worden waren, wurde bestimmt. Durch 60tägige Stratifikation wurde die Keimung am stärksten gefördert. Mit der Dauer der Stratifikation nahm auch die Auxinaktivität zu, um ihr Maximum in den keimenden Samen zu erreichen. Voll ausgereifte, frisch isolierte Samen zeigten keinerlei Gibberellin-artige Aktivität. Eine solche war in den Extrakten von Samen, die 60 Tage lang feucht stratifiziert worden waren, im Maximum vorhanden. In den Extrakten voll ausgereifter, frischer Samen trat ein Abscisinsäure-artiger Hemmstoff auf. Seine Konzentration nahm mit fortschreitender Stratifikation ab und erreichte ihr Minimum nach 45 und 90 Tagen. Aufgrund der vorliegenden Untersuchungen kann angenommen werden, daß die Samenkeimung bei Reben durch das verstärkte Auftreten wachstumsfördernder Substanzen wie Auxine und Gibberelline im Laufe der Stratifikation und den Rückgang Abscisinsäure-artiger Hemmstoffe reguliert wird. Auch bei anderen Rebensorten genügte durchweg eine 60tägige Stratifikation, um die bestmöglichen Bedingungen für die Samenkeimung zu schaffen.

Introduction

Recent studies on the dormancy of the seeds and the buds of a number of plants (Luckwill 1952, Vegis 1965, Wareing 1965, Amen 1968) have indicated the involvement of certain growth inhibiting and growth promoting substances in the dormancy mechanism of such organs. Walker (1970) proposed the balance concept between the two groups of compounds. If the seed contains more 'inhibitor units' than 'promoter units', then it remains dormant and does not germinate. When the promoter units outnumber the inhibitor units, the germination occurs under optimum environmental conditions.

As the stratification proceeds, gibberellins are synthesized or released from the bound form (Frankland and Wareing 1962, Mathur *et al.* 1971, Lin and Boe 1972). Luckwill (1952) observed that the ether extracts of matured apple seeds contained a substance which inhibited the germination of the seeds. During the after-ripening under moist conditions at 4 °C, disappearance of this acid inhibitor was noted and this disappearance took as much time as required for the dormancy to break.

This relationship between the growth promoting and growth inhibiting substances in the onset and the termination of grape seed dormancy was studied.

Material and Methods

Freshly extracted seeds of Anab-e-Shahi grapes were dried on filter paper after giving thorough washings. Seeds weighing 5 g were immediately placed in plastic

vial containing 10 ml of 80% methanol and were immediately placed in the deep freeze till needed for further work. Samples of 5 g seeds were kept for stratification by placing the seeds in alternate layers of moist river sand in a moisture box at a temperature of 4—5 °C. Batches of seeds were removed after an interval of 30, 45, 60, 75 and 90 days of stratification for the determination of auxin, gibberellin and inhibitor (ABA) content. The freshly extracted seeds and those stratified for respective periods were also sown in seed pans.

Detection and determination of auxins, gibberellins and inhibitors

The method adopted by SIRCAR (1971) was used for the extraction of auxin and for purification, the acetonitrile method of NITSCH (1956) was followed. Separation of the compounds was made by descending chromatographic technique with Whatman No. 1 chromatographic paper. *Avena* coleoptile bioassay test as described by WITHAM *et al.* (1971) was followed.

For the determination of gibberellins, 5 g seeds were macerated in small quantities of methanol, and extracted twice for 24 hours at 0 °C. Purification and fractionation were done by the method used by IWAHORI *et al.* (1968). Bioassay for gibberellins was done by means of barly half seed amylase method as given by NICHOLLS and PALEG (1963).

RUDNICKI's (1969) method was used for abscisic acid estimation. The separation was done by descending chromatographic technique. The chromatograms were developed in isopropanol : butanol : ammonia : water (6 : 2 : 1 : 2 v/v), synthetic abscisic acid was used for reference. The presence and activity of abscisic acid in the extracts were studied by cress seed (*Lepidium sativum* var. Curled) germination bioassay test (MASUDA 1962).

Results

Germination of seeds. — Freshly extracted seeds and those stratified for 30, 45, 60, 75 and 90 days were sown, the percentage seed germination is summarized below in the table.

Effect of period of stratification on percentage seed germination in different cultivars of grapes

Einfluß der Stratifikationsdauer auf die Samenkeimung (%) bei verschiedenen Rebensorten

Cultivars	Period of stratification (days)						
	Fresh	30	45	60	75	90	Average
Anab-e-Shahi	0.00	26.00	30.00	40.00	35.00	28.00	31.80
Early Muscat	0.00	14.00	33.00	51.00	37.00	—	42.75
Cardinal	0.00	0.00	8.00	28.00	7.00	—	14.37
Foster's White Seedling	9.00	17.00	35.00	34.00	25.00	30.00	38.33
Average	2.25	14.25	24.00	38.25	26.00	29.00	

There was an increase in the percentage seed germination with the extension in stratification period up to 60 days, after which it declined. Fresh seeds did not germinate at all, except for the Foster's White Seedling. The overall maximum germination was found in all the cultivars stratified up to 60 days, after which it declined.

Determination of auxins. — The activity in the immature and the freshly extracted seeds was mainly at Rf 0.1—0.3. However, when these were sprayed with 0.5% potassium permanganate, a yellow spot was observed at Rf 0.91 in all samples. Intensity of these spots was very faint in the immature and the fresh seeds, whereas these spots were very clear where the extracts of the stratified seeds were applied.

The quantity of auxins from all the Rf positions was pooled together and presented in Fig. 1 as IAA equivalent (µg/g fresh weight of seeds). The amount of

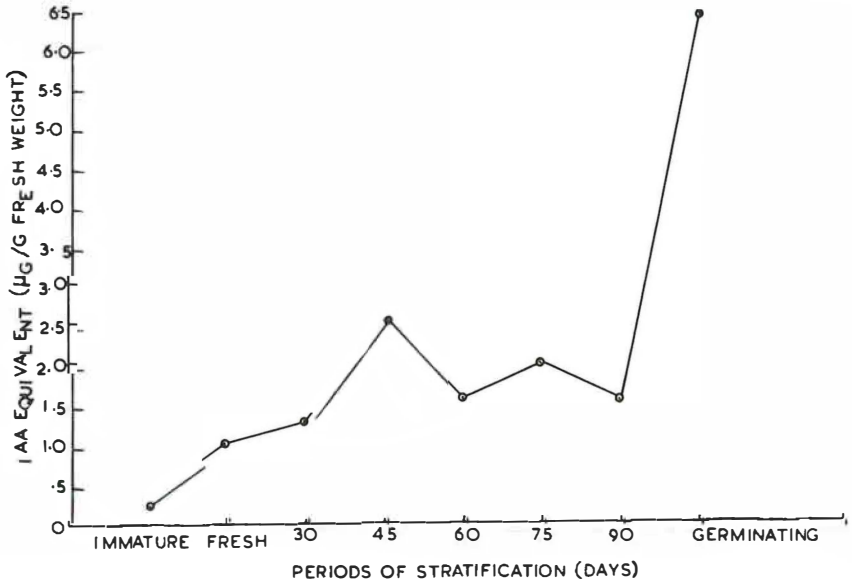

Fig. 1: Auxin content in Anab-e-Shahi grape seeds after stratification.
Auxingehalt in Samen von Anab-e-Shahi nach Stratifikation.

auxins was more in the stratified seeds than in the immature and fresh seeds. The maximum activity (2.64 µg/g) was observed in the seeds stratified for 45 days. However, the germinating seeds had many times more auxins (6.48 µg/g) than the non-stratified ones.

Determination of gibberellins. — The gibberellin-like activity was not seen in the freshly extracted seeds. The peak activity was observed in the stratified seeds, at Rf 0.0—0.2, 0.5—0.6 and 0.9—1.0, the maximum was after 60 days duration.

The total quantity of the gibberellin-like substances from all the Rf positions was pooled together as GA_3 equivalent (µg/g fresh weight of seed) and is shown in Fig. 2. No gibberellin-like substance was found in the freshly extracted seeds. However, it was extraordinarily high in the seeds stratified for 60 days (14.76 µg/g), the rise was rapid (Fig. 2), after which it declined. It was only 0.0005 µg/g as compared to 14.76 µg/g when the seeds were stratified for 75 and 60 days respectively. There was a rise again in the seeds stratified for 90 days and a further rise (0.007 µg/g) was observed in the germinating seeds.

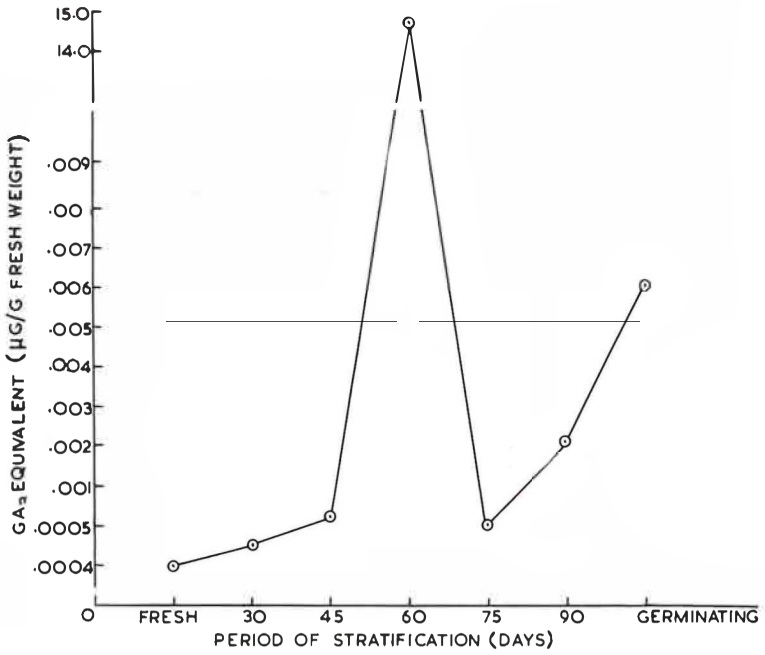

Fig. 2: Changes in gibberellin-like substances in Anab-e-Shahi grape seeds after stratification.

Veränderung der Gibberellin-artigen Substanzen in Samen von Anab-e-Shahi nach Stratifikation.

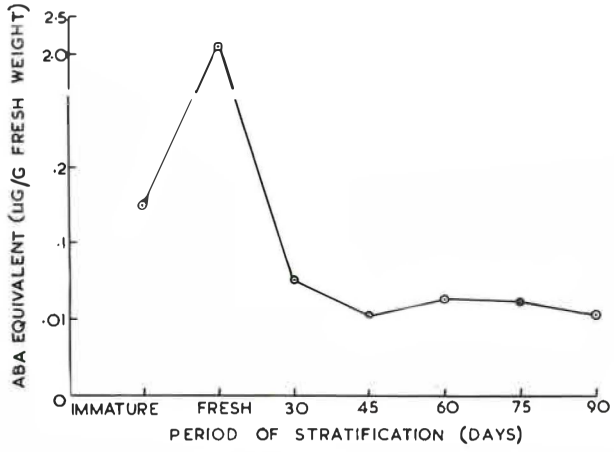

Fig. 3: Abscisic acid equivalent in Anab-e-Shahi grape seeds after stratification.

Abscisinsäure-Äquivalente in Samen von Anab-e-Shahi nach Stratifikation.

Determination of inhibitors. Quantitative changes were determined by eluting the chromatograms at Rf 0.6—0.8 and tested by the cress seed germination. The results expressed as ABA equivalent (μg/g fresh seeds) (Fig. 3) indicated that the maximum quantity of the inhibitors (2.19 μg/g) was present in the extracts of the fresh seeds. The immature seeds had much less quantity of the inhibitors than the fully ripe fresh seeds.

The quantity of the inhibtors in the seeds decreased with stratification. The minimum quantity (0.014 μg/g) was observed in the seeds stratified for 45 days. This level again rose in the seeds stratified for 60 days, but this quantity decreased further with the increase in the period of stratification and was only 0.015 μg/g in the extracts of the seeds stratified for 90 days.

Discussion

The dormancy of the seeds of a number of fruit species has been reported to be associated with growth inhibiting and growth promoting substances (Luckwill 1952, Vegis 1965, Lott 1968, Rudnicki 1969). During the moist stratification, there is synthesis of growth promoting substances like gibberellins and leaching of growth inhibiting substances like abscisic acid (Luckwill 1952, Lin and Boe 1972).

It has been indicated that the unstratified seeds had a higher amount of inhibitor like ABA and its level decreased as the period of stratification increased (Fig. 3). But the growth promoting substances as auxins and gibberellins increased (Fig. 1 and 2). It was significant to note here that, although inhibitor content was the lowest after 45 days of stratification, the seed germination was the maximum when the seeds were stratified for 60 days in all these cultivars (Table). This maximum seed germination after the period of 60-day-stratification coincided with the highest gibberellin activity (Fig. 2). These results showed that it was not only the level of the inhibitors which controlled the seed germination in the grapes but that it was also relative to the levels of the growth promoting substances. These results were further supported by the present findings that the seed germination was generally less in the seeds stratified for more than 60 days, although the level of the inhibitor was very low after 60 days and onward. This low seed germination in the longer periods of stratification seems to be due to the low level of gibberellins (Kachru *et al.* 1969, 1972). The information available so far justifies the formation of such a concept in the grape seed germination. Any of the treatments which either encourage the promotion activity or discourage the inhibitory activity would shift the balance to be favorable for the enhancement of seed germination.

Summary

Auxin, gibberellin and inhibitor contents were estimated in the freshly extracted seeds as well as in those moist stratified for 30, 45, 60, 75 and 90 days. The maximum seed germination was obtained when the seeds were stratified for 60 days. Auxin activity increased with the increase in the period of stratification and was found to be at its maximum in the germinating seeds. Fully ripe freshly extracted seeds did not show any gibberellin-like activity. It was maximum in the extracts of the seeds moist-stratified for 60 days. Abscisic acid-like inhibitor was present in the extracts of the fully ripe fresh seeds. Its quantity decreased with the period of

stratification and was minimum when the seeds were stratified for 45 and 90 days. From these studies, it may be postulated that the seed germination in grapes is regulated by the appearance of growth promoting substances like auxins and gibberellins during stratification and disappearance of inhibitors like abscisic acid. In all the cultivars, stratification for 60 days was sufficient to create these conditions for the best seed germination.

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Eingegangen am 29. 1. 1976

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