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Hormonal regulation of the lag phase in seeded and seedless grapes (*Vitis vinifera* L.)

by

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Die hormonale Steuerung der Phase des verlangsamten Beerenwachstums bei samenhaltigen und samenlosen Reben (*Vitis vinifera* L.)

Zusammenfassung. — In der ersten Phase des raschen Beerenwachstums (I) lagen hohe Konzentrationen auxinartiger Substanzen und niedrige Konzentrationen wachstumshemmender Substanzen vor. Während der anschließenden Phase des verlangsamten Beerenwachstums (II) wurden hohe Konzentrationen von Wachstumshemmern und niedrige Konzentrationen von wachstumsfördernden Substanzen festgestellt. Das erneut beschleunigte Beerenwachstum in der darauf folgenden Phase (III) war im allgemeinen mit erhöhten Konzentrationen gibberellinartiger Substanzen verbunden.

Introduction

The grape berries follow a biphasic growth pattern, having two distinct periods of active growth (stage I or pre-lag phase and stage III or post-lag phase). These two classic growth periods are separated by a slow growth period, stage II or lag phase (6, 7, 8, 15 and 20). Out of the many factors, growth regulators play an important role in controlling the various phases of berry growth.

Although the existence of auxins, gibberellins and abscisic acid-like substances in grape berries has been reported by many workers (1, 2, 3, 8, 9, 15 and 18), the opinion differs regarding the role of endogenous growth regulators in controlling the various phases of berry growth. The present investigation was, therefore, undertaken to find out the levels of various growth regulators and to correlate them with various phases of berry growth with particular reference to the lag phase both in seeded and seedless grapes.

Materials and Methods

The experimental plant material consisted of 14-year-old, four-arm kniffin-trained vines of Pusa Seedless and Tas (seeded) cultivars of grapes grown in the vineyard of the Division of Horticulture and Fruit Technology, Indian Agricultural Research Institute, New Delhi. The vines were cane-pruned and were given uniform cultural operations.

The levels of endogenous growth regulators, namely auxin-like substances, gibberellin-like substances and inhibitors were determined at different intervals before, during and after the lag phase, i.e. 3, 4, 4½, 5, 5½, 6 and 7 weeks after anthesis. This phase lasted from the 5th to the 6th week after anthesis in Pusa Seedless and from the 5th to the 7th week in Tas (6, 18). Duplicate samples each

containing 50 g berries were drawn from the middle of four different clusters from four different vines and were analysed separately as suggested by PANDEY and RAO (17). After analysis the average of four duplicate samples was taken and the data were presented accordingly. In case of Tas, the growth regulating substances were assayed both in seed and pulp separately.

The auxin-like substances from grape berry samples were extracted and purified as suggested by NITSCH (12). The berry extract was applied to the starting line of the paper strip by means of a tuberculin syringe in a stream of air to allow the rapid evaporation of the solvent as suggested by NITSCH and NITSCH (13). After complete drying, the paper strip was equilibrated over night in a solvent consisting of a mixture of isopropanol, 28 per cent ammonia and water (80 : 10 : 10 v/v) as recommended by STOWE and THIMANN (21). The presence of auxin-like substances was tested by the method of NITSCH and NITSCH (14) using straight growth of section of *Avena* (var. Algerian) first internode.

The extract used for bioassay to auxin-like substances was also used for detecting the presence of inhibitors by the cress (*Lepidium sativum*) seed germination method as suggested by MASUDA (11).

Gibberellin-like substances were extracted with 200 ml ethyl acetate for 8 hours, filtered and re-extracted with another 150 ml of ethyl acetate for 16 hours according to the method of COOMBE *et al.*, (5). After spotting, the chromatograms were equilibrated for 12 hours in 80% isopropyl alcohol and were run up to 20 cms. The presence of gibberellin-like substances was tested with the barley endosperm bioassay method as described by COOMBE *et al.* (4) using the huskless barley variety IB-65. The only modification made to this method was that the barley seeds were sterilized in 0.1% HgCl₂ solution (100 seeds/100 ml) for 15 minutes instead of 5% calcium hypochlorite solution (120 seeds/100 ml). The content of sugar was estimated by the method of PALLEG (16).

Results

1. Auxin-like substances

In Pusa Seedless berries (Fig. 1, A) the levels of auxin-like substances or growth promotive substances, respectively, at Rf 0.3 and 0.4 rose rapidly after the 3rd week and reached the peak value at the end of the 4th week. The auxin-like substances at these Rf zones rapidly declined to almost half of the value after 4½ weeks with a further decrease during the subsequent week of the lag phase (5th week). Although these substances maintained the same level at Rf 0.3 they showed a decrease at Rf 0.4 after 5½ weeks. Similarly auxin-like substances accumulating at Rf 0.5, 0.6, 0.7 and 0.8 during the pre-lag phase (at the end of the 4th week) declined during the lag phase and, at the end of the 7th week, their levels were approximately doubled as compared to the levels at the end of the 6th week.

In case of the seeded cultivar Tas, the values of auxin-like substances at Rf 0.3 and 0.4 in seeds (Fig. 2, A) were found to be at the highest level at the end of the 4th week. A slight reduction in the levels of these substances was observed after 4½ weeks and thereafter almost the same level was maintained up to the end of the 7th week (post-lag phase). Although the growth promotive substances at Rf 0.5, 0.6 and 0.8 were present during the lag phase, their levels were much lower as compared to the levels of these substances present at Rf 0.3 and 0.4. However, the auxin-like substances did not show any activity at Rf 0.1, 0.2, 0.7, 0.9 and 1.0 at any stage of berry development in the seed extract.

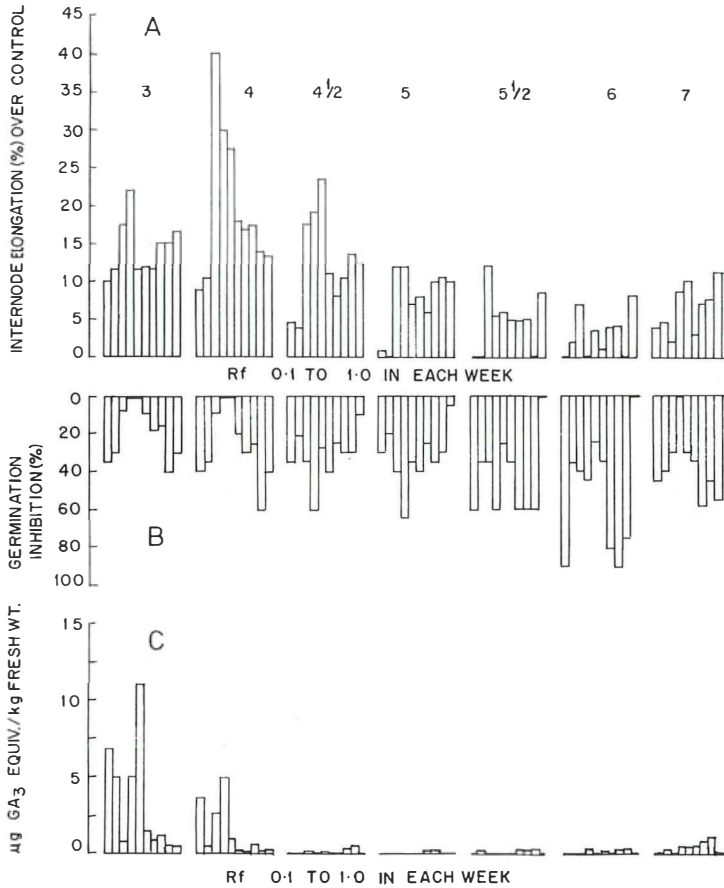


Fig. 1.: Levels of auxin-like substances (A), inhibitors (B) and gibberellin-like substances (C) in the developing Pusa Seedless grape berries (numbers represent weeks after anthesis).

Aktivität der auxinartigen Substanzen (A), der Inhibitoren (B) und der gibberellinsäureartigen Substanzen (C) während der Beerenentwicklung der Sorte Pusa Seedless. Zahlen = Wochen nach der Anthese.

The activity of auxin-like substances in the Tas berry pulp (Fig. 3, A) was also observed to be at the highest level at Rf 0.3 and 0.4 at the end of the 4th week followed by steady decrease during the lag phase. Thereafter, these substances again showed a higher level at the end of the 7th week (post-lag phase). Auxin-like substances at Rf 0.7 and 0.8 decreased steadily from week 4 to 5 and later on remained almost stationary up to the 6th week. No activity of auxin-like substances was observed at Rf 0.1 and 0.2 during the middle of the lag phase (5½ to 6 weeks after anthesis).

2. Inhibitors

Although in Pusa Seedless the growth inhibitory substances located at Rf 0.4 and 0.5 were completely absent during the pre-lag phase, they appeared in appreci-

able amount with the onset of the lag phase (Fig. 1, B). The levels of these inhibitors rose rapidly at the end of 4½ weeks with a further steady increase at the end of the 5th week. Substances particularly located at Rf 0.4 subsequently started decreasing after 5½ weeks and thereafter showed a still lower level at the end of the 6th week — the termination of the lag phase and finally disappeared at the end of the 7th week (post-lag phase). The values of inhibitors located at Rf 0.7 and 0.8 remained at higher levels throughout the lag phase and attained their maximum values at the end of the 6th week. These substances subsequently showed a decline with the beginning of the post-lag phase. Growth inhibitory substances located at Rf 0.1 and 0.2 first showed a slow decline in their levels from week 4 to 5 followed by a rise 5½ and 6 weeks after anthesis. However, at the end of the 6th week, substances located at Rf 0.1 showed the maximum value followed by a decrease again at the end of the 7th week.

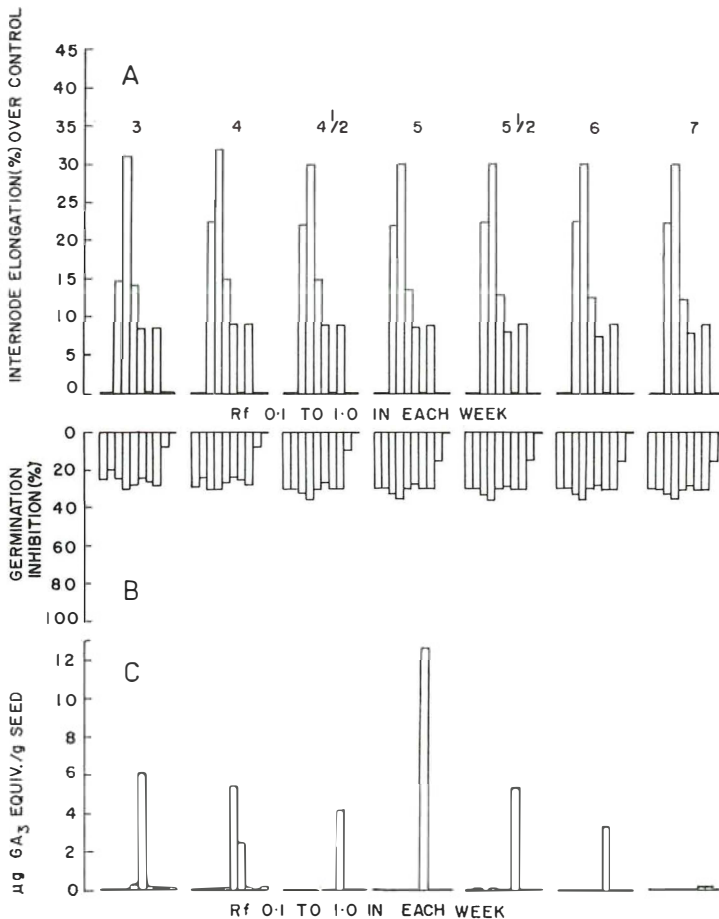


Fig. 2: Levels of auxin-like substances (A), inhibitors (B) and gibberellin-like substances (C) in the developing seed of Tas grapes (numbers represent weeks after anthesis). Aktivität der auxinartigen Substanzen (A), der Inhibitoren (B) und der gibberellinsäureartigen Substanzen (C) während der Samenentwicklung der Sorte Tas. Zahlen = Wochen nach der Anthese.

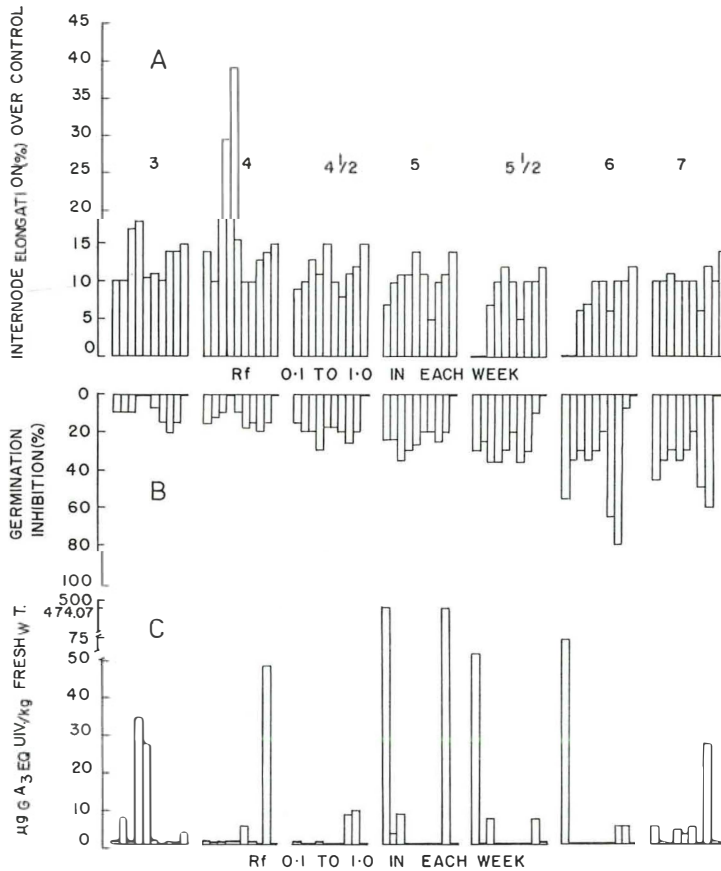


Fig. 3: Levels of auxin-like substances (A), inhibitors (B) and gibberellin-like substances (C) in the pulp of the developing Tas grapes (numbers represent weeks after anthesis). Aktivität der auxinartigen Substanzen (A), der Inhibitoren (B) und der gibberellinsäureartigen Substanzen (C) während der Entwicklung des Beerenfleisches bei der Sorte Tas. Zahlen = Wochen nach der Anthese.

In Tas seed extract (Fig. 2, B) the important inhibitors seem to be located at Rf 0.3 and 0.4 during the lag phase. The levels of growth inhibitory substances at these Rf zones showed a slight increase after 4½ weeks, thereafter they maintained almost the same level during and after the lag phase. No inhibitor was present at Rf 1.0 at any stage of berry development.

The levels of inhibitors in the Tas berry pulp (Fig. 3, B) in general recorded a steady increase during the lag phase. Growth inhibitory substances, particularly present at Rf 0.4, suddenly appeared with the start of the lag phase and maintained an approximately similar level up to the 5th week. Its level slightly increased after 5½ weeks followed by an approximately constant level throughout the lag and post-lag phases i.e. up to the 7th week in this cultivar. The inhibitors located at Rf 0.1, 0.2, 0.7 and 0.8 also rose gradually during the lag phase and recorded the highest level at the end of the 6th week followed by a decrease at the end of the 7th week. The level of inhibitor located at Rf 0.9 during week 4½ and 5 steadily declined during

the subsequent weeks (5½ and 6) of berry growth and finally disappeared at the end of the 7th week.

3. Gibberellin-like substances

The results obtained clearly indicated that the levels of gibberellin-like substances in Pusa Seedless grapes (Fig. 1, C) were higher during the pre-lag phase as compared to other phases of berry development. The levels of these gibberellins at Rf 0.4 to 0.6 abruptly declined at the beginning of the lag phase. However, slightly more gibberellin activity was observed at the end of the 7th week (post-lag phase) as compared to the lag phase.

The activity of gibberellin-like substances at Rf 0.6 in the seed extract of the Tas cultivar (Fig. 2, C) was found to be the highest during the pre-lag phase of berry development. In the subsequent weeks, i.e. during the lag and post-lag phases, it was observed to be present only in traces. The appearance of gibberellins at Rf 0.7 at the end of the 4th week showed a higher level after 4½ weeks followed by a sudden upsurge at the end of the 5th week, which gradually decreased during the subsequent stages i.e. at 5½ and 6 weeks. Finally the activity was noticed in traces at the end of the 7th week (post-lag phase).

The gibberellin-like substances in the pulp of Tas berries showed high activity at Rf 0.4 and 0.5 (Fig. 3, C) at the end of the 3rd week (pre-lag phase). This activity was reduced to very low level during the subsequent weeks of the lag phase with a slight reappearance of the activity at the end of the 7th week (beginning of post-lag phase). Another important zone of gibberellin-like substances was located at Rf 0.9 at the end of the 4th week. Thereafter, they showed very high activity at the 5th week and finally appeared only in traces at the end of the 7th week. Likewise, during the start of the lag phase (5th week) a very high gibberellin-like activity was also recorded at Rf 0.1. The levels of these substances decreased slowly after 5½ weeks showing slightly a higher value at the end of the 6th week. Later on, at the end of the 7th week, the activity at Rf 0.1 was reduced to a very low value.

Discussion

Although the degree or the time of the lag phase varies with the environmental factors, particularly temperature (7, 10), seed number (24) and time of flowering (6), its occurrence in grapes is a universal phenomenon, which cannot be eliminated but can be reduced to a certain extent (6, 19). The various phases of berry growth may also be affected by the rapid development of the seed in the seeded cultivars. This, in turn, may prolong the slow growth period of seeded berries as compared to seedless ones (15, 24). Perhaps because of the presence of seed in Tas berries the lag phase was prolonged in this cultivar as compared to Pusa Seedless (6).

In Pusa Seedless, the high levels of auxin-like substances present at Rf 0.3 and 0.4 at the end of the pre-lag phase (4th week) declined to very low levels during the subsequent weeks of the lag phase and attained the lowest values at the end of the 6th week. Low levels of growth promotive substances at these Rf zones and a corresponding increase in the levels of growth inhibitory substances might be responsible for the slow growth during stage II of the berry development. Similarly a decrease of promoters corresponded with an increase of inhibitors at Rf 0.7—0.8.

In contrast to inhibitors, very high levels of gibberellin-like substances noticed during the pre-lag phase rapidly fell down during the lag phase showing again a

slightly higher level during the subsequent phase of the berry development in Pusa Seedless and, to some extent, in Tas. This cultivar, however, had always a higher level of gibberellins during the lag phase of berry development as compared to Pusa Seedless berries.

Thus, the concentrations of growth promotive and growth inhibitory substances at various phases of berry development indicated some interesting results. The first rapid growth of the berries during the pre-lag phase was strongly correlated with high levels of growth promotive substances and low levels of growth inhibitory substances while the contrary was noticed during the lag phase. Similar results have also been reported by COOMBE (3) and NITSCH *et al.* (15) in some grapevine cultivars. These results have further been supported by the fact that exogenous application of gibberellin (50—100 ppm GA₃) during the lag phase greatly enlarged the Pusa Seedless berries as compared to Tas (seeded) berries (6). This may be due to the fact that Tas berries had higher gibberellin-like substances during the lag phase than Pusa Seedless berries. Thus, the exogenous application of GA₃ might have increased the endogenous levels of gibberellins to supraoptimal levels leading to an inhibitory effect on the growth of Tas berries. Although the level of ethylene has not been measured in grape berries during their various phases of development in the present investigation, exogenous application of ethrel (6) to Pusa Seedless berries during the lag phase increased their size and advanced the maturity by about a week, whereas Tas berries showed no response.

The lag phase was terminated with a marked fall in the levels of inhibitors and a slight increase in the levels of promotors when berries entered the post-lag phase, at least in Pusa Seedless. During the present studies, the higher levels of gibberellin-like substances in the post-lag phase as compared to the lag phase may help to trigger some mechanisms responsible for the entry of metabolites from leaves to the berries (6) through increasing the plasticity of the cell walls as gibberellins have been reported to act at membrane level (25). It also seems possible that the available gibberellin-like substances (in stage III) might have helped to increase the levels of auxins in berries (20, 22, 23) and thereby increased the berry growth in the post-lag phase by increasing the plasticity of cell wall leading to high osmotic attraction of water in berries due to influx of sugars.

Summary

The first rapid growth phase of grape berry (stage I) was associated with high levels of auxin-like substances and low levels of growth inhibitory substances. In the lag phase (stage II) of berry development high levels of growth inhibitors and low levels of growth promotors were observed. The berry growth in the post-lag phase (stage III) was generally associated with higher levels of gibberellin-like substances.

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