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Necrosis in grapevine buds (*Vitis vinifera* cv. Queen of Vineyard) III. Endogenous gibberellin levels in leaves and buds[1])

by

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Knospennekrosen bei der Rebsorte Queen of Vineyard (*Vitis vinifera*) III. Gehalt an endogenem Gibberellin in Blättern und Knospen

Zusammenfassung: Der Gehalt der endogenen freien und gebundenen Gibberelline (GA) in Blättern und Knospen der Rebsorte Queen of Vineyard wurde bestimmt. Die Achselknospen an den Trieben starkwüchsiger Reben zeigten ungefähr die doppelte Aktivität an freien GA wie Knospen von weniger wüchsigen Reben. Bei der Aktivität der gebundenen GA wurden keine Unterschiede gefunden. Der GA-Gehalt in den Blättern von Queen of Vineyard war einheitlich, unabhängig von der Wüchsigkeit der Reben. Die Beziehung zwischen Wüchsigkeit, GA-Gehalt und Knospennekrosen der Sorte Queen of Vineyard wird diskutiert; es wird eine kausale Abhängkeit vermutet.

Key words: gibberellic acid, disease, growth, bud, leaf, variety of vine, Israel.

Introduction

The development of necrotic buds in some grapevine cultivars during the spring was described previously (2). In cv. Queen of Vineyard, this necrosis was found only in buds of vigorous vines or occasionally in buds on vigorous shoots of regular vines (2, 6). The necrosis is induced during the early intensive growth period of the shoots, usually at or right after full bloom. It was shown that spray treatments or petiole feeding of gibberellic acid (GA) could produce the same type of necrosis (8). GA_3 treatments induced bud necrosis only when applied during bloom or right after. The necrosis occurs in the stem of the major bud of the grape eye — the bud for next year's development.

This necrosis of the center bud enables the active development of the secondary ones. Thus, the resulting eye and the necrotic center are clearly recognizable and have been described as 'split buds' (2, 7, 8). It was suggested that the GA_3 application causes a similar disorder as induced naturally by excess vigor (6, 8). In the present study, we tried to verify the role of gibberellin levels in inducing bud necrosis on shoots of vigorous vines. For this purpose, the activity of endogenous gibberellins was determined in leaves and buds of regular and vigorously growing vines of cv. Queen of Vineyard.

Materials and methods

The same regular and vigorously growing vineyards of cv. Queen of Vineyard grafted on 41 B rootstock in the continental Bet-Shean Valley, described in the first

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and second part of this study (6, 8), were used. Shoots of uniform vigor were tagged before bloom in both vineyards.

10 d after full bloom, sections of 5 nodes nos. 3—7 were sampled and packed in dry ice immediately after excision and kept frozen until extraction in the laboratory. In 1981, shoots were sampled on April 24, whereas in 1982 flowering was later and sampling was delayed. In the second year three field samplings were made: the first was in May, 5 d after full bloom; the second was 10 d later; and the third, on May 25, 30 d after full bloom. Five true replications of plant material were prepared and all chemical determinations and bioassays were made in duplicate from each repetition. Endogenous gibberellins were determined quantitatively on the basis of activity according to the method described by BEN-TAL and MARCO in 1980 (1). 25 g of leaves or 2.5 g buds were used for each extraction sample.

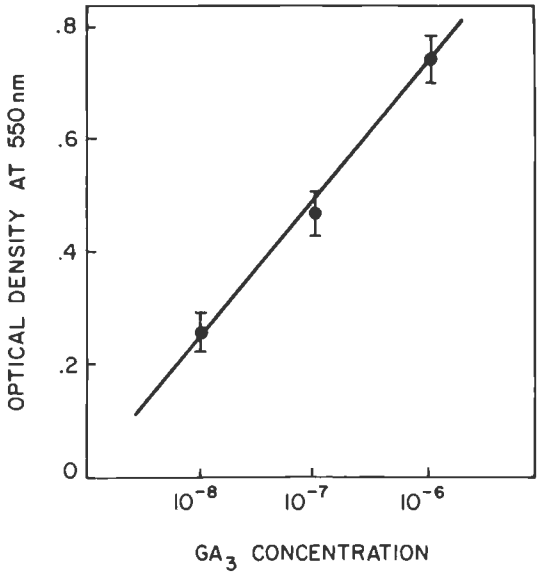

Fig. 1: The quantitative response of sugar release (expressed in optical density, OD) from barley half-seeds due to incubation with increasing concentration of GA_3.

Die Korrelation zwischen der Zuckerfreisetzung (als optische Dichte, OD, gemessen) aus halbierten Gerstenkörnern und ihrer Bebrütung mit zunehmenden GA_3-Konzentrationen.

The initial extraction was made with a 0.1 M phosphate buffer in 80 % methanol at pH 7. After filtering, reextraction and evaporation, the aqueous phase was adjusted to pH 8.3, and then cleaned repeatedly with petrol ether and ethyl acetate. The aqueous phase was acidified to pH 2.5 and reextracted 3 times with ethyl acetate. The combined ethyl acetate fraction was evaporated to dryness, the residue dissolved in 2 ml absolute ethanol, then used for determinations of free gibberellin-like activity (GLA).

The aqueous phase was also hydrolyzed with 0.4 M HCl, for 1 h at 60 °C, adjusted to pH 2.5 and reextracted with ethyl acetate for determination of possible bound GLA. The GA containing fractions were subjected to ascending paper chromatography and GA_3 markers were located by UV. The strips were than divided into Rf sections and incubated in a revolving drum with barley half-seeds for 36 h at 26 °C (3). After incubation, the amount of sugar was determined by the Sumner test (4) and expressed as

absorbance at 550 nm using a Spectronic 99 digital spectrophotometer. Three concentrations of GA_3, 10^{-8}, 10^{-7} and 10^{-6} M, were fractionated in a similar way and used as quantitative standards. A concentration-response curve was established (Fig. 1) for comparison with pooled active fractions of 1982 experiments.

The standard error (SE) was calculated for each sampling and presented either as bars or as values. When SE values obtained for different treatments of the same experiment were similar, they were presented as a mean standard error (MSE) expressed as percent of the mean value.

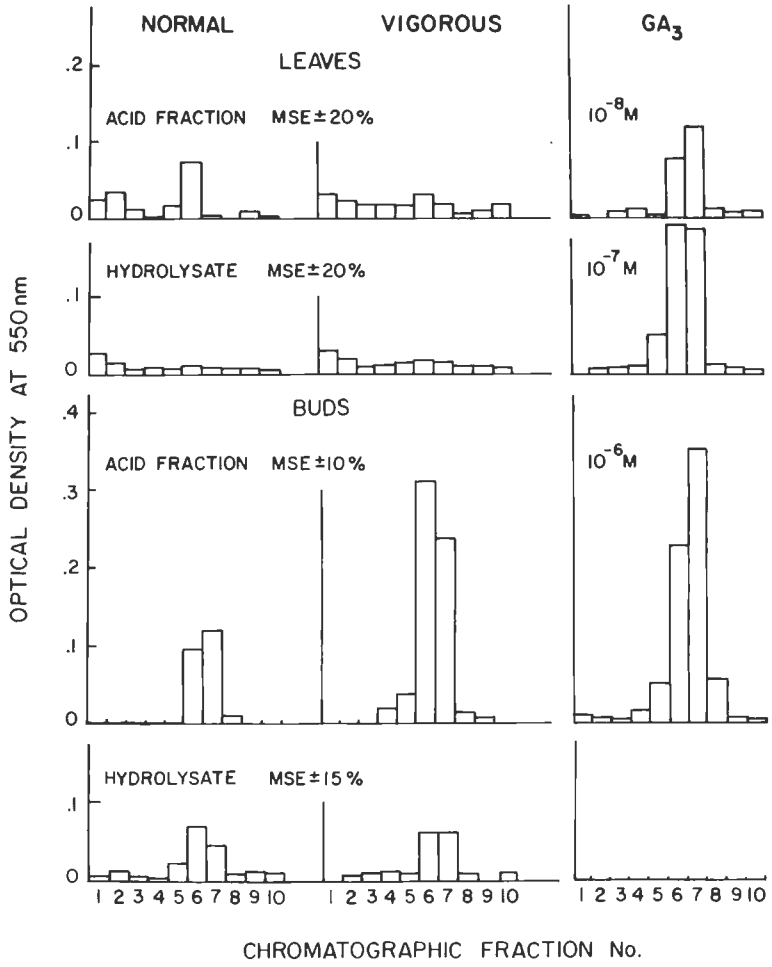

Fig. 2: The gibberellin-like activity (expressed in optical density, OD, for 1 g f. wt of tissue) in leaves and buds of regular and vigorously growing vines of cv. Queen of Vineyard 10 d after full bloom. 1981 experiments.

Die Gibberellin-artige Aktivität (als optische Dichte, OD, je g Frischgewicht) in Blättern und Knospen normal- und starkwüchsiger Reben der Sorte Queen of Vineyard 10 d nach der Vollblüte. Versuch 1981.

Results

Extractable GLA from leaves and buds on shoots of normal and vigorous Queen of Vineyard vines was determined. No significant difference in GLA was noted in leaves of shoots from regular and vigorous vines. This was true both for the acid-extractable free GLA and that released after hydrolysis (Fig. 2). Furthermore, no noticeable GLA was found in the hydrolysates of leaf extracts. The activity in the acid fraction was somewhat higher but also statistically insignificant. On the other hand, young buds on developing shoots had a marked GLA in the acid fraction and to a somewhat lesser extent also in the hydrolysate.

A highly significant difference in free GLA was found between buds of regular and vigorous vines. 10 d after full bloom, the buds on the vigorous vines had two to three times more GLA than those on regular vines (Fig. 2). Most of the activity was at Rf 0.6—0.7, which in our system was in the same range as the activity of a GA_3 marker. The amount of GLA in the acid fraction of 1 g of buds of the vigorous vines was at least approximately 10^{-6} M GA_3 while that of normally growing vines was in the range of 10^{-8} M.

In 1982, regular and vigorous vines were sampled three times, 5, 15 and 30 d after full bloom. In the leaves, no clear cut differences in GLA were noted between the regular and the vigorous plants except for a higher activity in the acid fraction of leaves from vigorous vines 15 d after full bloom. In the 1982 experiments, some GLA was noticed also in the hydrolysate at the two earlier sampling dates. At 30 d after full bloom, there was practically no GLA in the leaf hydrolysate fraction. Generally there was less GLA in the bound fraction of the leaves than in the acid fraction. The acid fraction had about the same level of GLA throughout the sampling period (Table).

Free and bound gibberellin-like activity (GLA) in leaves and buds on shoots of regular and vigorously growing Queen of Vineyard vines 5, 15 and 30 d after full bloom · 1982 experiments (Results are expressed as absorption data of the Sumner test of the fraction-treated barley endosperms, for 1 g f. wt of tissue ± SE)

Freie und gebundene Gibberellin-artige Aktivität (GLA) in Blättern und Knospen normal- und starkwüchsiger Reben der Sorte Queen of Vineyard 5, 15 und 30 d nach der Vollblüte · Versuch 1982 (Die Daten sind Absorptionswerte nach dem Sumner-Test an Gerstenendosperm, sie beziehen sich auf 1 g frisches Rebengewebe ± SE)

d after full bloom	Free GLA fraction		Bound GLA fraction	
	Regular	Vigorous	Regular	Vigorous
		Leaves		
5	76 ± 8	67 ± 7	33 ± 4	41 ± 8
15	69 ± 5	118 ± 4	29 ± 4	27 ± 4
30	68 ± 6	66 ± 7	6 ± 1	7 ± 2
		Buds		
5	332 ± 18	778 ± 24	187 ± 12	217 ± 14
15	404 ± 14	864 ± 16	317 ± 23	375 ± 17
30	185 ± 17	265 ± 12	425 ± 22	459 ± 13

The GLA of the buds was very different in the regular and vigorous vines at all three sampling dates. The acid-extractable GLA was high 5 and 15 d after full bloom and lower after 30 d. However, even at the latter date it was three to four times higher (on a fresh-weight basis) than in the leaves. The amount of acid-extractable GLA was more than double in the buds of vigorous vines than in those of normal ones. This was true both 5 and 15 d after full bloom, but thereafter both the activity and the difference decreased.

The bound GLA in the buds was considerably lower than the acid extractable GLA on the 5th and 15th d after full bloom. At the third sampling date, 15 d later, the bound GLA was higher than that in the acid fraction. It seems that the bound GLA increases gradually in the buds over the sampling period. A reverse tendency was noted with the bound GLA in the leaves (Table). The bound GLA in the buds of the vigorous and regular vines was similar, but still the higher GLA in the buds of vigorous plants at the two early sampling dates was statistically significant (16 and 18 % higher, respectively). At the late sampling, 30 d after full bloom, the difference was considerably less (8 %) and nonsignificant. The free GLA in the buds of the 1982 experiments reached the same level (comparable to 10^{-6} M GA_3) as in 1981, at relatively similar sampling dates. The bound GLA in the buds was somewhat higher in 1982 and increased to levels comparable to 10^{-7} M GA_3/g buds 30 d after full bloom.

Discussion

In previous studies (6, 8), we have shown that the occasional necrosis of lateral buds on canes of cv. Queen of Vineyard is positively correlated with vine vigor. Furthermore, the same type of necrosis occurs on this cultivar also due to GA_3 treatments between full bloom and full set. The enhanced growth of young vegetative shoots and elongation of reproductive organs due to GA_3 treatments is well known in seeded *V. vinifera* cultivars (7). Abnormal rapid shoot elongation due to GA_3 treatments of cv. Queen of Vineyard has been observed at many sites in Israel since 1960 (5). It was suggested that the level of endogenous GA in vigorously growing shoots is higher than in shoots on the regularly growing vines. The present work confirmed this hypothesis, showing high GLA in the lateral buds of the vigorous shoots. Furthermore, it was quite clear that the GLA was considerably higher at the early stages of shoot development, during rapid growth, than later in the season. As the high GLA was in the soluble fraction, it is suggested that a supraoptimal concentration of GA reaches sensitive organs. Such a high concentration might prevent lignification of tissues, e. g. of the developing bud, and cause abnormal development. As both GA_3 applications and high vigor, which is linked to high GLA, lead to necrosis in lateral buds of cv. Queen of Vineyard, it can be concluded that high GA levels are a cause of this bud malformation. The uniform and low levels of GLA in the leaves of both regular and vigorous vines could be expected as the leaves are terminal organs and not directly connected to the continuous growth of the shoots. On the other hand, it was quite clear that the initial high level of free GLA was slowly deactivated by binding and formation of an insoluble, and thus not readily active fraction. This binding of the GA might explain the normal development of the younger lateral buds in the grapevine eye, a development which leads to the formation of split buds (2, 8).

The actual gibberellins involved and the metabolic pathway in the Queen of Vineyard buds are not yet established. It may be concluded, however, that a high level of

GA, whether the GA is applied exogenously or transported endogenously in vigorous shoots, is a major factor in leading to abortion of the central bud in cv. Queen of Vineyard's lateral eyes.

Summary

The endogenous content of free and bound gibberellins (GA) in leaves and buds of Queen of Vineyard grapevines was determined. Lateral buds on shoots from vigorous vines had about double the free GA activity of buds from less vigorous vines. No difference in the activity of bound GA was found. The GA content in the leaves of Queen of Vineyard was uniform, regardless of the vigor of the plants. The relation between vigor, GA content and bud necrosis in Queen of Vineyard vines is discussed and a causal dependence is suggested.

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