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Effects of Application Date of Antibiotic on Seedlessness and Berry Size in 'Muscat of Alexandria' and 'Neo Muscat' Grapes

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A study on the effects of the application date of antibiotic on seedlessness and berry size was conducted using 'Muscat of Alexandria' (MOA) and 'Neo Muscat' (NM) grapevines. Two antibiotics were used as seedlessness inducer, streptomycin (SM) and spectinomycin (SE) applied in the concentration of 200 mg/L by dipping the clusters. The tested dates of application were 6 and 3 days before full bloom, at full bloom (50% anthesis), and 5 days after full bloom. The 3-days before full bloom application gave the highest percentage of seedless berry within each antibiotic in both cultivars. Application later or earlier than 3 days before full bloom increased production of empty-seeded berries, though the later application was able to increase the berry size and berry weight.

Key words : Streptomycin, spectinomycin, date of application, seedlessness

Introduction

It is known that the antibiotics could be used as seedlessness inducer in grapevine[3,6,7,10,14]. It gave a promising alternative method to produce seedless berry in seeded *vinifera* cultivars that were responsive to the negative effect of GA, the common seedless inducer in grapes[8,9,10]. Because of its negative effects such as decrease in berry size, increase of shot berry formation, berry drop after harvest, and lignified and thickened rachis[12,13], GA cannot be applied for certain *vinifera* cultivars, especially for 'Muscat of Alexandria'. 'Muscat of Alexandria' has a very severe response to the effect on lignifying and thickening of peduncles after GA treatment.

However, it was also evidenced that antibiotics decreased the berry size and berry weight of the seedless berry[14]. On the other hand, the early period of berry development takes place as cell division for about 2 weeks[4], and the antibiotics induce seedless berry by inhibiting endosperm nuclei division[3,14]. Therefore, it was suggested that suspending application of antibiotic could increase the berry size. Meanwhile until recently, it was known that the effective date of application of antibiotic to induce seedlessness in grapes is 3 days before full bloom[2,6]. The object of this experiment was to study the effects of date of application of antibiotic on seedlessness induction in grapes. Moreover, the effects of the date of application on the berry size and berry weight were also observed.

Materials and Methods

Plant materials

The experiment was initiated on March 1998 at research vineyard of Okayama University using individual-bed planted vines of 4-year-old 'Muscat of Alexandria' (MOA) and a collective-bed planted with 3-year-old 'Neo Muscat' (NM)

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vines. Fifteen vines of each cultivar were used. MOA vines were cultivated under green house conditions, while NM vines were cultivated in field conditions under plastic tunnel.

Treatment and Sampling

Two antibiotics were used: streptomycin (SM) as AGREPT of Meiji Pharmaceutical industry containing 25% of streptomycin and spectinomycin (SE). Both antibiotics were applied in the concentration of 200 mg/L by dipping the clusters in the antibiotic solutions using four dates of application, examined, 6 and 3 days before the full bloom (6-dBB and 3-dBB), at full bloom (50% anthesis) and 5 days after bloom (5-dAB). Clusters with no antibiotic treatment were used as the control. The treatments were done using 8 clusters for each. This experiment was conducted in completely randomized design.

At harvest, the proportion of seedlessness, and the harvest parameters including berry-weight, berry length and berry width, and °Brix of berry juice were determined using 5 clusters for MOA and 4 clusters for NM as replication. These variables were statistically analyzed with F-test and Duncan's Multiple Ranges Test (DMRT).

Results

Seedlessness

The effects of application date of SM and SE on MOA grape are shown in Table 1. It was seen that SM was generally more effective than SE on seedless berry induction. All of the SM treatments produced more than 80% seedless berries, while SE produce more than 80% seedless berries when it was applied before blooming. Blooming or post-blooming treatment of SE only produced seedless berries in percentages of 50% or less. The highest percentage of seedless berry was gained in SM applied at 3 days before full bloom (3-dBB) followed by the 3-dBB treatment of SE. Empty-seeded berries were observed in lowest percentage in 3-dBB treatment of SM, also followed by 3-dBB treatment of SE. These facts indicate that 3 days before blooming was the best date of application for SM or SE to induce seedlessness for MOA.

If the values of seedless berry percentage were added to the values of empty-seeded berry percentage, which then expressed the percentage of berry with abnormal seed formation, it could be seen that SM was more effective than SE on the inhibitory effect of seed development. All of the SM treatments produced no normal-seeded berries, while the blooming treatment and the 5-dAB treatment of SE still produced 9% and 3% of seeded berries, respectively. However, the seed number of those seeded berries from SE treatments was only half or less than that of the

Table 1 Effects of application date of antibiotic on seedlessness induction in 'Muscat of Alexandria' grape

Treatment[a]	Seeded berries (%)	Empty seeded berries (%)	Seedless berries (%)	Seed number of seeded berries	Empty seed number[b]
Control	86.5a[c]	0.5b	13.0c	2.3	0.42c
SM 200; 6-dBB	0.0c	14.0b	86.0a	0.0	0.80c
SM 200; 3-dBB	0.0c	7.0b	93.0a	0.0	0.60c
SM 200; fB	0.0c	19.0b	81.0a	0.0	0.80c
SM 200; 5-dAB	0.0c	13.0b	87.0a	0.0	0.93bc
SE 200; 6-dBB	0.0c	15.0b	85.0a	0.0	0.69c
SE 200; 3-dBB	0.0c	11.8b	88.2a	0.0	0.40c
SE 200; fB	9.0b	41.0a	50.0b	1.2	1.53ab
SE 200; 5-dAB	3.0bc	50.0a	47.0b	0.2	1.78a

[a] Control: without any treatment; SM 200: streptomycin 200 mg/L; SE 200: spectinomycin 200 mg/L; dBB: days before full bloom; fB: full bloom (≤ 50% anthesis); dAB: days after full bloom

[b] Counted in the berries containing empty seeds

[c] Mean separation in columns by Duncan's Multi Range Test, 5% level

control.

Table 2 shows the effects of the application date of SM and SE on seedlessness in NM grape. In NM grape, SM was also observed to be more effective than SE. The effect of application date of SM in NM grape was in a similar pattern to that in MOA grape. For NM grape, it was also found that 3-dBB treatment of SM gave the highest result on seedless berry percentage. In SE case, the effect of the application date was of a similar pattern but weaker than that in MOA grape. Once again, 3-dBB treatment of SE expressed the highest percentage of seedless berry though it only reached 54%.

More empty-seeded berries were produced in SE treatments than in SM treatments for NM grape. As in MOA grape, 3-dBB treatment within each antibiotic kind resulted in the lowest percentage of empty-seeded berry. We observed one interesting phenomenon concerning empty-seeded berry production based on antibiotic kind. Within SM treatments, the highest percentage of empty-seeded berry was produced by blooming treatment and followed by 6-dBB treatment, while within SE treatments, the highest percentage of empty-seeded berry was produced 5-dAB and followed by full-bloom treatment.

Berry Size and Total Soluble Solid

The effects of the application date of SM and SE on berry size and Total Soluble Solid (TSS) of berry juice are shown in Table 3 for MOA and Table 4 for NM. All of the antibiotic treatments decreased the berry size and berry weight either in MOA or MN, but increased TSS in MOA and only slightly increased it in NM.

Berry length, berry width and berry weight of the SM treatments in MOA grape were ranged in 59-67%, 65-71% and less than one-third of those of the control. SE treatments showed weaker effects on the decrease of berry size and berry weight than SM. The ranges of berry length, berry width and berry weight of SE treatments were 67-87%, 53-88% and 30-63% of those of the control. The early treatments of either SM or SE presented stronger effects in the decreasing of berry length and berry weight than the late treatments, but the SE also presented a similar pattern on berry width. The different effect between SM and SE on the berry width caused a different result of both antibiotic treatments on berry shape. SM treatments of full bloom and 3-dBB treatments produced rounder berries compared to the other treatments included the SE treatments. It seemed that SE had no effect on the berry shape.

The effects of SM and SE on berry size and TSS in NM grape were weaker than in MOA grape. The ranges of berry length, berry width

Table 2 Effects of application date of antibiotic on seedlessness induction in 'Neo Muscat' grape

Treatment[a)]	Seeded berries (%)	Empty seeded berries (%)	Seedless berries (%)	Seed number of seeded berries	Empty seed number[b)]
Control	97.2	5.00c[c)]	0.28c	1.7	1.10a
SM 200; 6-dBB	0.0	27.44bc	72.56ab	0.0	1.44a
SM 200; 3-dBB	0.0	13.33c	86.67a	0.0	0.50b
SM200; fB	0.0	44.79b	52.51b	0.0	1.25a
SM 200; 5-dAB	0.0	19.17bc	81.67ab	0.0	1.14a
SE 200; 6-dBB	0.0	74.17a	25.84c	0.0	1.28a
SE 200; 3-dBB	0.0	45.84b	54.16b	0.0	1.21a
SE 200; fB	0.0	79.17a	20.83c	0.0	1.51a
SE 200; 5-dAB	0.0	83.34a	16.67c	0.0	1.59a

a) Control: without any treatment; SM 200: streptomycin 200 mg/L; SE 200: spectinomycin 200 mg/L; dBB: days befere full bloom; fB: full bloom (≤ 50% anthesis); dAB: days after full bloom

b) Counted in the berries containing empty seeds

c) Mean separation in columns by Duncan's Multi Range Test, 5% level

Table 3 Effects of application date of antibiotic on berry size, berry weight and TSS of berry juice in 'Muscat of Alexandria' grape

Treatment[a)]	Berry length (mm)	Berry width (mm)	Berry shape[b)]	Berry weight (g)	°Brix (%)
Control	23.76a[c)]	20.68a	1.169a	6.58a	14.74d
SM 200; 6-dBB	13.97d	13.91d	1.134abc	1.67d	19.882
SM 200; 3-dBB	14.68cd	14.77cd	1.074c	1.73d	20.22a
SM 200; fB	15.91cd	14.66cd	1.084bc	2.26d	20.14a
SM 200; 5-dAB	15.06cd	13.52d	1.113abc	1.93d	20.32a
SE 200; 6-dBB	16.53c	14.89cd	1.106abc	2.35d	19.28bc
SE 200; 3-dBB	15.89cd	14.27d	1.111abc	2.02d	20.17a
SE 200; fB	20.73b	18.28b	1.133abc	4.19b	19.07c
SE 200; 5-dAB	19.65b	16.93bc	1.158ab	3.20c	20.03ab

a) Control: without any treatment; SM 200: streptomycin 200 mg/L; SE 200: spectinomycin 200 mg/L; dBB: days befere full bloom; fB: full bloom (≤ 50 % anthesis); dAB: days after full bloom

b) Berry shape = length: width ratio

c) Mean separation in columns by Duncan's Multi Range Test, 5 % level

Table 4 Effects of application date of antibiotic on berry size, berry weight and TSS of berry juice in 'Neo Muscat' grape

Treatment[a)]	Berry length (mm)	Berry width (mm)	Berry shape[b)]	Berry weight (g)	°Brix (%)
Control	21.26a[c)]	19.26a	1.103a	4.83a	18.95b
SM 200; 6-dBB	15.10d	14.16d	1.070bc	2.35de	19.38ab
SM 200; 3-dBB	15.38d	14.48d	1.062c	2.10e	19.68ab
SM 200; fB	16.23cd	15.42cd	1.052c	2.68cd	20.72a
SM 200; 5-dAB	15.63d	14.73d	1.062c	2.29de	19.75ab
SE 200; 6-dBB	18.57b	17.19b	1.081abc	3.41b	20.24ab
SE 200; 3-dBB	15.51d	14.77d	1.050c	2.49de	19.56ab
SE 200; fB	18.79b	17.15b	1.096ab	3.50b	19.98ab
SE 200; 5-dAB	17.56bc	16.34bc	1.075abc	3.04bc	19.94ab

a) Control: without any treatment; SM 200: streptomycin 200 mg/L; SE 200: spectinomycin 200 mg/L; dBB: days befere full bloom; fB: full bloom (≤ 50 % anthesis); dAB: days after full bloom

b) Berry shape = length: width ratio

c) Mean separation in columns by Duncan's Multi Range Test, 5 % level

and berry weight of the SM treatments in NM grape were 71-76 %, 73-80 % and 43-55 %, while those of the SE treated berries were 73-89 %, 69-81 % and 52-72 % of those of the control. All of the SM treatments produced rounder berries than the control in NM grape, while within the SE treatments, only the berries from 3-dBB treatment were rounder than the control berries. The date of application of SM and SE did not affect TSS of berry juice, except that the full bloom application of SM increased TSS significantly.

Discussion

Among the four tested dates of application of SM and SE, It was shown that the 3-days before full bloom (1 days after the first anthesis) treatment resulted in the highest percentage of seedless berry within each antibiotic either in MOA or NM grapes. This was in agreement with our previous experiment[14]. It was also in agreement with Kimura *et al.*[3] and Ogasawara[6] who had examined the use of SM on seedlessness induction in 'Muscat Bailey A'.

Further, if the percentage of seedless berry was added to the percentage of empty-seeded berry, and expressed as the percentage of the total berry with abnormal seeds, either SM or SE was very effective for inhibition of ovule development. Therefore, both antibiotics had a similar effect on the inhibition of the development of internal parts of the seed. In this case, SM was stronger than SE. This effect was also dependent on the date of application. The effect of SE became weaker when applied later, while SM presented a constant effect, i.e. there was no normal seeded berry produced from all of the SM treatments.

The interesting phenomenon observed in this experiment was the empty-seeded berry production. It seemed that the pattern of the effect of SM and SE on empty-seeded berry production was not dependent on the cultivar. The lowest percentage of empty-seeded berry was produced by 3-dBB treatment within each antibiotic. In the case of SM treatment, the highest percentage of empty-seeded berry was gained on full bloom treatment, while for the SE treatment it was on 5-dAB treatment. However, within SE treatments, there was no significant different on empty-seeded berry percentage between full bloom and 5-dAB treatment.

This phenomenon maybe had some connection to the working of antibiotics on the inhibition of ovule development. It was known that SM inhibited the seed development by its inhibitory effect on the endosperm nuclei development[3,14]. In another of our other experiments, it was also evidenced that SE also affected the endosperm nuclei division severely, leading to ovule abortion in MOA (unpublished). So, it was suggested that in the pre-bloom treatments, the 6-dBB presented a higher percentage of empty-seeded berry than the 3-dBB because the activity of the antibiotics had weakened. But the highest percentage of empty-seeded berry of full bloom treatment, especially in SM treatments, could not be explained clearly in this experiment.

Since the NM grape produced a higher percentage of empty-seeded berries than MOA, it could be suggested that the external part of the ovule of MOA was more responsive to antibiotics than that of NM. On the other hand, since there was no normal-seeded berry produced in antibiotic treated NM, while it was still produced in MOA, it also could be concluded that NM was more responsive to the inhibitory effect of seed development on the internal parts of the seed than MOA.

In this experiment, the berry size was increased by suspending the date of application. This fact was clearly seen on SE treatments, either in MOA or NM. Although suspending application of SE could increase the berry size of NM, it became useless in the seedlessness induction effort, because suspending the application also increased the percentage of empty-seeded berry. The empty-seeded berry was not considered as a seedless berry, due to the hardness of the perceptible seed traces[1,5]. However, there was an interesting physiological phenomenon that could be observed in this experiment, i.e. the relationship between the berry size and the seed number in the berry, though the seeds were empty. The berries grew in size with any increase in seed number. It was in agreement with Bosseli *et al.*[2].

Based on the results above, our next experiments are designed to find methods of berry enlargement for seedless berries induced by antibiotics applied at 3 days before full bloom. The use of plant growth regulators should possibly be observed more intensively to overcome the berry size problem of those seedless berries.

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ブドウ‘マスカット・オブ・アレキサンドリア’，‘ネオ・マスカット’の無核化と果粒サイズに及ぼす抗生物質の処理時期の影響

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‘マスカット・オブ・アレキサンドリア’と‘ネオ・マスカット’について，無核化のために行う抗生物質処理の時期がその効果と果粒の肥大に及ぼす影響を調査した．無核化剤としてストレプトマイシンとスペクチノマイシンを用い，満開日（小花の50％が開花）とその6，3日前，および5日後に200mg/MLで果房の浸漬処理を行った．両品種とも，3日前に各抗生物質を処理すると無核化率が最も高かった．それより遅い処理では果粒サイズは大きかったが，空洞種子（empty seed）を含む果粒が多く着生した．