EFFECT OF TWO FUNGICIDES WITH CYTOKININ LIKE ACTIVITY ON SHOOT PROLIFERATION *IN VITRO* OF TWO COMMERCIAL SWEET CHERRY ROOTSTOCKS

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Abstract

The fungicides imazalil and carbendazim were used in the *in vitro* studies. Imazalil was used at 5, 10 and 20 mg/l concentrations for the rootstock CAB-6P and 2.5, 5 and 10 mg/l for the cherry rootstock Gisela 6. The fungicides were applied alone or in combination with 0.5, 1 and 2 mg/l benzyl-adenine (BA). Carbendazim was applied at 0.5 and 1 mg/l and combined with BA in all the tested rootstocks. The maximum number and length per shoot were 15,67 and 154,33 mm in the rootstock CAB-6P and was produced with 5 mg/l imazalil plus 1 mg/l BA. The respective numbers for Gisela 6 were 13,4 and 198 mm, in the precence of 5 mg/l imazalil plus 0.5 mg/l BA. Different ratio of carbendazim / BA concentration for each tested rootstock affected differently the number of produced shoots. Both fungicides showed a cytokinin like – activity only in the presence of BA. The efficiency of the two fungicides in combination with BA concerning shoot proliferation followed the order CAB-6P > Gisela 6.

Key words: Carbendazim, fungicides, Imazalil, *in vitro*, *Prunus avium* rootstocks

INTRODUCTION

Imazalil belongs to the group of imidazole fungicides with diasystemic action. As concerns the mechanism of its action, it acts as an inhibitor of demethylation at ^{14}C. So, it interferes with membrane permeability, by accumulation of sterols instead of ergosterol.

The interference of gibberellin biosynthesis attributed to inhibition of cytochrome P-450 dependent enzyme of ent-kaurene oxidase, which catalizes the oxidation of ent-kaurene into kaurenic acid, a precursor of gibberellin biosynthesis.

Carbendazim belongs to the category of benzymidazole fungicides.

Concerning the mechanism of action, carbendazim interferes with the growth and development, by regulating the process of mitosis and cell division.

In the literature, there are many reports, Werbrouck and Debergh (1995), the Debergh and colleagues (1993), the Baskaran and Jayabalan (2008) and the Nandi et al, (2006), who indicate that the above two fungicides, show cytokinin like activity in diferent species. Therefore, the aim of this work was to verify their organogenetic action in tissue culture experiments.

MATERIALS AND METHODS

It was studied the effect of imazalil at three increasing concentrations of 5, 10 and 20mg/l for the cherry rootstock CAB-6P (*Prunus cerasus*), and at 2.5, 5 and 10mg/l for the rootstock, Gisela 6 (*Prunus cerasus x Prunus canescens*). Imazalil was applied alone or in combination with three concentrations of BA (0.5, 1 & 2 mg/l). Carbendazim was added in the culture medium at 0.5 and 1mg/l concentrations, individually or combined with the same concentrations of BA as above, in the rootstock Gisela 6. In CAB-6P, carbendazim applied only in combination with BA.

The culture medium that used was the full strength MS (Murashige and Skoog, 1962). The culture medium also supplemented with 30g/l sucrose. Agar 6g/l was added after adjusting the pH of the medium to 5.8 and afterwards the medium was autoclaved for 20 minutes at 121°C.

Shoot apices 2 to 2.5cm long from previous in vitro culture were used. The explants were transferred aseptically into 25x100mm test tubes, containing 10 ml of medium. Each treatment consisted of 10 replicates. Explants were transferred to a growth chamber with a temperature of 21°C ± 2°C and 16 hour photoperiod of 90μmol $m^{-2}s^{-1}$ light intensity, provided by cool-white fluorescent lamps.

When seven weeks elapsed, we measured number, length (mm), fresh weight (g) of shoots and afterwards the explants were dried in an oven at 65°C for 24 hours and the total dry weight of the explants of each treatment was recorded.

Statistical analysis

The calculation of the average shoot number, fresh and dry weight of shoots, shoot length and the percentage of shoots per treatment as well as the comparison of the means conducted with the statistical programme SPSS (version 16.0). Statistical analysis was performed by one-way ANOVA for significance level ≤ 5% and the comparison of means was based on the Duncan's multiple range test. In the tables that follow, the numbers with different letters differ statistically significantly at the level of P≤0.05.

RESULTS

Effect of imazalil and BA on shoot proliferation

The maximum number and length per shoot were 15,67 and 154,33mm in the rootstock CAB-6P and was produced with 5mg/l imazalil plus 1mg/l BA. The respective numbers for Gisela 6 were 13,4 and 198mm respectively in the presence of 5mg/l imazalil plus 0,5mg/l BA.

Table 1

Effect of BA and imazalil concentration, alone or combined on the average number and length of shoots/explant in the rootstock CAB-6P.

Treatments (mg/l)	Number of shoots / explant	Shoot length (mm)
Control	1,00 a	28,55 a
0,5 BA	10,33 cde	119,25 de
1 BA	9,33 cde	82,75 bcd
2 BA	7,27 bcd	62,18 abc
5 Imazalil	1,00 a	30,88 a
5 Imazalil+0,5 BA	11,33 e	104,42 cd
5 Imazalil+1 BA	15,67 f	154,33 e
5 Imazalil+2 BA	5,00 b	48,11 ab
10 Imazalil	1,00 a	28,50 a
10 Imazalil+0,5 BA	10,73 de	100,55 cd
10 Imazalil+1 BA	10,45 cde	87,91 bcd
10 Imazalil+2 BA	5,73 b	50,82 ab
20 Imazalil	1,00 a	25,13 a
20 Imazalil+0,5 BA	7,00 bc	66,67 abc
20 Imazalil+1 BA	7,33 bcd	65,33 abc
20 Imazalil+2 BA	5,60 b	50,80 ab

Table 2

Effect of BA and imazalil concentration, alone or combined on the average number and length of shoots per explant in the rootstock Gisela 6.

Teatments (mg/l)	Number of shoots / explant	Shoot length (mm)
Control	2,00 a	23,89 a
0,5 BA	5,75 b	54,50 ab
1 BA	5,88 b	59,75 ab
2 BA	5,88 b	64,00 ab
2,5 Imazalil+0,5 BA	11,09 de	170,45 c
2,5 Imazalil+1 BA	12,50 e	163,00 c
2,5 Imazalil+2 BA	8,90 bcd	82,50 ab
5 Imazalil+0,5 BA	13,40 e	198,00 c
5 Imazalil+1 BA	10,70 de	133,50 bc
5 Imazalil+2 BA	8,11 bcd	67,20 ab
10 Imazalil+0,5 BA	10,11 cde	133,33 bc
10 Imazalil+1 BA	6,90 bc	72,50 ab
10 Imazalil+2 BA	8,80 bcd	80,00 ab

In the rootstock CAB-6P, increase of BA had as a result a decrease in the number and length of shoots (Table 1). The percentage of shoots raised from 8,33% to 100% when the concentration of BA increased from 0,5 to 1mg/l, but was constant when BA was 2mg/l (data not shown). Application of imazalil in the absence of BA, had absolutely no effect in the induction of multiple shoots. Imazalil combined with 2mg/l BA, gave the minimum mumber and length of shoots (Table 1). In both rootstocks, CAB-6P and Gisela 6, in the treatments, where imazalil was combined with BA, the percentage of shoots was maximum (100%) (data not shown).

In the rootstock Gisela 6, there were no statistically significant differences between treatments, concerning the number and length of shoots, as BA concentration raised. For a certain concentration of imazalil, when BA concentration increased, the shoot length decreased. At the lowest concentration of imazalil (2,5mg/l), an increase, of BA concentration from 0,5 to 1mg/l, leaded also to an increase in number and length of shoots, but the above two characteristics decreased by doubling the BA concentration up to 2mg/l (Table 2).

Effect of carbendazim and BA on shoot proliferation

Table 3

Effect of BA and carbendazim concentration, alone or combined on the average number and length (mm) of shoots per explant in the rootstock Gisela 6.

Treatments (mg/l)	Number of shoots / explant	Shoot length (mm)
Control	1,00 a	26,00 a
0,5 BA	5,75 bc	53,88 ab
1 BA	5,88 bc	61,63 b
2 BA	5,88 bc	64,00 b
0,5 Carbendazim	1,00 a	28,00 a
0,5 Carbendazim+0,5 BA	6,38 bc	76,38 b
0,5 Carbendazim+1 BA	5,63 b	54,00 ab
0,5 Carbendazim+2 BA	8,75 c	67,88 b
1 Carbendazim	1,00 a	28,00 a
1 Carbendazim+0,5 BA	5,25 b	60,25 b
1 Carbendazim+1 BA	7,57 bc	75,86 b
1 Carbendazim+2 BA	7,88 bc	66,63 b

In the rootstock Gisela 6, the shoot length raised, as BA concentration increased. However, no statistically significant differences were recorded concerning the number of shoots. Also, the percentage of shoots was 100% and did not altered. Application of carbendazim in the absence of BA, had absolutely no effect in the induction of multiple shoots. (Table 3).

The combination of the lower concentration of carbendazim (0,5mg/l) and the higher of BA (2mg/l) presented the maximum number of shoots per explant (8,75). The maximum shoot length (76,38mm) was recorded in the treatment of 0,5mg/l carbendazim plus 0,5mg/l BA (Table 3).

Table 4

Effect of BA and carbendazim concentration alone or combined on average number and length (mm) of shoots per explant in the rootstock CAB-6P.

Treatments (mg/l)	Number of shoots / explant	Shoot length (mm)
Control	2,00 a	40,00 a
0,5 BA	10,33 bcd	119,25 abc
1 BA	9,33 bcd	82,75 ab
2 BA	7,27 b	62,18 a
0,5 Carbendazim+0,5 BA	13,90 cde	166,00 bcd
0,5 Carbendazim+1 BA	22,50 f	215,00 d
0,5 Carbendazim+2 BA	8,70 bc	71,50 a
1 Carbendazim+0,5 BA	28,20 g	446,50 e
1 Carbendazim+1 BA	16,80 e	190,00 cd
1 Carbendazim+2 BA	14,45 de	107,30 abc

In the rootstock CAB-6P, when BA concentration increased, it resulted in a reduction of the number and length of shoots(Table 4). The percentange of shoots was low only (8,33%) at the lowest concentration of BA but increased and was maximum (100%) at higher concentrations (data not shown).

In the rootstock CAB-6P, at the lowest concentration of BA (0,5mg/l), as the concentration of carbendazim increased, the number of shoots doubled from 13,90 to 28,20 and the shoot length tripled from 166mm to 446,5mm. At 1mg/l of BA, concentration, there was a negative correlation between carbendazim concentration and the two above characteristics. The same applies also for the two higher concentrations of BA (Table 4). In the treatments where carbendazim was combined with BA, the percentage of shoots was 100% but it was reduced to 11,11% as BA concentration increased. This happened at the low applied concentration of carbendazim (0,5mg/l). However, at the high concentration of 1mg/l, the percentage was 100% and remained stable (data not shown). The maximum shoot number and length per shoot were 28,2 and 446,5mm and was produced with 1mg/l carbendazim plus 0,5mg/l BA (Table 4).

DISCUSSION

A different response to organogenesis of the two rootstocks to BA concentration alone or in combination with imazalil and carbendazim was reported in this work. Benzyladenine exerted a positive effect on shoot proliferation by breaking dominance of the apical meristem and by inhibiting the auxin polar transport from the base of the explant (Vogiatzis and Koukourikou – Petridou, 2003). Imazalil and carbendazim alone had not a cytokinin activity. In the rootstocks CAB-6P and Gisela 6, imazalil of 2.5-5mg/l increased the positive effect of BA concerning shoot proliferation.

In *Spathiphyllum floribundum "Petite* an average of 127 shoots / explant developed when 2.5 mg/l BA was combined with 16 mg/l imazalil. Doubling the BA concentration had no significant effect on shoot induction. When imazalil was applied without BA, the number of roots and total root length/plant were reduced, but no new shoots developed (Werbrouck and Debergh, 1995). The Debergh and colleagues (1993), in *Coryline terminalis* and *Prunus avium* and the Baskaran and Jayabalan (2008) in *Bacopa monniera* and *Psoralea corylifolia*, found the same upward trend concerning the number and length of shoots.

On the other hand, carbendazim reinforces the stimulating effect of BA on shoot proliferation regardless of its concentration, especially in the rootstock CAB-6P and to a lesser extent in Gisela 6. In the Indian medicinal plant *Bacopa monniera*, carbendazim at a concentration of 30μg/l leaded to a significant increase in the number and length of shoots (Baskaran and Jayabalan, 2008). In an other medicinal plant, *Psoralea corylifolia*, 100mg/l carbendazim combined with BA, kinetin, 2-isopentenyladenine and the pesticide trimethoprim, increased significantly the number of shoots per explant (85,2±0,9) after 4 weeks of cultute *in vitro* (Baskaran and Jayabalan, 2008).

Another factor indicating that imazalil and carbendazim did not act like a cytokinin in the absence of BA on the culture medium, is that they caused rooting. Hence imazalil caused rooting in all the tested concentrations in the CAB-6P rootstock and carbendazim in Gisela 6 in the lowest tested concentration of 0,5mg/l. The rooting percentage in the case of imazalil was high and ranged between 75-100% but lower (37,5%) in the case of carbendazim (data not shown).

The Nandi and colleagues (1996) in *Taxus baccata* and Tiwari et al (2006) in *Bacopa monniera*, also indicate high rates of rooting, up to 80%.This is in agreement with the findings of Werbrouck and Debergh (1995), who concluded that imazalil does not influence the inhibitory effect of BA on rooting.

CONCUSIONS

The two fungicides, imazalil and carbendazim, showed cytokinin like activity only when applied on the culture medium with BA, in both rootstocks, CAB-6P and Gisela 6.

The rootstocks reaction concerning the number of produced shoots as affected by the two fungicides in combination with BA, follows the order CAB-6P>Gisela 6. As concerns the shoot length, the CAB-6P rootstock reacted better on the combination imazalil plus BA while Gisela 6 on carbendazim plus BA.

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