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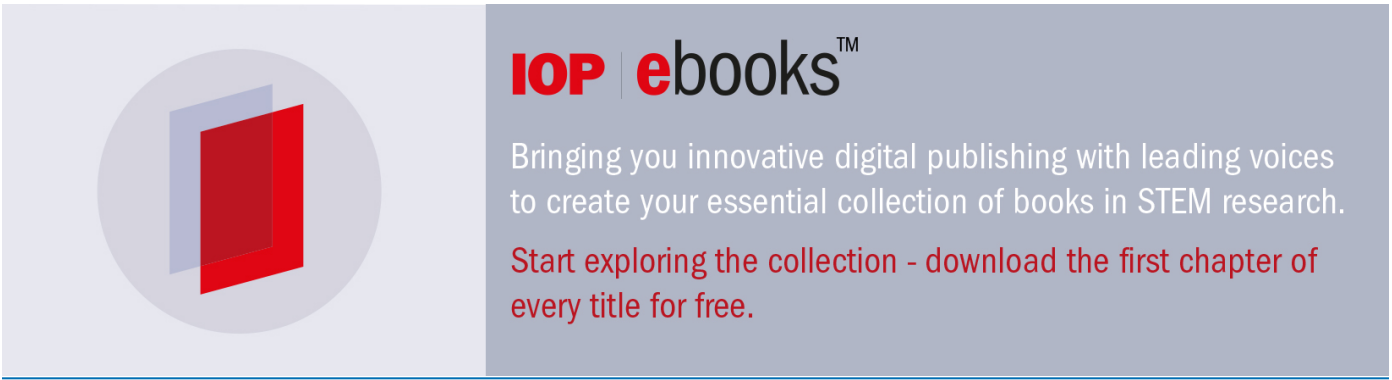

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The Effects of Carbenicillin on Growth and Differentiation of Three Berries

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Abstract. The research aimed to provide references for further study of gene transformation of blueberry, raspberry and cranberry in Agrobacterium-mediated transformation. Effects of carbenicillin on the growth and differentiation of various explants of three berries were studied, and the inhibitive effects of carbenicillin upon Agrobacterium tumefaciens also were studied. The results showed that the optimum carbenicillinon's concentration was 200～300 mg/kg in the differentiating stage of blueberry and raspberry, and the time of subculture was 7 days; the optimum carbenicillinon's concentration was 400 mg/kg in the differentiating stage of cranberry, and the time of subculture was 7～14 days; The optimum carbenicillin's concentration was under 200 mg/kg in the rooting stage of three berries.

1. Introduction

The Blueberry (Vaccinium spp) of Vaccinium of Ericaceae is a perennial deciduous plant or evergreen shrubs, Cranberry (Vacinium macrocarpon) of Vaccinium of Ericaceae is evergreen shrub, Raspberry (Raspberries) of (Rosacea) Rubus L.of Rosaceae is Semi-shrub tree. The berries, which were known as the third generation of fruit-trees, have the unique flavor, possessing excellent taste, rich aroma and high fruit juice. Besides, These fruit-trees, which contains abundant vitamin E, some flavonoids, polyphenols and a certain ornamental value, has been planted a lot in recent years [1-4].. However, the annual output and quality of these fruits did not meet the requirements of current market [5-7]. A few of varieties was dominating reason to cause this. Therefore, the breeding work needs be done according to the market and conditions [8]. The most varieties of berry are derived from wild species in spite of a few of germplasm resources. Introducing the new characte is difficult by conventional hybridization. However, modern molecular breeding technology is feasible to increase their adaptability of fruit-trees for extensive cultivation[9].

However, there were the low conversion frequency, poor repeatability and other defects caused by the many influential factors and the possible interaction between factors in plant transgenic technology system. The antibiotic as resistance selection agent and Agrobacterium growth inhibitor in selective transformation system is an important factor to determine whether the system can be built. The functions of the antibiotic are to Inhibit the growth of Agrobacterium, and prevent pollution from the excessive growth of Agrobacterium [10]. The large difference in plant sensitivity to antibiotics is

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existed among different varieties of the same plant, and even the same specie of different genotypes [11]. Therefore, the concentration of antibiotic need to be done for mutant selection accurately [12]. The effects of different concentration of carbenicillin on the differentiation, growth of plants and inhibition to Agrobacterium tumefaciens were analyzed with the stems of blueberry, raspberry and cranberry to provide reference data for future gene transformation.

2. Materials and methods

2.1 Materials

The tissue-culture seedlings of blueberry, raspberry and cranberry were offered by landscape plant laboratory of Tianjin Agricultural University. The Agrobacterium tumefaciens LBA4404 and C58 were preserved by the laboratory. The antibiotics were purchased from Dingguo Biological Co., Ltd.

2.2 Methods

2.2.1 Medium. The differentiation medium from stem-segments of Blueberry, raspberry and cranberry was： MS+KT 0.25 mg/L +IAA 0.5 mg/L +sucrose 3%+agar 0.65%. The elongation medium was:1/2MS +IAA 0.25 mg/L +sucrose 3%+agar 0.65%. The rooting medium was: 1/2MS+NAA 0.2 mg/L +sucrose 3%+agar 0.65%. The antibiotic were added into mediums before solidification after mediums were autoclaved and temperature was reduced to 50 ° C, then mediums were shaked well and condensd for application.

2.2.2 Effects of different concentration of carbenicillin on stem-segments differentiation and rooting of blueberry, raspberry and cranberry. The stem-segments and test-tube plantlets of blueberry, raspberry and cranberry of wild seedlings were inoculated on the differentiation and rooting medium of different concentration of carbenicillin gradient. Besides, the their growing status were respectively observed, and a survey statistics was conducted after 21 days. The concentration of carbenicillin was 0, 200, 400, 600 and 800 mg / L. Each treatment was 5 bottles (repetition) ,and 10 stem-segments were in every bottle under 28 ℃ and 24 h / d lighting with 2 000 lx intensity. The growth status of explants was observed regularly.

*2.2.3 The effects of different concentration of carbenicillin on Inhibiting two kinds of Agrobacterium.*The single bacteria colony of Agrobacterium tumefaciens LBA4404 and C58 was inoculated respectively into YEB medium and cultivated overnight on shaker. The mediums were taken out when the OD value of the bacteria liquid was 0.4. The explants of blueberry, raspberry and cranberry were taken out and dried with filter paper after they were soaked in YEB medium with Agrobacterium for 10 minutes. Then, the explants were inoculated into the differentiation mediums with different concentration of carbenicillin to cultivate for 40 days. The growing status of Agrobacterium was observed and recorded on a daily basis.

3. Results and Analysis

3.1 Effects of carbenicillin on the differentiation from stem-segments of blueberry, raspberry and cranberry

The differentiation from stem-segments of blueberry, raspberry and cranberry was shown in Table 1 after 21 days. The differentiation rate of the three varieties reached 100%, and the new buds were also normal when the concentration of carbenicillin was less than 200 mg / kg. But, the situation was changed when the concentration of carbenicillin was higher than 200 mg / kg, the differentiation and growth of only cranberry were not affected, but the blueberry and raspberry were affected to different degree, leading the largest change on raspberry. Besides, the differentiated buds began to die when the

concentration of carbenicillin increased to 600 mg / kg. Therefore, the dosage of carbenicillin of blueberry, raspberry was less than 200 mg / kg, and cranberry was properly increased to 400 mg / kg.

Table 1. Effects of Carbenicillin on Bud Differentiation from Stem Segment of Three Kinds of Berries

variety	Carbenicillin concentration / mg • kg $^{-1}$				
	0	200	400	600	800
Blueberry	100(+)	100(+)	78(0)	23(0)	12(0)
Raspberry	100(+)	100(+)	34(0)	21(-)	0
Cranberry	100(+)	100(+)	100(+)	100(+)	100(0)

Notice: “ + ” Indicating the differentiated buds grow normal; “ 0 ” Indicating the buds stop growing; “ -” Indicating the buds were wilting or death.

It could be seen from Table 2, the rooting rate of the three varieties reached 100%, and the growing of plants were also normal when the concentration of carbenicillin was less than 200 mg / kg. But, they changed when the concentration of carbenicillin was higher than 200 mg / kg, the differention rate of raspberry only were not affected, the blueberry and cranberry were affected to different degree, leading the largest change on blueberry when the concentration of carbenicillin was 400 mg / kg. Besides, the rooting rate of blueberry began to decline when the concentration of carbenicillin reached 200 mg / kg. Therefore, the dosage of carbenicillin of blueberry, cranberry was less than 200 mg / kg, and raspberry was properly increased to 200 ~ 400 mg / kg.

Table 2. Effects of Carbenicillin on Rooting Rate of Three Kinds of Berries

variety	Carbenicillin concentration / mg • kg $^{-1}$				
	0	200	400	600	800
Blueberry	100(+)	67(+)	73(0)	11(0)	0
Raspberry	100(+)	100(+)	100(0)	56(-)	0
Cranberry	100(+)	100(+)	89(+)	23(+)	0

Notice: “ + ” Indicating the differentiated buds grow normal; “ 0 ” Indicating the buds stop growing; “ -” Indicating the buds were wilting or death.

3.2 Inhibitory effect of different concentration of carbenicillin on Agrobacterium tumefaciens

The explants of three varieties, which were treated by Agrobacterium, grew on the differentiation mediums for 40 days, the time of appeared clones of Agrobacterium was gotten by observing with eyes in Table 3 or 4. In table 3, the Inhibitory effect of different concentration of carbenicillin on Agrobacterium tumefaciens LBA4404 was different, and the antibacterial effect on blueberry and cranberry was better than that of raspberry. From the appearance of bacterial colonies on each plant, the duration inhibiting the bacterial colonies for three varieties did not exceed 7 days when the concentration of carbenicillin was less than 200 mg / kg. However, the duration was exceed 14 days for three varieties when the concentration of carbenicillin reached 400 mg / kg. Therefore, 400 mg / kg of carbenicillin was used to the plant transformation cultivation with 14 days interval of subculture. Although the duration was 30 days under treatment of 600 mg /kg, the growth of explants were the basic shutoff or death.

Table 3. Inhibitory effect of carbenicillin on Agrobacterium tumefaciens LBA4404

variety	Carbenicillin concentration / mg • kg $^{-1}$				
	0	200	400	600	800
Blueberry	2	7	20	30	32
Raspberry	1	7	16	19	19
Cranberry	2	5	23	32	33

Notice: the data in the table for the number of days of bacterial colony.

It could be seen from table 4, the inhibitory effect of different concentration of carbenicillin on Agrobacterium tumefaciens C58 was basic same in three varieties, and the antibacterial effects of blueberry and cranberry was a little better than raspberry. From the appearance of bacterial colonies on each plant, the inhibition time of bacterial colonies did not exceed 7 days for all varieties when the concentration of carbenicillin was less than 200 mg / kg. However, the inhibition time was about 14 days when the concentration of carbenicillin reached 400 mg / kg. Therefore, the appropriate concentration of carbenicillin was 400 mg / kg in the transformation cultivation, the time of subculture was below 10 days. Although the inhibition time of bacterial colonies was postponed to 20 days under 600 mg / kg, the growth of explants were the basic shutoff or death.

Table 4. Inhibitory effect of carbenicillin on Agrobacterium tumefaciens C58

variety	Carbenicillin concentration / mg • kg $^{-1}$				
	0	200	400	600	800
Blueberry	1	5	15	26	30
Raspberry	1	4	13	20	21
Cranberry	1	5	16	28	33

Notice: the data in the table for the number of days of bacterial colony.

4. Discussion

It could be seen from the results, the effects of carbenicillin were different on the bud differentiation and rooting of three varieties, which could be relative to degree of tolerance of different plants to carbenicillin[13-15]. Therefore, the tolerance of explants to carbenicillin was bound to inspect in the gene experiment to obtain accurate dosage and increase the transformation rate. Since the response of plant to carbenicillin in rooting medium was stronger than that in bud differentiation medium, the dosage of carbenicillin could be appropriately decreased in the stage.

LBA4404 was easier to be inhibited by carbenicillin compared with Agrobacterium tumefaciens C58[16]. Therefore, the concentration of carbenicillin and the time of subculture should be adjusted according to Agrobacterium type in the gene transformation. Besides, the factors that affected the inhibitory effect of carbenicillin on Agrobacterium wer a lot, such as the concentration of infecting bacteria, soaking time and time of co-cultivation [17].

5. Conclusion

the stem- segments of three berries were taken as explants in the process of gene transformation, the optimum carbenicillinon's concentration was 200～300 mg/kg in the differentiating stage of blueberry and raspberry, and the time of subculture was 7 days; the optimum carbenicillinon's concentration was 400 mg/kg in the differentiating stage of cranberry, and the time of subculture was 7～14 days; The optimum carbenicillin's concentration was under 200 mg/kg in the rooting stage of three berries. Besides, the agrobacterium was basically inhibited in the rooting stage,the time of subculture could be appropriately extended to facilitate rooting and transplant.

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