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The effect of γ ray and early blight fungitoxin on the growth of tomato calli

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Summary Calli derived from tomato leaves were treated with different γ ray doses and cultured in medium with and without early blight fungitoxin. The experiment indicated that γ ray had obvious influence on the growth of tomato calli, with a stronger inhibition at 3000 rad dose. The fungitoxin could be used as selection pressure in the screening of disease resistant mutant.

Key words γ ray, fungitoxin, callus

Introduction

On the basis of the somaclonal variation theory, some practical development was achieved in screening disease resistant mutants and breeding disease resistant varieties by use of fungitoxin as selection pressure (Jiang Youyi et al., 1988).

Only few papers were published on disease resistant mutation of tomato. Shepherd et al. (1986) carried out this research several years ago. Recently, tomato plants resistant to *Fusarium* were obtained by Shahin et al. (1986) using fusaric acid, and analyses of resistance segregation in the progeny were also done.

In this study, resistant plants were selected and regenerated through different culture way of leaf explants on the medium containing *Alternaria solani* toxin. These plants displayed higher disease resistance than the control. In order to find good mutagenic effect, calli derived from leaf explants were irradiated at different γ ray doses, and then inoculated to the media with and without the toxin. Effects of γ ray and the toxin treatments on callus growth were compared and their relation was studied.

Materials and methods

Tomato cultivar Jiafen 10 was used. Its seeds were sown in small plastic pots. After emergence, the youngest, fully expanded leaves were used as explants. These leaflets were sterilized for 10 min. in 0.1% mercuric chloride solution. Subsequently, they were rinsed 4—5 times with sterile distilled water, then leaf discs (0.6—0.8 cm in diameter) were inoculated immediately to the inducing medium (MS+BA1mg/L+NAA 2mg/L). A total of 6 discs were inoculated to each flask. After 20 days of incubation in the culture room, the full-grown, light yellow and approximately round calli emerged from the leaf explants on this medium. These calli were irradiated at different γ ray doses including 0, 500, 1000, 2000, 3000, 4000, 6000 and 8000 rad.

Each 24 calli in 4 flasks were treated at one dosage, and after irradiation, each callus was cutted into 3 pieces, so 72 pieces would be amounted to at every irradiation level. These calli were inoculated to differentiating medium (MS+ZT 2mg/L) with 3% early blight fungitoxin and without it. After two weeks, all calli were inoculated to the same medium without the fungitoxin, then subcultured at one week intervals. The fresh weight

of the callus was weighed and statistically analysed during each transfer.

Results and discussion

The data on fresh weight changes were summarized on Table 1, which indicated that after culture the fresh weight of callus without irradiation and toxin treatment increased by 7.19 times, but that without irradiation but with toxin treatment only increased by 4.53 times. Comparison with the controls revealed that callus growth was inhibited in the toxin medium. On the other hand, the fresh weight of the calli treated with 8000 rad irradiation but without toxin increased by 2.09 times, while that of calli treated with 8000 rad irradiation and toxin increased by 1.97 times only. It suggested that the inhibition of irradiation was stronger than that of toxin. At higher irradiation dosage, calli grew worst regardless of the presence of the toxin, and browning was apparent many calli.

Table 1. The fresh weight changes of tomato calli different treatments

γ irradiation dose (rad)																
treatment	No. of weighing	0		500		1000		2000		3000		4000		6000		8000	
		Fresh weight (g)	times	Fresh weight (g)	times	Fresh weight (g)	times	Fresh weight (g)	times	Fresh weight (g)	times	Fresh weight (g)	times	Fresh weight (g)	times	Fresh weight (g)	times
toxin free	1	0.72±0.14		0.81±0.03		0.65±0.07		0.60±0.09		0.78±0.11		0.92±0.14		0.98±0.23		0.74±0.07	
	2	3.66±0.79	5.08	3.06±0.8	3.78	3.32±0.53	5.10	1.88±0.20	3.13	1.83±0.45	2.35	2.33±0.32	2.53	1.97±0.27	2.01	1.36±0.17	1.38
	3	4.75±1.40	6.59	4.80±1.63	5.92	4.65±1.35	7.15	2.42±0.31	4.03	2.02±0.58	2.59	2.62±0.29	2.85	2.32±0.40	2.37	1.50±0.18	2.02
	4	5.18±2.16	7.19	5.95±2.21	7.34	5.69±2.45	8.75	2.88±0.27	4.80	2.07±0.64	2.65	2.72±0.35	2.96	2.46±0.54	2.51	1.55±0.21	2.09
toxin	1	0.57±0.11		0.77±0.12		0.78±0.24		0.80±0.05		0.79±0.07		0.73±0.09		0.85±0.19		0.77±0.11	
	2	1.55±0.55	2.72	1.86±1.72	2.42	1.77±0.50	2.27	1.67±0.35	2.09	1.67±0.23	2.11	1.57±0.26	2.15	1.73±0.13	2.04	1.34±0.16	1.74
	3	2.20±1.10	3.85	2.44±1.10	3.17	2.15±0.59	2.76	2.05±0.59	2.56	1.92±0.38	2.43	1.72±0.29	2.36	1.85±0.20	2.18	1.39±0.17	1.81
	4	2.58±0.77	4.53	2.94±1.37	3.82	2.53±0.70	3.24	2.28±0.73	2.85	2.09±0.55	2.65	1.81±0.51	2.48	1.92±0.53	2.26	1.52±0.89	1.97

At lower dosage (less than 2000 rad) the weight gain of the calli treated with the toxin was lower than that treated without the toxin, but at higher dosage (more than 3000 rad) that of the calli treated either with or without toxin were similar. It was considered that the toxin was the main inhibiting factor for the calli under the condition of low γ dosage treatment, while irradiation acted as the main factor when the γ dosage was over 3000 rad. It was believed that the 3000 rad dosage was the critical value for inhibition effects.

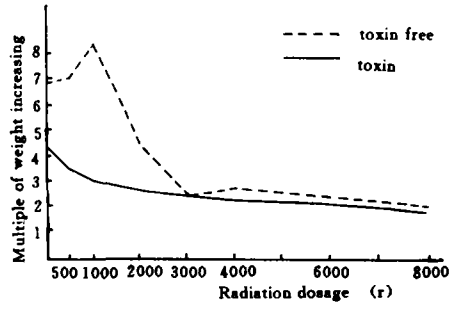

Fig. 1 The relations between the tomato calli and the two influencing factors (γ ray and toxin)

In Fig. 1, the dotted curve indicated that the weight gain of calli (low dose, toxin free) was greater than that of the control (0 dose, toxin free). Under toxin free conditions, the low dose (500 or 100 rad) irradiation stimulated cell multiplication. Inhibition of irradiation became stronger with dosage increase (above 2000 rad). In the toxin supplemented medium, both toxin and irradiation treatments gave rise to certain effects on callus

growth. And even at low doses, the toxin inhibition was greater than the irradiation stimulation. As a result, the solid curve came down smoothly.

Conclusion

1. The irradiation of γ ray has obvious influence on the growth of calli derived from tomato leaves, so it can be used in mutation treatment. Generally speaking, the 3000 rad dose may produce stronger inhibition. Therefore, it can be regarded as the critical value.
2. The fungitoxin has strong inhibition and can used as selection pressure in the screening of disease resistant mutants.
3. In the toxin-supplemented medium, the higher the γ ray dose is, the stronger the inhibition will be to callus growth. In the toxin free medium, low dose irradiation may display certain stimulation to callus growth, while high dose may show strong inhibition to it.

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References

Jiang Youyi et al. (1988) *Beijing Agricultural Sciences*, 5:6—8
Shahin EA et al. (1986) *Thero Appl Genet*, 73:164—169
Shepherd SLK et al. (1986) IV International Congress of Plant Tissue and Cell Culture, Abstracts, 248