INDUCTION OF MUTATIONS IN GARLIC BY COMBINED USE OF GAMMA-RAYS AND TISSUE CULTURE

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Abstract

Callus cultures were initiated from leaf explants of garlic on MS medium supplemented with 1.0 mg/l KIN + 1.0 mg/l IAA + 2.0 mg/l 2,4-D. Plantlets were induced from leaf calli on MS medium with 2.0 mg/l BA + 2.0 mg/l IAA. Bulblets were induced from plantlets on MS medium containing 3.0 mg/l ITA. Callus growth and plantlets induction were remarkably inhibited with irradiation doses of 8 and 10 Gy. It was found that doses of 3 and 5 Gy were suitable to induce variation. Somatic embryos were induced on MS medium supplemented with 2.0 mg/l 2,4-D + 500 mg/l casein, 1000 mg/l yeast extract and 3 to 5% sucrose.

1. INTRODUCTION

Garlic is an important crop in China. The objective of this research project is to induce mutants for tolerance to high temperature stress and resistance to garlic mosaic virus (GMV) by using *in vitro* techniques and radiation. For this purpose, *in vitro* regeneration of garlic plantlets and bulblets was studied from leaf explants, and different media were tested to obtain somatic embryogenesis. The effect of different doses of gamma-rays on callus growth of irradiated garlic leaves was investigated.

2. MATERIALS AND METHODS

The local variety of garlic, cv. 'Ga ding' was used in the experiments. Young leaves from germinating bulbs were used as explants. The protective leaves and storage leaves were taken from cloves, surface-sterilized in a solution of 70% alcohol for 10 sec., then in a solution of 0.1% mercuric chloride for 8 min., and rinsed 3 times with sterile distilled water. The lower parts of leaves were cut into 2 mm long pieces and used as explants. The explants were cultured on basal MS medium supplemented with various combinations of growth regulators such as kinetin (KIN), NAA, IAA, and 2,4-D, and maintained at 25°C under photoperiod of 16/8 hr light/dark with light intensity of 3000 lux. Nine different media, A1 to A9 (Table I) were used for callus induction from leaf explants. Eight media, S1 to S8 were used for plantlet induction from callus cultures (Table II). Three media R1-R3 were used for bulblet induction from plantlets. These were R1: MS + 1 mg/l ITA, R2: MS + 2 mg/l ITA. and R3: MS + 3 mg/l ITA. In addition, media G1-G5 and E1-E7 were tested for induction of somatic embryos from calli (Table III and IV).

^{60}Co was used as radiation source. The distance from the radiation source to material was 2.8 m and the radiation rate was 0.258 Gy/min. Doses of 1, 3, 5, 8 and 10 Gy were used in the present experiments.

TABLE I. MEDIA FOR CALLUS INDUCTION FROM LEAF EXPLANTS.

Medium	KIN mg/l	NAA mg/l	IAA mg/l	2,4-D mg/l
A1	1	-	-	2.0
A2	2	0.5	-	-
A3	2	-	10	2.0
A4	-	-	-	3.0
A5	1	-	1	-
A6	2	-	2	1.0
A7	-	-	-	2.2
A8	2	1.5	5	-
A9	1	-	1	2.0

TABLE II. MEDIA USED FOR PLANTLET INDUCTION FROM CALLI OF GARLIC.

Medium	KIN (mg/l)	BA (mg/l)	IAA (mg/l)	NAA (mg/l)
S1	-	-	-	-
S2	-	2.3	-	-
S3	2	-	2	-
S4	6	-	2	-
S5	2	-	6	-
S6	2	-	-	2
S7	-	2	-	2
S8	-	2	2	-

TABLE III. MEDIA USED FOR THE INDUCTION OF SOMATIC EMBRYOS FROM CALLI OF GARLIC.

Medium	P-CPA (mg/l)	2,4-D (mg/l)	Kinetin (mg/l)	IAA (mg/l)
G1	0.95	0.45	0.1	-
G2	0.95	0.90	0.1	-
G3	1.90	0.45	0.1	-
G4	1.90	0.90	0.1	-
G5	-	-	4.3	1.8

TABLE IV. MEDIA FOR THE INDUCTION OF SOMATIC EMBRYOS FROM CALLI OF GARLIC.

Medium	2,4-D (mg/l)	6-BA (mg/l)	Casein hydrolysate (mg/l)	Yeast extract (mg/l)
E1	1	-	-	-
E2	1	0.5	-	-
E3	1	1	-	-
E4	2	-	-	-
E5	2	0.5	-	-
E6	2	1	-	-
E7	2	-	500	1,000

3. RESULTS AND DISCUSSION

Callus induction from leaf explants

The results showed that all the six media A1, A3, A4, A6, A7, A9, were able to induce callus from the leaf explants. All media contained 2,4-D. This suggested that 2,4-D was effective in inducing calli from leaf explants. Among nine media, the medium A9 was the best to induce callus. The callus growth was rapid, callus size was about twice the size of explant after 45 days culture.

TABLE V. EFFECT OF VARIOUS MEDIA ON CALLUS INDUCTION FROM LEAF EXPLANTS.

Medium	Explant number	Callus induction frequency (%)
A1	20	100
A2	20	-
A3	20	-
A4	20	85
A5	20	-
A6	20	100
A7	20	90
A8	20	-
A9	20	100

To obtain calli with rapid growth and excellent texture, it was necessary to supplement MS medium containing 2,4-D with other auxins and kinetin. The medium A9 (MS medium containing 2 mg/l 2,4-D, 1 mg/l IAA and 1 mg/l KIN) was very effective in inducing calli, and the frequency of callus induction was 100%. The calli were characterized by rapid growth and excellent texture (Table V).

Plantlet induction from leaf callus

The results showed that five media combinations (A6-S7, A6-S8, A7-S2, A7-S7, A9-S4) were effective in inducing plantlets from calli. The frequency of induction in all cases was more than 80%. This suggested that the calli induced on the MS medium containing 2,4-D when transferred to MS medium lacking 2,4-D, produced plantlets. The plantlet induction frequency was affected by the composition of medium used for callus induction. For example, when the calli were induced on the media A6, A7, A9, respectively, and were transferred to the medium S8, the frequency of plantlet induction was 82.8, 57.1 and 77.1 per cent, respectively. This suggested that the plantlet induction was influenced by callus induction medium indirectly. Improved frequency of plantlets and number of plantlets per callus were obtained on media A6-S7, A7-S2. Two media, S2 and S7 which contained BA were effective in inducing plantlet formation (Table VI).

TABLE VI. EFFECT OF MEDIA ON PLANTLET INDUCTION FROM GARLIC LEAF CALLI.

Media		Plantlet		Mean No. of Plantlets per callus
medium for inducing callus	medium for inducing plantlets	No.	Frequency (%)	
	S1	1	2.8	0.1
	S2	14	40.0	1.5
	S3	19	54.3	1.4
	S4	8	22.8	0.5
A6	S5	16	45.7	0.6
	S6	20	57.1	1.9
	S7	31	88.6	3.9
	S8	29	82.8	2.9
	S1	-	-	-
	S2	31	89.6	4.5
	S3	23	65.7	1.9
	S4	9	25.7	0.6
A7	S5	14	40.0	1.6
	S6	16	45.7	1.6
	S7	29	82.8	2.5
	S8	20	57.8	1.8
	S1	1	2.8	0.1
	S2	17	48.6	1.6
	S3	23	65.7	3.5
	S4	30	85.7	3.0
A9	S5	20	57.1	1.2
	S6	25	71.4	2.7
	S7	25	71.4	2.9
	S8	27	77.1	3.7

The bulblet induction from plantlets

The results showed that plantlets cultured on media S1, R1, R2, R3 were able to produce bulblets *in vitro*. ITA in the media R1, R2, R3 was effective in accelerating bulblet formation.

TABLE VII. EFFECT OF VARIOUS LEVELS OF ITA ON BULBLET INDUCTION.

Medium	Number of explants	Bulblet induction frequency (%)	Number of bulblets per callus
S1	60	58.3	1.7
R1	60	40.0	0.8
R2	60	78.3	1.9
R3	60	71.7	2.3

The bulblet number increased with the increase of ITA in medium. The number of bulblets induced on medium R3 (containing 3 mg/l ITA) was four times more than that on the medium R1 (containing 1 mg/l ITA). But the bulblets induced on the media containing ITA were smaller than those on MS (S1) medium without any auxin.

Somatic embryo induction from leaf calli

When leaf explants were cultured on media G1-G4 for 45 days, and then transferred onto the medium G5, or on media E1-E6 for 120 days, no somatic embryos were induced. Only E7 medium (MS medium containing 2 mg/l 2,4-D + 500 mg/l casein hydrolysate, 1000 mg/l yeast extract, with high sucrose, 3% to 5%) was effective in inducing somatic embryos. The calli formed after 14 days of culture, and the somatic embryos began to form after 40 days of culture. After 70 days of culture, surface of calli was covered by many small globules with smooth surface, some of these could be easily isolated from calli. After 110 days culture, all calli were able to produce somatic embryos which were easily isolated from calli (Table VIII).

TABLE VIII. INDUCTION OF EMBRYOGENIC CALLI AND SOMATIC EMBRYOS FROM LEAF CALLI.

Number of explants	Embryogenic calli		Number of somatic embryos	
	Number	Frequency (%)	Total	Mean per callus
40	40	100	489	12.2

The mean number of somatic embrys per callus was 12.2 after 110 days culture. The number of somatic embryos on single callus ranged from 1 to 60.

Effect of gamma rays on growth of calli from irradiated leaves

Leaves were irradiated with ^{60}Co gamma rays, with doses 1, 3, 5, 8 and 10 Gy. The leaves were cut into 4 x 4 mm pieces and used as explants and cultured on MS medium containing 1 mg/l KIN, 1 mg/l IAA, 2 mg/l 2,4-D. After 50 days culture, the formed calli were weighed. Analysis of variance gave an F value of 5.707, and at 0.01 per cent level (5,195) of 3.11; the differences between the treatments were highly significant. LSD test showed that the callus growth was promoted with 1 and 3 Gy, and inhibited with 8 and 10 Gy. Especially, the mean weight of individual calli in the treatment with 10 Gy was 76.3% less than that in the non-irradiated control (CK). Therefore, we used 5 Gy dose to induce mutants from irradiated leaves.

TABLE IX. EFFECT OF GAMMA RAYS ON GROWTH OF CALLI FROM IRRADIATED LEAVES.

Doses (Gy)	Number of explants	Mean weight of single callus (mg)	In comparison with CK (%)
CK	40	0.0885	
1	40	0.0975	+ 10.17
3	40	0.0965	+ 9.04
5	40	0.0858	- 0.03
8	40	0.0600	- 32.20
10	40	0.0210	- 76.27

Effects of different doses of gamma rays on plantlet differentiation

Leaves were irradiated with ^{60}Co gamma rays with 1, 3, 5, 8 and 10 Gy. The irradiated leaves were cut into 4 x 4 mm piece and used as explants. And then the explants were cultured on the MS medium containing 1 mg/l KIN, 1 mg/l IAA and 2 mg/l 2,4-D for inducing calli. After 35 days, the calli were cultured on the MS medium containing 6 mg/l KIN and 0.5 mg/l IAA for 70 days to induce plantlet. The results showed that the frequency of plantlet induction in the non-irradiated control (CK), 1 and 3 Gy dose were 85.5, 83.3 and 77.7%, respectively, but those in the higher dose (5, 8 and 10 Gy) treatments were only 75.9, 64.3 and 73.5%, respectively. The number of plantlets per callus in the non-irradiated control (CK), 1 and 3 Gy treatments were 3.9, 3.8 and 3.1, respectively, but those in the higher dose (5, 8 and 10 Gy) treatments were 2.2, 2.4 and 2.5, respectively. Therefore, we could induce garlic variant lines by irradiating garlic leaves at 5 GY dose and using tissue culture method.

TABLE X. EFFECTS OF GAMMA RAYS ON SEEDLING DIFFERENTIATION FROM IRRADIATED GARLIC LEAVES.

Dose (GY)	Number of explants	Calli with seedlings		Number of differentiated	
		Number	Frequency (%)	Total	Per explant
CK	28	24	85.7	108	3.9
1	30	25	83.3	113	3.8
3	27	21	77.7	83	3.1
5	29	22	75.9	65	2.2
8	28	18	64.3	68	2.4
10	34	25	73.5	85	2.5

Effect of gamma rays on plantlet differentiation from garlic leaf calli.

Leaf explants were cultured to initiate callus on the MS medium containing 1 mg/l KIN, 1 mg/l IAA and 2 mg/l 2,4-D. After 35 days culture, the calli were irradiated with 1, 3, 5, 8 and 10 Gy gamma rays, and the irradiated calli were cultured on the MS medium containing 6 mg/l KIN and 0.5 mg/l IAA for 70 days to regenerate plants. The results showed that plant induction was inhibited in the treatments with irradiation at all doses. In case of higher gamma ray doses, the frequencies of plant regeneration was markedly reduced. The frequency of plant induction in the non-irradiated control (CK) and 1 and 3 Gy dose was 100, 90 and 95%, respectively; but in those give higher doses, 5, 8 and 10 Gy, it was 55, 55 and 30%, respectively. The mean number of plants per callus in the non-irradiated control (CK), 1 and 3 Gy was 10.3, 5.4 and 6.5, respectively; but in the treatments with higher doses, 5, 8 and 10 Gy was 2.5, 2.6 and 1.3, respectively. Therefore, 3 or 5 Gy dose appeared to be optimal to induce mutant from irradiated leaf calli.

TABLE XI. EFFECT OF GAMMA RAYS ON PLANT REGENERATION FROM GARLIC LEAF CALLI.

Irradiation dose (Gy)	Number of explants	Calli with seedlings		Mean No. of seedlings differentiated	
		Number	Frequency (%)	Total	Per explant
CK	20	20	100	205	10.3
1	20	18	90	107	5.4
3	20	19	95	123	6.5
5	20	11	55	49	2.5
8	20	11	55	52	2.6
10	20	6	30	25	1.3