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Callus Induction and Plant Regeneration in Chinese Kale

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Abstract. Using the cotyledon and hypocotyl of Chinese kale 'Sijicutiao' as explants, the effects of MS medium supplemented with different types and concentrations of growth regulators (2, 4-D, 6-BA, NAA) on the callus induction and regeneration of Chinese kale were studied. The results showed that different hormone combinations of 2,4-D, 6-BA and NAA play a key role in the callus induction and regeneration culture of Chinese kale, and hypocotyl has a better effect than cotyledon with petiole as an explant. MS + 0.1 mg·L-12, 4-D + 0.02 mg·L-1 6-BA was the optimal combination in the process of induction, the hypocotyl explant induction rate was 80%. During the regeneration culture, the best combination was MS + 0.75 mg·L-1 6-BA + 0.03 mg·L-1 NAA, the differentiation rate of adventitious buds was 94%, and the rooting rate was 67%. These results could provide a theoretical reference value for the study of cell suspension culture of Chinese kale.

INTRODUCTION

Chinese kale (Brassica oleracae var. alboglabra Bailey) belongs to the Brassica species of the Brassicaceae family. It is an economically important vegetable crop rich for vitamin C and bioactive compounds like glucosinolates, and widely cultivated in South China and Southeast Asia [1].

Genetic engineering and tissue culture protocols are efficient tools for complementing traditional breeding programs and producing cultivars with higher yields, higher tolerance/resistance to viral and fungal diseases and better adapted to local environmental conditions [2]. The most important factors affecting plant regeneration are the explant type, the genotype and the growth regulator combination used in the culture medium. Besides, callus differentiation and plant development are determined by growth regulators [2-5].

To our knowledge, the effect of different plant growth regulators on callus induction and regeneration of Chinese kale has not been conducted. Therefore, the objective of the present study was to develop a procedure which allows to obtain a system for callus production giving cell masses that could be used in studies of cell suspension culture as well as to establish an efficient protocol to regenerate plants from callus of Chinese kale [6-7].

MATERIALS AND METHODS

Plant Materials

Seeds from 'Sijicutiao' of white-flowered Chinese kale was used in the experiment. They were surface-sterilized by dipping into 75% (v/v) alcohol for 30 s and disinfected with 0.1% (w/v) mercury chloride (HgCl2) for 6 min, respectively, then immersed in sterile distilled water 3-5 times and dried off with sterilized filter paper. The sterilized seeds were germinated on 1/2 murashige and skoog (MS) medium [8] [2% (w/v) sucrose, 0.7% (w/v) agar, pH 5.8] at 25 °C with 16 h light period and 8 h dark period.

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Callus Induction

Callus initiation was carried out by using cotyledon and hypocotyl from seedlings above as source of explants. For callus induction, the explants were cultured on MS solid medium supplemented with different concentrations and combinations of growth regulators as described in Table 1. All media were adjusted to pH 5.8 with 1 M HCl or 1 M NaOH, then 7 g·L-1 of agarose and 20 g·L-1 sucrose were added before autoclaving for 20 min at 121 °C. Subsequently, they were incubated at (25±1) °C in dark. The experiment was carried out with three replicates and with 36 explants in each replication. The frequency of callus formation was investigated after culture for a total of 30 days.

Shoot and Root Regeneration

In regeneration experiment, callus were transferred to MS solid medium supplemented with 6-BA (0, 0.75, 1.5 mg·L-1) in combination with NAA (0, 0.03, 0.06 mg·L-1) to identify the optimal medium compositions for callus regeneration (Table 2). Cultures were maintained at (25±1) °C with 16 h light period and 8 h dark period. The frequency of shoot and rooting was recorded at 20 days after transferring the calluses to regeneration medium.

Statistical Analysis

All experimental data were statistically analyzed by one-way analysis of variance (ANOVA) using the protected least-significant-difference (LSD) test ($P<0.05$), and data were evaluated using ananalysis of variance from which mean ± standard error values were computed for comparison between treatments.

RESULTS

Effects of 2,4-D and 6-BA Combination on Callus Induction

Calluses from cotyledon with petiole and hypocotyl were induced in all media tested. The percentage of explants producing calluses as affected by growth regulators and explant type was shown in Table 1. Among all treatments, callus induction frequencies of cotyledon with petiole and hypocotyl varied from 43.3% to 66.6% and 30% to 80%; the rates of browning were 36.67% to 66.6% and 23.3% to 53.3%, respectively. The highest frequency (80%) of callus formation was obtained with hypocotyl explants when cultured on MS with 0.1 mg·L-12, 4-D and 0.02 mg·L-1 6-BA, and the rate of browning was the lowest. The callus type were also noticed. Calluses induced with cotyledon were mostly watery and yellow or white in color, while calluses induced with hypocotyl were mostly friable or soft and yellowish in color (Table 1 and Fig. 1).

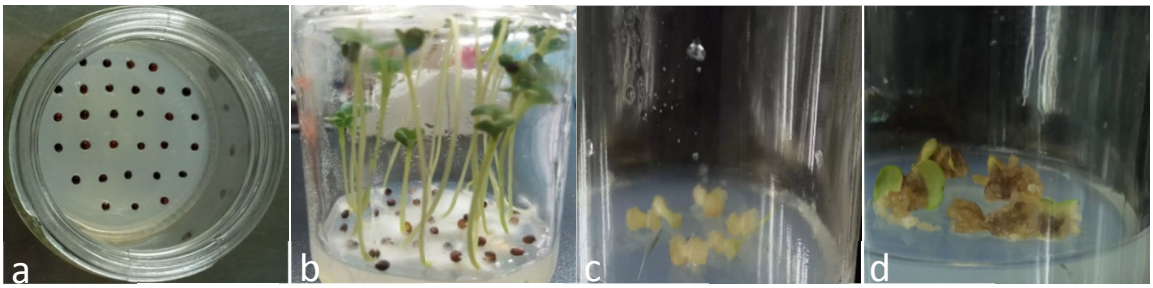

FIG 1. Callus induction from explants of Chinese kale. (a) Seeds cultured on 1/2 MS medium; (b) Plantlets; (c) Callus induced from hypocotyl; (d) Callus induced from cotyledon

TABLE 1. Effects of 2, 4-D and 6-BA combination on callus induction of cotyledon with petiole and hypocotyl

Growth regulators ($mg \cdot L^{-1}$)		Cotyledon with petiole				Hypocotyl			
2,4-D	6-BA	Callus time (days)	Callus induction (%)	Browning (%)	Callus status	Callus time (days)	Callus induction (%)	Browning (%)	Callus status
0.05	0.02	14	46.6 ± 11.6ab	36.67 ± 5.8b	Yellow, soft	13	30.0 ± 10.0c	33.3 ± 15.3ab	Yellow, soft
	0.1	12	46.6 ± 15.3ab	43.3 ± 20.8ab	Yellow, compact	13	53.3 ± 11.6bc	36.6 ± 15.3ab	Yellow, soft
	0.5	12	50.0 ± 10.0ab	50.0 ± 17.3ab	Yellow, compact	12	46.6 ± 25.2bc	43.3 ± 15.3ab	Yellowishcompact
0.1	0.02	14	66.6 ± 15.3a	50.0 ± 20.0ab	Yellowishcompact	10	80.0 ± 10.0a	23.3 ± 5.8b	Yellowishfriable
	0.1	11	50.0 ± 20.0ab	43.3 ± 11.6ab	Yellowish, soft	10	46.6 ± 15.3bc	43.3 ± 15.3ab	Yellow, friable
	0.5	11	53.3 ± 11.6ab	60.0 ± 20.0ab	White, watery	10	50.0 ± 20.0bc	40.0 ± 10.0ab	Yellowishcompact
0.5	0.02	11	43.3 ± 11.6b	43.3 ± 5.8ab	White, watery	12	33.3 ± 11.6c	40.0 ± 17.3ab	Yellow, soft
	0.1	11	43.3 ± 15.3b	66.6 ± 11.6a	White, watery	12	63.3 ± 15.3ab	46.6 ± 11.6ab	Yellow, soft
	0.5	10	46.6 ± 5.8ab	66.6 ± 15.3a	White, watery	11	46.6 ± 5.8bc	53.3 ± 15.3a	Yellow, compact

Different letters indicate significant difference at the 0.05 probability level.

Effects of 6-BA and NAA Combination on Callus Regeneration

The actively growing embryogenic calli were transferred on MS basal media containing various combinations of growth regulators to evaluate shoot and root regeneration (Table 2). BA, alone or in combination with NAA, has previously been reported as being efficient in promoting shoot differentiation in several species. In our experiment, MS supplemented with 0.75 mg·L-1 6-BA and 0.03 mg·L-1 NAA gave the highest shoot proliferation rate (94%) (Table 2 and Fig. 2) which had significant differences with the other treatments and rate of roots (67%).

TABLE 2. Effects of 6-BA and NAA combination on shoot and root regeneration from the hypocotyl induced callus

Growth regulators ($mg \cdot L^{-1}$)		Callus number	Adventitious bud differentiation (%)	Rooting (%)
6-BA	NAA			
0.0	0.0	6	6.0 ± 9.8f	100.0 ± 0.0a
	0.03	6	12.0 ± 8.1ef	67.0 ± 0.0b
	0.06	6	28.0 ± 9.2de	77.7 ± 9.2ab
0.75	0.0	6	39.0 ± 9.8cd	38.7 ± 9.8c
	0.03	6	94.0 ± 9.8a	67.0 ± 0.0b
	0.06	6	50.0 ± 17.0bc	72.3 ± 9.2b
1.5	0.0	6	44.3 ± 9.8bcd	16.7 ± 16.5c
	0.03	6	61.3 ± 9.8b	33.3 ± 16.5c
	0.06	6	61.3 ± 9.8b	33.7 ± 28.9c

Different letters indicate significant difference at the 0.05 probability level.

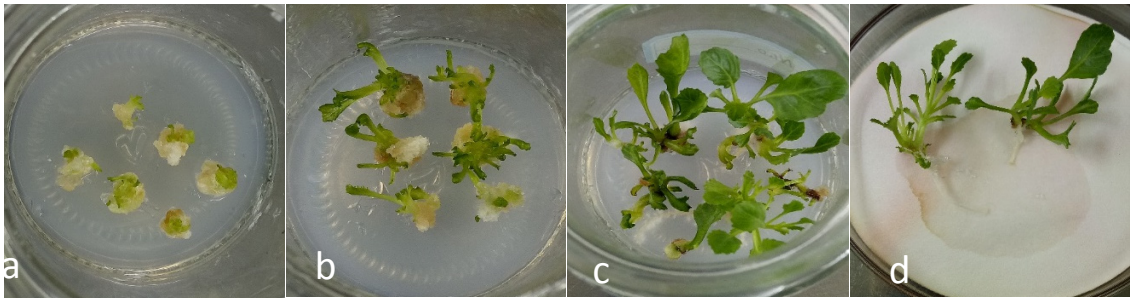

FIG 2. Different stages of callus regeneration cultured on MS media with 0.75 mg·L-1 6-BA and 0.03 mg·L-1 NAA. (a) Callus; (b) Shoot regeneration from callus; (c) Shoot elongation and proliferation; (d) Root regeneration

SUMMARY

There are many published reports for plant regulators affecting callus and in-vitro regeneration [9-10], but different genotypes or plants differ significantly in callus induction and regeneration.

In the present study, we have optimized a suitable media for production of callus and regeneration in Chinese kale. For callus induction and regeneration, medium combination gave maximum production of 80% and 94% respectively. The findings of the study could be used in Chinese kale cell suspension culture, in-vitro regeneration and genetic transformation studies.

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