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Effects of Plant Growth Regulators on Callus Proliferation of Chinese Kale

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Abstract. Endogenous and exogenous plant growth regulators play essential role in the growth and proliferation of calluses. To establish an efficient protocol of callus proliferation, various concentrations of a-naphthaleneacetic acid (NAA) (0.4, 0.5, 0.6 mg·L^{-1}) in combination with 6-benzylaminopurine (6-BA) (1.5, 2.0, 2.5 mg·L^{-1}) on callus proliferation of Chinese kale were evaluated. The results showed that the best callus proliferation was observed on MS medium with 0.4 mg·L^{-1} NAA and 2.5 mg·L^{-1} 6-BA after 30 d of culture, and the morphology of the callus was yellowish in colour and friable. This study provides a basis for future studies on genetic improvement for Chinese kale.

Key words: Chinese kale, callus proliferation, 6-benzyladenine, a-naphthalene acetic acid.

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].

Plant biotechnology, such as plant tissue culture techniques offer a powerful tool for germplasm conservation and mass multiplication of many plant species, and also offer an alternative to chemical synthesis or natural harvesting for the production and extraction of plant secondary metabolites within controlled laboratory environments[2]. A suitable callus proliferation medium was required to obtain healthy and regenerable calluses. Different genotypes or plants differ significantly in callus proliferation even when cultured using the same plant growth regulators. The degree of cell proliferation is also particularly influenced by the concentration of growth regulators in the culture medium [4, 5]. The medium compositions ignificantly affected callus induction and callus proliferation of C. nutans. Murashige and Skoog (MS) [3] medium with agarose as a gelling agent containing 0.50 mg·L-1 2, 4-Dichlorophenoxyaceticacid (2, 4-D) was best for callus proliferation [4].

To our knowledge until present, the effect of different plant growth regulators on callus proliferation of Chinese kale has not been conducted. In this study, the effects of growth regulators including a-naphthaleneacetic acid (NAA) and 6-benzylaminopurine (6-BA) on callus proliferation of Chinese kale were performed to establish an efficient protocol of callus proliferation, which will be useful for conservation, sustainable development and utilization, and provide a foundation for cell suspension culture of Chinese kale and future transgenic biotechnological applications.

MATERIALS AND METHODS

Plant Materials

Callus was used as initial materials in this study, it was obtained on MS solid medium supplemented with 0.1 mg·L-1 2, 4-D and 0.02 mg·L-1 6-BA using hypocotyl of Chinese kale as explant material.

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Callus Proliferation

Calluses were cultured on MS solid medium supplemented with NAA (0.4, 0.5, 0.6 mg·L-1) in combination with 6-BA (1.5, 2.0, 2.5 mg·L-1) to identify the optimal medium compositions for callus proliferation (Fig. 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 and 60-70% relative humidity in the dark, and subcultured every 7 d. Calluses were harvested from different medium after 30 d of culture and recorded fresh weight, browning rate and callus status respectively. The experiment was carried out with three replicates, with each replicate containing six bottles.

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

For the callus proliferation experiments, the effect of various concentrations of NAA (0.4, 0.5, 0.6 mg·L-1) in combination with 6-BA (1.5, 2.0, 2.5 mg·L-1) was performed on the MS medium. The multiplication rate of callus was presented in Table 1. After 30 days of culture, the effect of callus proliferation was very significant. A particular interaction was observed when callus cultured on MS medium supplemented with 0.4 mg·L-1 NAA in combination with 2.5 mg·L-1 6-BA, fresh weight of callus increased to 3.23 g from initial incubated weight 1.03 g after 30 d of culture, the multiplication rate reached at 212.74%, much higher than other treatments and this callus was yellowish and mostly friable in structure (Table 1 and Fig.1). Although the browning rate was 35.29%, but had no significant differences with other treatments except for the combination of 0.4 mg·L-1 NAA and 1.5 mg·L-1 6-BA.

TABLE 1. Effects of different growth regulator combinations on callus proliferation from Chinese kale

Growth regulators ($mg·L^{-1}$)		Browning rate (%)	Fresh weight(g)	Fresh weight (g)	Multiplicationrate (%)	Callus status
NAA	6-BA					
0.4	1.5	25.00±3.93c	1.24	1.95	57.23±1.84f	yellowish, friable
0.5	1.5	32.35±4.16abc	0.95	2.20	132.55±5.77c	yellowish , friable and few compact
0.6	1.5	38.89±0a	0.92	1.69	84.73±0.04e	yellowish , friable
0.4	2.0	26.67±9.43bc	1.26	2.37	88.05±3.35e	yellowish , compact
0.5	2.0	26.67±0bc	1.24	2.74	120.14±2.81d	yellowish and white, friable
0.6	2.0	30.56±3.93abc	1.23	3.33	171.77±0.80b	yellowish, friable
0.4	2.5	35.29±0ab	1.03	3.23	212.74±12.39a	yellowish, friable
0.5	2.5	27.78±0bc	1.13	2.19	93.31±2.49e	yellowish, compact
0.6	2.5	31.25±0abc	0.86	2.33	172.31±3.27b	yellowish, friable

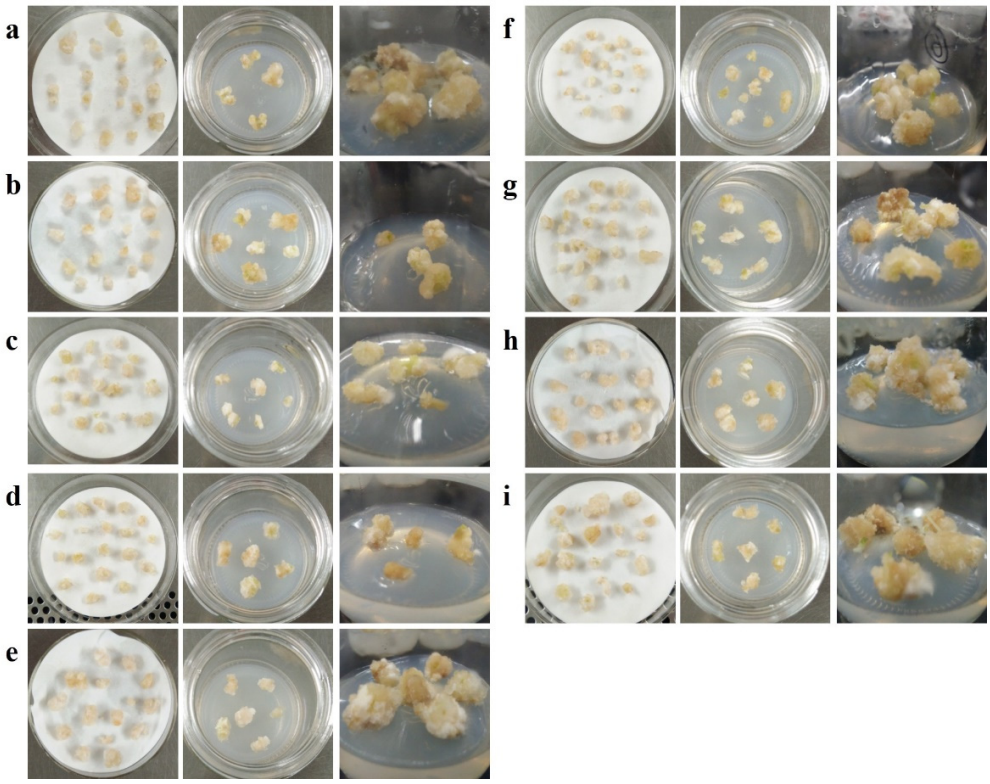

FIG 1. Callus status cultured on MS medium supplemented with different concentrations of NAA and 6-BA

(a) 0.4 mg·L-1 NAA+1.5 mg·L-1 6-BA; (b) 0.5 mg·L-1 NAA+1.5 mg·L-1 6-BA; (c) 0.6 mg·L-1 NAA+1.5 mg·L-1 6-BA; (d) 0.4 mg·L-1 NAA+2.0 mg·L-1 6-BA; (e) 0.5 mg·L-1 NAA+2.0 mg·L-1 6-BA; (f) 0.6 mg·L-1 NAA+2.0 mg·L-1 6-BA; (g) 0.4 mg·L-1 NAA+2.5 mg·L-1 6-BA; (h) 0.5 mg·L-1 NAA+2.5 mg·L-1 6-BA; (i) 0.6 mg·L-1 NAA+2.5 mg·L-1 6-BA.

SUMMARY

For a rapid creation of plants propagules for cultivation, an efficient callus proliferation system was necessary [6, 7]. Several studies had been reported regarding the effects of plant growth regulators on callus growth and proliferation of different plant species [4, 5, and 8]. In the present study, we found that the fastest callus proliferation occurred on MS supplemented with 0.4 mg·L-1 NAA in combination with 2.5 mg·L-1 6-BA after 30 d of culture. In addition, the morphology of the callus was yellowish in color and friable (few compact) in structure (Table 1 and Fig. 1). Indicating that this callus proliferation medium was optimal for establishing a cell suspension culture. This study might provide a protocol for rapid mass propagation of Chinese kale to produce uniform seedling in short time.

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