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ACTIVE SHOOT REGENERATION IN CALLUS CULTURE OF KIWI FRUIT (*ACTINIDIA CHINENSIS* PLANCH.)

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キウイフルーツ (*Actinidia chinensis* Planch.)のカルス培養における活発な不定芽分化

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Hormonal and nutritional factors affecting the callus formation and adventitious shoot regeneration from stem segment callus of kiwi fruit were investigated.

Extremely active callus proliferation and shoot regeneration were found on the medium containing 4PU, compared with 2iP, BA or kinetin. NAA or 2, 4—D supplemented to the medium containing 0.1 mg/ℓ of 4PU suppressed shoot regeneration. The presence of 2, 4—D in the medium severely inhibited callus growth and shoot regeneration even after the callus was transferred onto the 2, 4—D free medium containing 4PU. The callus induced by 2, 4—D resumed the regenerative potential on the medium containing 4PU, after 4 weeks of culture with NAA.

Prolific callus formation was achieved on MS or B5 medium, whereas the shoot regeneration occurred more actively on WPM medium, followed by B5, Lepoivre and MS.

By IBA treatment, the regenerated shoots could be rooted easily under non-sterile condition and grown into plantlets.

キウイフルーツの節間カルスからのカルス増殖及び不定芽分化に及ぼす植物生長調節物質及び基本培地の種類の影響について検討した。

2iP及びBA, kinetinと比較して，4PUはカルスの増殖及び不定芽の分化を著しく促進した。4PU 0.1 mg/ℓを含む培地に添加した2, 4—D及びNAAは不定芽の分化を抑制した。特に2, 4—Dの抑制効果は大きく，カルスを4PUのみを含む培地に移植した後にも維持された。しかし，2, 4—Dにより誘導されたカルスも，NAAを含む培地で4週間培養することで不定芽分化の能力を回復した。

カルスの増殖はMSおよびB5培地で優れたが，不定芽の分化はWPM培地でより活発で，以下B5, Lepoivre, MS培地の順であった。

分化した不定芽は，IBA浸漬処理により非無菌条件において良好な発根を示し，幼植物に生長した。

Introduction

Because of the dioeciousness, sexual hybridization among cultivars that have desirable fruit charateristics is impracticable for kiwi fruit. Induction of somaclonal variation or somatic hybridization by protoplast fusion, therefore, has much significance for its breeding and improvement. As the plant regeneration from callus or suspended cells should be essential for that purpose, a simple and reliable method needs to be established. As for kiwi fruit, several works have dealt with the regeneration from callus[1-4,6], and its potentiality of differentiation from callus appears to be relatively higher than the other kinds of deciduous fruit trees.

For the adventitious shoot induction of kiwi fruit, purine compound such as zeatin, 6-benzyl amino purine (BA) or 6-(r, r-dimetylallyl-amino)-purine (2iP) was usually used[1,2,4], but their effectiveness were not always stable. Recently, some of the urea compounds have been found to show marked cytokinin activity[6], and the application of these chemicals to tissue culture for intractable woody species is now greatly expected.

This report describes the effect of several cytokinins including urea compound on adventitious shoot regeneration from stem segment derived callus of kiwi fruit. And the other hormonal and nutritional factors affecting the efficiency of regeneration were also studied.

Materials and Methods

Two-year-old potted vines cv. Hayward were grown in 20℃ growth chamber. Emerging shoots were harvested when they attained a length of about 15 cm. Internodes from the shoots were dipped briefly in 70% ethanol and immersed in 1% sodium hypochlorite for 15 min. After being rinsed 5 times in sterile water, the internodes were sectioned into 2 mm lengths and placed in test tube containing 15 mℓ of Murashige and Skoog (MS) medium supplemented with 1.0 mg/ℓ of 2iP and naphthalene acetic acid (NAA), 30 g/ℓ sucrose and 8 g/ℓ agar for callus induction. The callus formed on the cut surface was dissected and subcultured on the same medium every 4 weeks.

The pH of the medium was adjusted to 5.8 before autoclaving for 15 min at 120℃. The cultures were kept at 27℃ under 2000lux light intensity by white fluorescent tubes with 16 h light.

Experiment 1. Effect of cytokinin on shoot regeneration from callus.

Subcultured callus was sectioned into 3 mm^3 in size and placed on the MS medium supplemented with 0.5-5.0 mg/ℓ of BA, 2iP, 6-furfurylamino purine (kinetin) and 0.001-5.0 mg/ℓ of 2-chloro-4-pyridil urea (4PU) (KYOWA HAKKO KOGYO CO., LTD.) individually.

Experiment 2. Effect of auxin on shoot regeneration from callus.

NAA or 2, 4-D (0.01-5.0 mg/ℓ) was added to the MS medium containing 0.1 mg/ℓ of 4PU. The procedures for callus preparation and planting were the same as described above. Six weeks after planting, the callus formed on each medium was sectioned into 3 mm^3 in size and transferred to medium containing 0.1 mg/ℓ of 4PU. In another experiment, the callus induced with 1.0 mg/ℓ of 2, 4-D was transferred to the medium containing 1.0 mg/ℓ of NAA and cultured for 4 weeks. Then, the callus was transferred again to the medium supplemented with 0.1 mg/ℓ of 4PU.

Experiment 3. Effect of basal medium on shoot regeneration from callus.

Four kinds of basal media; MS, Gamborg and Eveleigh (B5), Woody Plant Medium (WPM) and Lepoivre[8], were prepared and supplemented with 0.1 mg/ℓ of 4PU. The procedures for callus preparation and planting were the same as experiment 1.

In all experiments, the degrees of callus formation and the activity of shoot regeneration were evaluated 6 weeks after planting by the indexes described in the footnote of each table.

Results and Discussion

Experiment 1. Callus could survive even on the cytokinin free MS medium as well as on all media containing cytokinin.

Callus growth was, however, greatly varied with the kind or concentration of cytokinin applied to the medium. At concentrations in excess of 0.1 mg/ℓ, 4PU markedly stimulated callus growth. Although higher concentrations of 2 iP (3.0-5.0 mg/ℓ) also induced callus formation, its efficacy was considerably less than that of 4 PU. On the medium containing BA or kinetin, callus could developed only slightly. The calli induced on all media tested had consistent green color and compact characteristic (Table 1, Fig. 1).

Active regeneration of adventitious shoots was found only in the cultures containing 4 PU at the concentrations in excess of 0.1 mg/ℓ. The shoots generated from almost whole periphery of the callus, and some

Table 1. Effect of cytokinin on callus proliferation and adventitious shoot regeneration of kiwi fruit

treatment	mg/ℓ	callus proliferation[z]	shoot regeneration[y]
hormone free		+	−
BA	0.5	−	−
	1.0	−	−
	3.0	+	−
	5.0	+	−
2iP	0.5	+	−
	1.0	+	−
	3.0	+++	−
	5.0	++	+
KIN	0.5	+	−
	1.0	+	−
	3.0	+	−
	5.0	+	−
4PU	0.001	+	−
	0.01	++	−
	0.1	+++++	++++
	1.0	+++++	+++
	5.0	++++	++

z: −=no callus proliferation～+++++=abundant callus proliferation
y: −=no shoot regeneration～+++++=active shoot regeneration

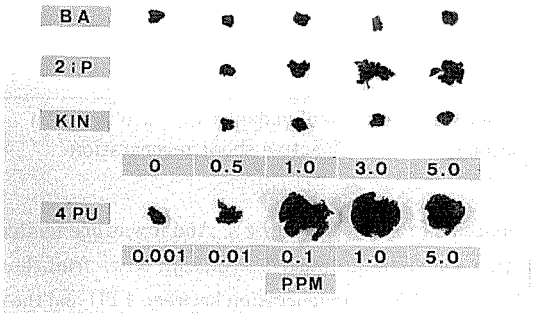

Fig. 1. Morphological response of kiwi fruit calli to several cytokinins

of them had expanded small leaves. But the elongation of the internodes did not occur, so that the shoots showed rosette form. In the other cultures, no adventitious shoot was formed except when 3.0 or 5.0 mg/ℓ of 2 iP was supplied to the medium (Table 1, Fig. 1).

In *in vitro* culture of stem and root segments of kiwi fruit, Harada[4] found that zeatin was most effective for the differentiation of shoot buds among several growth substances, and it was also effective in inducing bud formation in subcultured callus. On the other hand, it was also reported that 2 iP at 1.0 mg/ℓ was similarly promotive for plantlet regeneration from stem segments of var. hispida as well as zeatin at 1 mg/ℓ[3].

In this experiment, 4 PU was found to be extremely effective for both callus proliferation and adventitious shoot regeneration of kiwi fruit, among the cytokinins tested and this agrees with the results reported by Shimura et al[6]. In the assey with tobacco callus, 4 PU was shown to have the maximum of promotive effect

Table 2. Effect of auxin application on callus proliferation and adventitious shoot regeneration of kiwi fruit

treatment	mg/ℓ		callus proliferation[z]	shoot regeneration[y]
4PU	0.1		+++++	+++++
4PU	0.1	+2,4−D 0.01	+++++	−
		0.1	++	−
		1.0	+	−
		5.0	+	−
4PU	0.1	+NAA 0.01	+++++	+++
		0.1	+++++	+
		1.0	+++++	−
		5.0	+++++	−

z −=no callus proliferation ~ +++++=abundant callus proliferation
y −=no shoot regeneration ~ +++++=active shoot regeneration

Table 3. Aftereffect of auxin on callus proliferation and shoot regeneration on the medium containing 0.1 mg/ℓ of 4PU

treatment	mg/ℓ		callus proliferation[z]	shoot regeneration[y]
4PU	0.1		+++++	+++
4PU	0.1	+2,4−D 0.01	+++++	+
		0.1	+	−
		1.0	+	−
		5.0	+	−
4PU	0.1	+NAA 0.01	+++++	+++++
		0.1	+++++	++++
		1.0	+++++	++++
		5.0	+++++	++++

z −=no callus proliferation ~ +++++=abundant callus proliferation
y −=no shoot regeneration ~ +++++=active shoot regeneration

for callus proliferation at the concentration of 0.001 mg/ℓ. And it was one tenth of BA concentration at which the same degree of proliferation could be attained[5]. As with kiwi fruit, however, the difference in the effectiveness for callus proliferation and shoot regeneration between 4 PU and the other kinds of cytokinins was greater than in tobacco callus, and it might be qualitative rather than quantitative.

Experiment 2. The callus showed considerably different response to kind and concentration of auxin supplemented to the medium containing 0.1 mg/ℓ of 4 PU. Callus proliferation was severely suppressed by 2, 4-D at the concentrations in excess of 0.1 mg/ℓ. And furthermore, shoot regeneration was completely inhibited by 2, 4-D even at lowest concentration (0.01 mg/ℓ) (Table 2). The calli formed on the medium containing 2, 4-D were whitish and fragile.

On the other hand, NAA (0.01-5.0 mg/ℓ) did not repressed callus proliferation, but considerably inhibited shoot regeneration, especially, at higher concentrations (Table 2). With NAA, callus had green color and compact structure.

The calli induced on medium containing NAA could regenerate shoots actively after they were transferred onto NAA free medium containing 4 PU. In contrast, the presence of 2, 4-D in the medium severely inhibited callus growth and shoot regeneration even after the callus was transferred onto the 2, 4-D free medium containing 4 PU (Table 3).

The fragile calli proliferated on the medium containing 1.0 mg/ℓ of 2, 4-D were changed its structure to compact and nodular, when they were transferred onto the medium containing 1.0 mg/ℓ of NAA. And these compact calli could actively generate adventitious shoot on the medium containing 0.1 mg/ℓ of 4 PU (Fig. 2).

The inhibition of adventitious shoot regeneration by 2, 4-D on the medium supplemented with zeatin has already reported[4]. In this experiment, the similar results were obtained with the medium containing 4 PU. The potential of the callus to regenerate shoot was found to be suppressed by auxin and the extent of the suppression was severer with 2, 4-D than NAA. And it was also observed that the callus induced by 2, 4-D needs to be cultured for a certain period on the medium supplied with NAA to resume the regenerative potential.

The callus proliferated on the medium with 2, 4-D was much fragile and easily disintegrated to form a suspension of cells in a liquid medium. This characteristic also seemed to suit for protoplast isolation directly from callus.

Experiment 3. Both callus proliferation and adventitious shoot regeneration occurred on all basal media tested, when these were supplemented with 0.1 mg/ℓ of 4 PU. The prolific callus growth was found on MS and B5 medium, whereas, the shoot regeneration occurred most actively on WPM medium, followed by B5,

Table 4. Effect of basal medium on callus proliferation and adventitious shoot regeneration of kiwi fruit[x]

basal medium	callus proliferation[z]	shoot regeneration[y]
M S	+++++	++
B 5	+++++	+++
WPM	++	+++++
Lepoivre	++	+++

z : −=no callus proliferation～+++++=abundant callus proliferaton
y : −=no shoot regeneration～+++++=active shoot regeneration
x : Each medium contained 0.1 mg/ℓ of 4PU

Fig. 2. Effect of subculture with NAA on adventitious shoot regeneration from the callus induced by 2,4−D
From left to right: callus induced with 2,4−D, subcultured callus with NAA (1.0 mg/ℓ) for 4 weeks, shoot regeneration from the callus previously subcultured with NAA (1.0 mg/ℓ) on the medium containing 4PU (0.1 mg/ℓ)

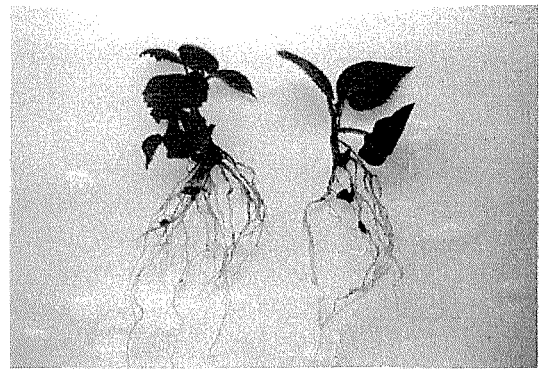

Fig. 3. Rooting of adventitious shoots by IBA treatment
Basal end of the shoot was dipped with 1000 mg/ℓ of IBA.

Lepoivre and MS (Table 4). These results suggest that lower salt and sucrose concentrations suit the shoot regeneration, while higher concentrations of them could stimulate callus growth.

Although, as mentioned above, the adventitious shoots regenerated on the medium with 4 PU showed stunted growth, these shoots started to elongate when they were transferred onto the hormone free medium. By planting the excised shoot onto the medium with 0.1-1.0 mg/ℓ of IBA or planting onto hormone free medium after dipping the basal end into higher concentration of IBA (1000 mg/ℓ), severe callus formation occurred at the basal end and only poor root formation could be attained. On the other hand, the shoots could produce active roots abundantly without any callus formation, when they were inserted directly into vermiculite medium under non-sterile condition after dipping the basal end into IBA solution of 1000 mg/ℓ (Fig. 3). These rooted shoots could be domesticated without difficulty and developed into plantlets.

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