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武庫川女子大紀要（自然科学）

Callus Induction from *Hibiscus manihot* Seed and Leaf

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Hibiscus manihot are used as a viscidity of manufacture of Japanese paper. In this paper, we report successful results in the induction of callus from seed and leaf of *H. manihot*. In the case of seed, the better results were obtained with the medium containing 1μM of 2, 4-D and 1μM of kinetin. On the other hand, in the case of leaf, the better results were obtained with the medium containing more than 0.1μM of 2, 4-D in spite of concentration of kinetin or BA.

Introduction

Hibiscus manihot L. are used as a viscidity of manufacture of Japanese paper.

In this paper, we report the induction of callus from seed and leaf of *H. manihot*.

Materials and Methods

Induction and culture of callus

Seeds and leaves of this plant were rinsed in 70%(v/v)ethanol for 30 seconds, sterilized by immersion for 5 minutes in 5% sodium hypochlorite solution containing 0.01ml/L of Tween 80, and rinsed three times in sterilized distilled water. The sterilized seeds and leaves were cut into 1mm x 3mm piece with a surgical knife and placed on Murashige and Skoog medium(MS medium)[1] supplemented with 3% sucrose, various concentration(0, 0.01, 0.1, 1, 10μM)of 2, 4-dichlorophenoxy- acetic acid(2, 4-D), 6-benzyladenine(BA)and kinetin. The medium was solidified by 0.2% Gelrite. The culture were incubated in dark at 25℃.

After 20 days of culturing on MS growth regulator-free medium, somatic embryos formed in calli were transferred to the plant regeneration medium containing half-strength MS salts and vitamins, 5% sucrose and 0.2% Gelrite.

Results and Discussion

Induction and culture of callus

Table 1. shows the frequency of callus formation from seed after 23 days culture. In particular, in the case of seed, the better results were obtained with the medium containing 1μM of 2, 4-D and 1μM of kinetin.

Table 1. Effects of concentration of 2, 4-D and kinetin on callus formation in *H. manihot* seed.

2, 4-D (μM)	Callus formation(%) Kinetin(μM) 0	0.01	0.1	1	10
0	0	0	0	0	0
0.01	0	0	0	23	0
0.1	0	50	48	42	21
1	60	50	42	100	21
10	60	60	60	72	58

Callus was observed after 23 days culture.

Two types of calli were formed: one on the medium contained 1μM of 2, 4-D and 1μM of kinetin was transparent(white)and friable(Fig. 1.)and the other on the medium contained 10μM of 2, 4-D and 0.01μM of kinetin was trans-

parent(yellow)and friable(Fig. 2.).

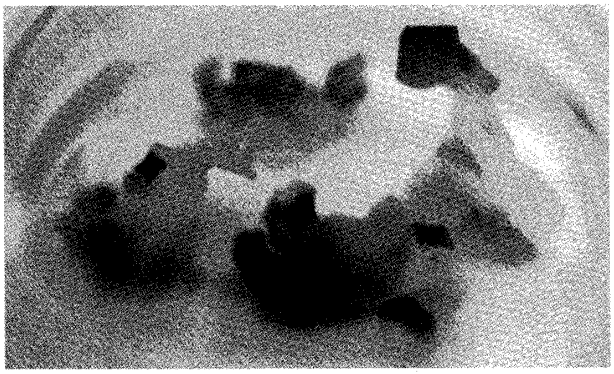

Fig. 1. Callus on the medium contained 1μM of 2, 4-D and 1μM of kinetin from a peeled seed of *H. manihot*.

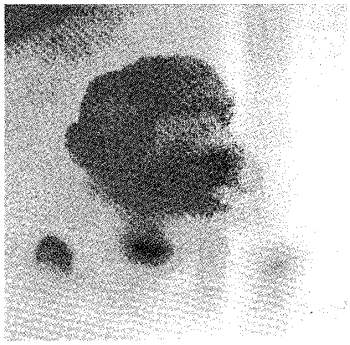

Fig. 2. Callus on the medium contained 10μM of 2, 4-D and 0.01μM of kinetin from a peeled seed of *H. manihot*.

Since somatic embryos developed slowly and occasionally ceased to grow on MS growth regulator-free medium, they had to be transferred onto the plant regenaration medium to develop tubers from the embryos(Fig. 3. and Fig. 4.). When calli from seeds were transferred to the medium contained 0.1μM of 2, 4-D without kinetin, they developed roods after 5 days of incubation. On the other hand, when calli from seeds were transferred to the medium containing 0.01μM of 2, 4-D and 10μM of kinetin, they developed leaves after 14 days of incubation(Fig. 5.).

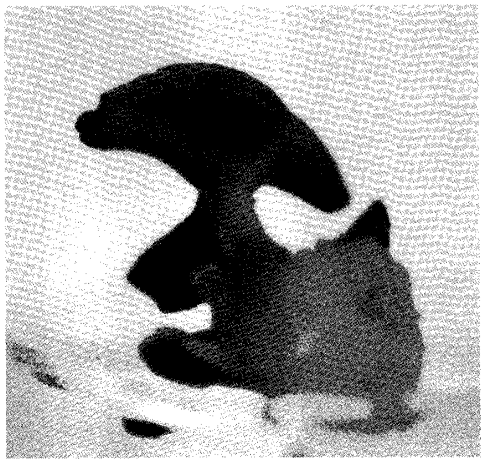

Fig. 3. Plantlet growing on plant growth medium. Plantlet was derived from callus on the medium containing 0.1μM of 2, 4-D and 1μM of kinetin.

Fig. 4. Plantlet growing on plant growth medium. Plantlet was derived from callus on the medium containing 0.1μM of 2, 4-D without kinetin.

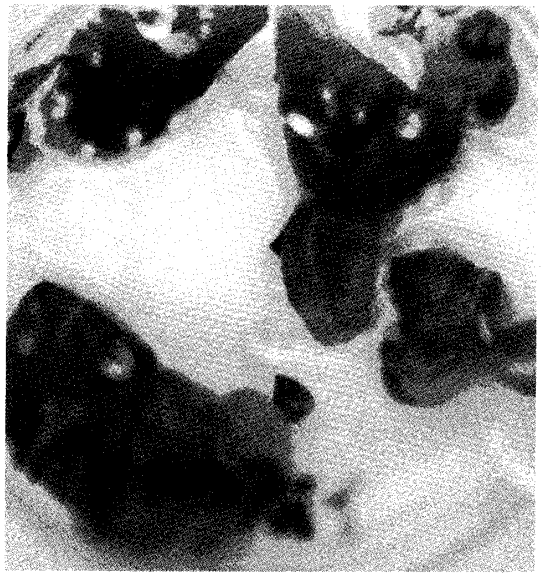

Fig. 5. Development of leaves on the medium contained 0.01μM of 2, 4–D and 10μM of kinetin.

Table 2. shows the frequency of callus formation from leaf after 36 days culture. The better results were obtained with the medium containing more than 1μM of 2, 4–D in spite of concentration of kinetin or BA. Two types of calli were formed;one on the medium contained 1μM of 2, 4–D and 0.01μM of BA was transparent(white)and friable(Fig. 6.(a))and the other on the medium contained 10μM of 2, 4–D and 0.01μM of BA was transparent(yellow)and friable(FIg. 6.(b)).

Table 2. Effects of concentration of 2, 4–D, kinetin and BA on callus formation in *H. manihot* leaf.

	Callus formation(%)									
2, 4–D	Kinetin(μM)					BA(μM)				
(μM)	0	0.01	0.1	1	10	0	0.01	0.1	1	10
0	0	0	0	0	0	0	0	0	20	25
0.01	0	0	0	0	20	0	0	0	60	25
0.1	0	50	100	100	100	100	100	50	80	70
1	100	100	100	100	100	100	100	100	100	100
10	100	100	100	100	100	100	100	100	100	100

Callus was observed after 36 days culture.

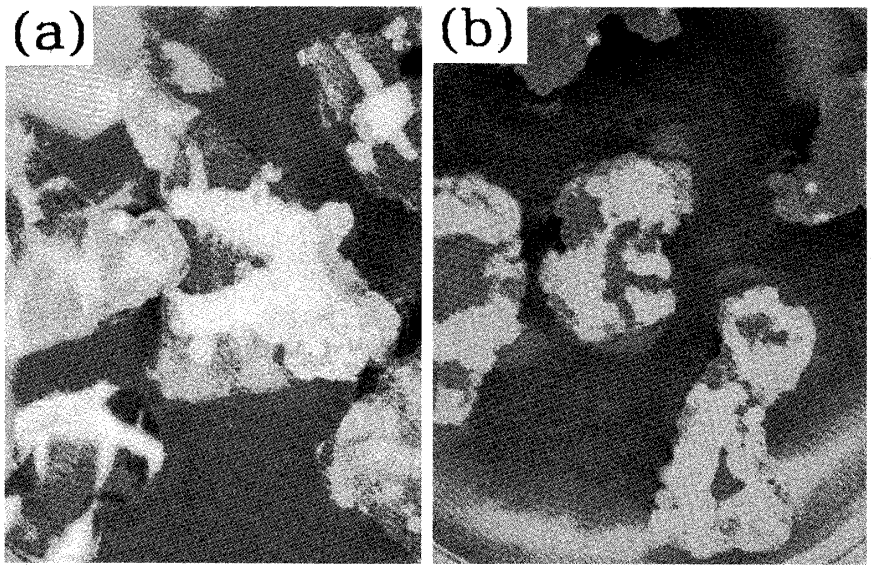

Fig. 6. Callus from leaf of *H. manihot*.
(a) Transparent(white)and friable callus(the medium contained 1μM of 2, 4–D and 0.01μM of BA).
(b) Transparent(yellow)and friable callus(the medium contained 10μM of 2, 4–D and 0.01μM of BA).

Acknowledgments

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References

1) Murashige, T. and Skoog, F., *Physiol. Plant.*, **15**, 473–497(1962).