Bull. Mukogawa Women's Univ. Nat. Sci., **47**, 47－50(1999)
武庫川女子大紀要（自然科学）

Callus Formation and Plant Regeneration from *Basella rubra* Leaf and Stem Cultures

Tokumitsu Okamura, Aki Yuguchi, Shiho Matsuo,
Takako Shinmi and Mikiko Tabuchi

Department of Food Science and Nutrition,
School of Human Environmental Sciences,
Mukogawa Women's University, 6-46, Ikebiraki-cho, Nishinomiya 663-8558, Japan.

The leaves and stems of *Basella rubra* are used as a medicine for fever and diuretic agent. In this paper, we report successful results in the induction of callus and plant regeneration from leaf and stem cultures. The media containing 2, 4-D and kinetin effectively induced callus. Six types of calli were formed. The better results were obtained with the medium containing 3μM of 2, 4-D and 3μM of kinetin.

Introduction

The leaves and stems of *Basella rubra* L. are used as a medicine for fever and diuretic agent. And also the leaves and stems are used as a healthy vegetable.

In this paper, we report the induction of callus and plant regeneration from leaf and stem cultures of *B. rubra*.

Materials and Methods

Induction and culture of callus

Leaves and stems of *B. rubra* (Fig. 1.) 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 leaves (5mm × 5mm) and stems (20mm × 5mm) were cut into pieces or blocks with a surgical knife and placed on Murashige and Skoog medium (MS medium)[1] supplemented with 3% sucrose, various concentration (0, 0.01, 0.02, 0.05, 0.1, 0.5, 1, 2, 3μM) of 2, 4-dichlorophenoxy-acetic acid (2, 4-D) and kinetin. The medium was solidified by 0.8% agar. The culture were incubated in dark at 25℃.

Fig. 1. *Basella rubra* L.

Plant regeneration from somatic embryo

After 36 days of culturing on MS growth regulator-free medium, somatic embryos formed in stem calli were transferred to the plant regeneration medium containing half-strength MS salts and vitamins, 5% sucrose and 0.8% agar. Plantlets derived from somatic embryos were transferred to the growth medium containing one-half strength MS medium and 0.8% agar. Somatic embryos and regenerated plantlets were incubated at 25℃ under cool white fluorescent light (6,000lux) with a 16 hour-photoperiod.

Results and Discussion

Induction and culture of callus

Callus formation from leaf and stem explants was observed within 17–36 days of culture. The media containing 2, 4-D and kinetin effectively induced callus. Six types of calli were formed: opaque green and compact callus (Fig. 2.(a)), transparent green and friable callus (Fig. 2.(b)), transparent white and friable callus (Fig. 2.(c)), opaque white and compact callus (Fig. 2.(d)), opaque green and red and compact callus (Fig. 2.(e)) and opaque brown and friable callus (Fig. 2.(f)).

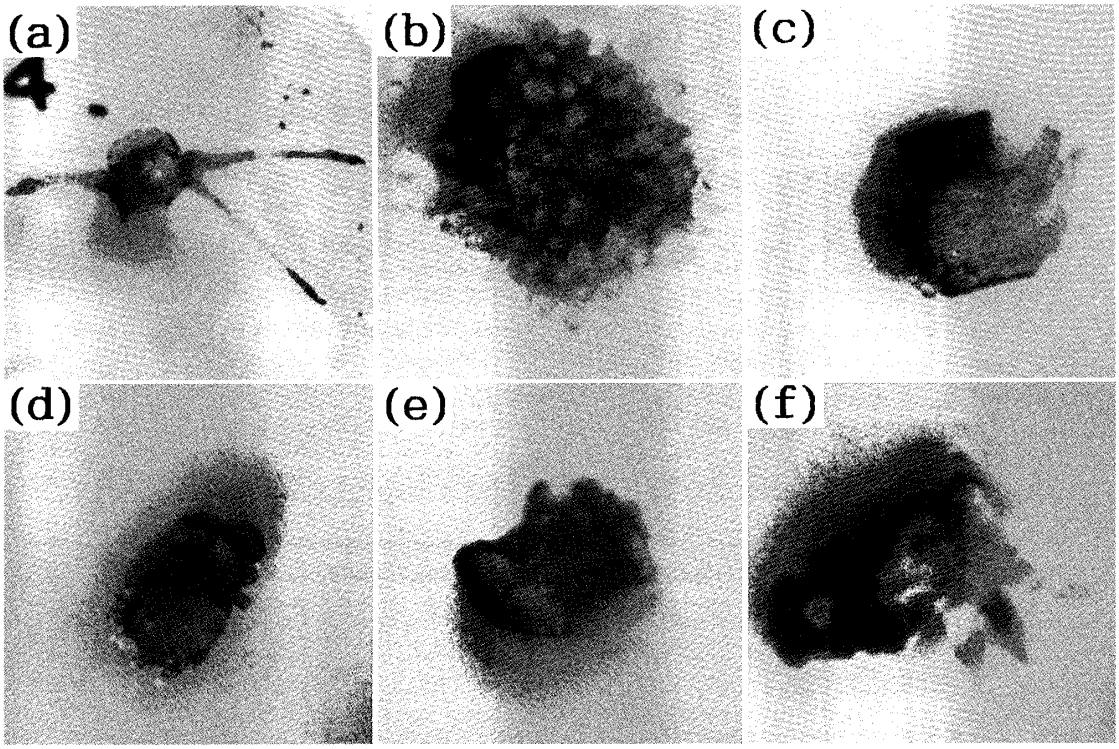

Fig. 2. Callus from a peeled stem of *B. rubra*.

(a) Opaque green and compact callus (the medium contained 0.05μM of 2, 4-D and 0.01μM of kinetin).

(b) Transparent green and friable callus (the medium contained 0.5μM of 2, 4-D and 0.5μM of kinetin).

(c) Transparent white and friable callus (the medium contained 1μM of 2, 4-D and 0.5μM of kinetin).

(d) Opaque white and compact callus (the medium contained 1μM of 2, 4-D and 1μM of kinetin).

(e) Opaque green and red and compact callus (the medium contained 0.05μM of 2, 4-D and 0.5μM of kinetin).

(f) Opaque brown and friable callus (the medium contained 0.01μM of 2, 4-D and 0.5μM of kinetin).

Table 1. shows the frequency of callus formation from leaf and stem explants after 36 days culture. In particular, in the case of leaf, the better results were obtained with the medium containing 3μM of 2, 4-D and 3μM of kinetin. In the case of stem, the better results were also obtained with the same medium.

Table 1. Effects of concentration of 2, 4-D and kinetin on callus formation in *B. rubra* leaf and stem.

	2, 4-D(μM)	Kinetin(μM)	Callus formation
Leaf	0	0	−
	0.03	0.03	+
	0.3	0.3	+
	3	3	+++
	0	3	+
Stem	0	0	−
	0.03	0.03	+
	0.3	0.3	+
	3	3	++
	0	3	−

−, none; +, normal; ++, good; +++, very good.

Plant regeneration from somatic embryo

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.(a), (b)). When tubers with buds and roods were transferred to the growth medium, they further developed shoots and roods(Fig. 3.(c), (d)). Approximately 90% of them developed into mature plants in a growth chamber, after plantlets of 10 stem were transferred to pots.

Regenerated plants were transferred to pots containing a 2:1, vermiculite and perlite mixture. These potted plants were maintained at 25℃ under a 16 hour-photoperiod(6000lux)in a growth chamber. And then, 10 plants have grown in a greenhouse, and have exhibited no morphological variation(Fig. 4.).

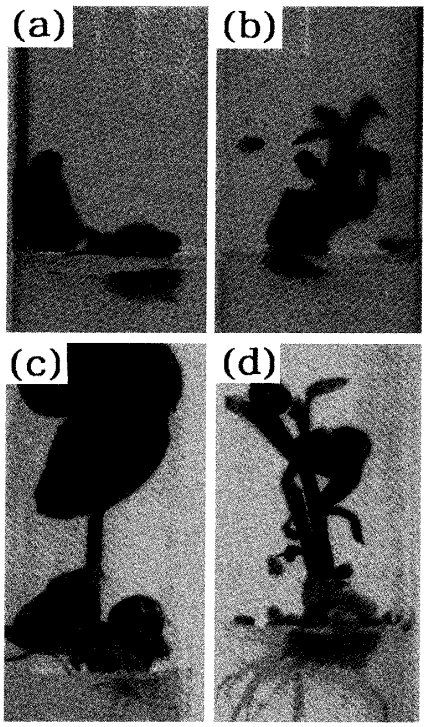

Fig. 3. Plantlet growing on plant growth medium. Plantlet((a)and(b))was derived from callus on the medium containing 0.02μM of 2, 4-D and 2μM of kinetin. Plantlet((c)and(d))was derived from callus of(a)and(b)on the regulator-free medium, respectively.

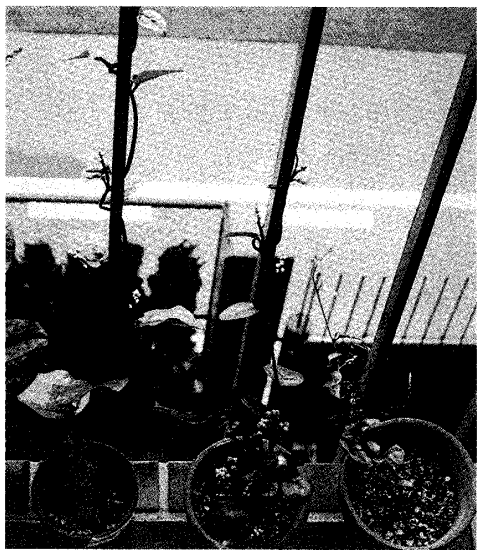

Fig. 4. *B. rubra* via callus. Plants were grown in a greenhouse.

Acknowledgments

We are grateful to Dr. Tsuneo Tomita for his valuable advice throughout this work.

References

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