

## Effects of Various Factors (Hormone Combinations, Genotypes and Antibiotics) on Shoot Regeneration from Cotyledon Explants in *Brassica rapa* L.

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High frequency shoot regeneration is one of the pre-requisites for the application of *Agrobacterium*-mediated genetic transformation. Various subspecies of *Brassica rapa* L. are oilseed, turnip, leaf vegetables and Chinese cabbage. *B. rapa* is known to be one of the most recalcitrant of *Brassica* cultivars in cell and tissue culture response<sup>1-5</sup>. In a recent report, Hachey *et al.*<sup>6</sup> showed that a high frequency of shoot regeneration could be obtained from some oilseed cultivars of *B. rapa*. In our study, shoot regeneration from cotyledon explants was evaluated in sixty cultivars of *B. rapa*. Effects of antibiotics generally used in the process of *Agrobacterium*-mediated transformation was also investigated.

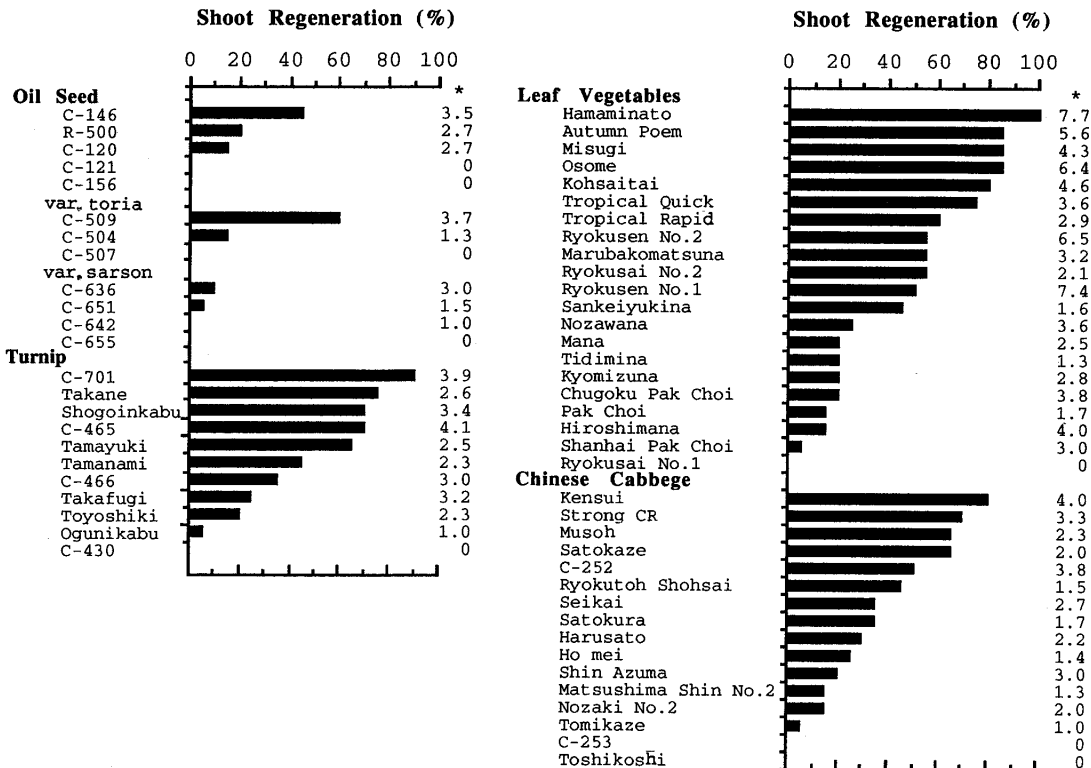
Seeds of sixty genotypes of *B. rapa* were obtained from several seed companies and the genetic stocks in Tohoku University. Seeds were sterilized<sup>7</sup> and placed on Murashige and Skoog medium (MS medium<sup>8</sup>) containing 3% sucrose and 0.8% agar. Cotyledons with petiole of 2 mm in length were excised from 4-day-old seedlings and transferred to MS medium supplemented with 2% sucrose, 0.7% agar and various concentrations of NAA (0, 0.5, 1, 2 and 4 mg/l) and BA (0, 1, 2, 4 and 8 mg/l). Ten cotyledons were placed in a petri-dish (90 × 20 mm) with three replications. Petri-dishes were sealed with gas-permeable tape (Surgical tape; 3 M), unless otherwise stated. They were maintained at 25°C under fluorescent light (16 h photo period, 30-40 μEm<sup>-2</sup>s<sup>-1</sup>). Shoot regeneration frequency was evaluated after 3 weeks of culture as a ratio of the number of cotyledons producing shoots to total explants. The number of shoots per explants were also counted. Carbenicillin (Geopen; Pfizer), Cefotaxime (Claforan; Hoechst) or Vancomycin (Vancomycin; Shionogi) was added for eliminating *Agrobacterium*.

Optimum concentrations of NAA and BA for shoot regeneration were first examined in three cultivars, namely, Kohsaitai, Osome and Strong CR. After 2-3 weeks of culture, many adventitious shoots were visually observed from the cut ends of cotyledonary petioles. The highest frequency of shoot regeneration was achieved on the medium containing 1 mg/l NAA and 2 mg/l BA in Kohsaitai (60%) and Strong CR (47%), while 0.5 mg/l NAA and 2 mg/l BA in Osome (83%). The number of shoots per cotyledon on this medium was 3-4 in Kohsaitai, 5-6 in Osome and 2-3 in Strong CR (Table 1). On the basis of these results, we chose a combination of 1 mg/l NAA and 2 mg/l BA for evaluating shoot regeneration ability in sixty cultivars.

**Table 1.** Effect of NAA and BA combinations on frequency of shoot regeneration from cotyledon explants in three cultivars of *B. rapa*.

Cultivars	NAA mg/l	Shoot regeneration frequency(%)				
		0	1	2	4	8
Kohsaitai	0	0(0)	0(0)	0(0)	0(0)	0(0)
	0.5	0(0)	37(4.3)	30(3.1)	30(1.7)	27(2.9)
	1	0(0)	43(5.0)	60(4.3)	37(2.5)	7(2.5)
	2	0(0)	33(2.7)	43(2.5)	7(2.3)	10(2.0)
	4	0(0)	17(1.4)	17(1.2)	0(0)	0(0)
Osome	0	0(0)	0(0)	0(0)	0(0)	0(0)
	0.5	0(0)	56(5.8)	83(6.2)	63(5.3)	33(2.7)
	1	0(0)	77(4.5)	77(6.5)	50(4.3)	7(4.0)
	2	0(0)	47(5.4)	47(3.9)	33(4.6)	17(2.4)
	4	0(0)	23(2.3)	33(3.3)	23(2.7)	3(1.0)
Strong CR	0	0(0)	0(0)	0(0)	0(0)	0(0)
	0.5	0(0)	23(2.4)	40(2.2)	30(2.4)	0(0)
	1	0(0)	23(2.3)	47(2.5)	17(3.2)	0(0)
	2	0(0)	3(3.0)	0(0)	0(0)	3(0)
	4	0(0)	0(0)	0(0)	0(0)	0(0)

Number of regenerated shoots per cotyledon was also shown in parenthesis.

**Fig. 1** Effect of genotypes on frequency of shoot regeneration and number of shoots from cotyledon explants in 60 genotypes of *B. rapa*.

\* indicates number of regenerated shoots per cotyledon.

**Table 2.** Effect of various antibiotics on frequency of shoot regeneration from cotyledon explants in eight cultivars of *B. rapa*.

Cultivars	Shoot regeneration(%)			
	None	Cb	Cef	Van
Osome	97(8.5)	97(8.5)	53(3.7)	90(6.7)
Strong CR	83(3.7)	27(2.1)	0(0)	30(2.7)
Kohsaitai	70(4.2)	40(4.2)	0(0)	23(2.4)
Hakuyoh No. 1	70(3.6)	63(4.3)	6(3.0)	53(2.8)
Misugi	70(4.9)	87(5.0)	0(0)	60(4.0)
Hamaminato	80(5.8)	80(3.7)	NT	NT
Autumn Poem	70(4.5)	60(3.8)	NT	NT
Takane	60(2.5)	3(2.0)	NT	NT

Antibiotics was added at a concentration of 500 mg/l for carbenicillin(Cb) and cefotaxime(Cef) and 750 mg/l vancomycin(Van). Number of regenerated shoots per cotyledon was also shown in parenthesis. NT : not tested.

Shoot regeneration frequency differed widely from 0% to 100% among 60 genotypes. Seven cultivars, namely, C-701, Hamaminato, Autumn poem, Misugi, Osome, Kohsaitai and Kensui exhibited shoot regeneration frequencies higher more than 80%. These cultivars also produced 4-8 shoots per cotyledon(**Fig. 1**).

Shoot regeneration was reduced to 25-50% when petri-plates were sealed with parafilm in place of gas-permeable tape(data not shown). Gas-permeable tape might be effective for removing ethylene that was reported to inhibit shoot regeneration<sup>9,10</sup>.

Addition of 500 mg/l carbenicillin, 500 mg/l cefotaxime or 750 mg/l vancomycin greatly reduced the shoot regeneration in 8 cultivars tested. Deteriorative effect on shoot regeneration of cefotaxime and vancomycin was stronger than that of carbenicillin. Shoot regeneration was not significantly reduced in cultivars, Osome, Hakuyoh No. 1 and Misugi under the condition of 500 mg/l carbenicillin. Osome was least affected by the three antibiotics, and exhibited the highest shoot regeneration frequency and the highest number of shoots per cotyledon(**Table 2**).

In conclusion, Osome was found to be the most suitable cultivar for the study of *Agrobacterium*-mediated transformation among 60 tested cultivars in *B. rapa* in our experiment.

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## 《和文要約》

*Brassica rapa* 子葉片からの不定芽形成に及ぼすホルモン組成、遺伝子型と抗生物質の影響

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*B. rapa* 3品種の子葉片からの不定芽形成に最適な培地について検討した。その結果、NAA 1 mg/l と BA 2 mg/l を含む培地で最も高い不定芽形成率を示すことが判明した。次に、60品種の再分化能を評価したところ、80%以上の不定芽形成率を示す7品種を見出すことができた。アグロバクテリウムの除菌に使用される抗生物質の添加により、いくつかの品種で不定芽形成の減少が見られたが、おそめはその影響を受けなかった。今後アグロバクテリウムによる遺伝子導入を行う際におそめは最も適した材料であると考えられる。