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Ability of Callus Growth and Shoot Regeneration in the Wild Species of Brassicaceae

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Ability of callus proliferation and plant regeneration was surveyed for 79 strains of about 50 wild species in Brassicaceae. Leaf segments were cultured on Murashige and Skoog (MS) medium with 1 mg/*l* 2, 4-dichlorophenoxyacetic acid to produce calli, and the growth of calli was evaluated. Response to a liquid culture was also examined in some species. Although the growth of calli was not so vigorous in many species, some species produced vigorous calli on solid medium and produced cell suspension in liquid one. Shoot regeneration ability from leaf segments and primary callus was evaluated on MS medium supplemented with 1 mg/*l* α-naphthaleneacetic acid, 1 mg/*l* 6-benzylaminopurine and 100 mg/*l* casamino acids. Some species showed high shoot forming ability. Data for all strains examined are listed.

The family Brassicaceae includes many important species for human consumption, e. g. genus *Brassica* and *Raphanus* are used as vegetables, oil crops and animal feeds all over the world. It is also known in this taxa that interspecific and intergeneric hybridization by the ordinary method has been successfully utilized for the improvement of cultivars.[1] Recently, successful cases of somatic hybridization have been accumulating in this taxa.[2-4] We believe that somatic hybridization with wild species will be very effective to broaden the genetic diversity of these cultivars. For a further stage of development, it may be desirable to collect informations about the response of wild germplasms to tissue culture.

In the present survey, the ability of callus growth and shoot regeneration was evaluated for about 50 wild species that were closely related to *Brassica* in Brassicaceae.

Materials and Methods

Seventy nine strains of about 50 wild species in the tribe Brassiceae and some other tribes in Brassicaceae (**Table 1**) were taken for experiments from the genetic stocks preserved in the Laboratory of Plant Breeding, Tohoku University. The material plants were grown in pots in a greenhouse. Young leaves of about 2-month-old seedlings were sterilized by dipping them successively in a 75% ethanol solution for a few seconds, a 2% sodium hypochlorite solution for 20 min and sterile water twice. About 5 mm square segments around the mid rib of the leaves were used as explants. Three pieces of the leaf segments were innoculated on an agar (0.8%) medium consisted of MS medium[5] and 1 mg/*l* 2, 4-dichlorophenoxyacetic acid (D 1 medium) in a test tube. Callus culture in test tubes was carried out under fluorescent light (12.5 w/m^2) at 25℃. After one month of culture on D 1 medium, the primary callus was transferred to fresh D 1 medium and then subcultured every one month. The growth of subcultured callus was evaluated as follows: ++; grow vigorously, +; grow a little and −; no more growth. Color and friability of callus were also observed.

Table 1. Callus growth on an agar medium (A) and that in a liquid medium (B), and shoot regeneration (C) in the Brassicaceae.

Species[a]	A[b]	B[b]	C[c]
Tribe BRASSICEAE			
Subtribe Brassicinae			
Brassica			
adpressa-111	+	−	−
adpressa-112	+	−	−
alboglabra-201	+		−
amplexicaulis-4	+		
barrelieri-108	+		−
campestris var. *toria*-506	+		
carinata-104	+		−
deflexa-1	++	+	−
erucastrum-102	+		−
fruticulosa-103	+	−	−
fruticulosa-104	+		−
fruticulosa-401	+		−
fruticulosa			
subsp. *cossoneana*-201	+		−
subsp. *radicata*-502	+		+
gravinae-1	+		
juncea-113	++	−	−
maurorum-1	−		−
manrorum-5	+		−
maurorum-6	++		−
napus-111	+		−
nigra-116	++	+	−
nigra-138	++	−	−
nigra-141	+		
oleracea-166 (wild kale)	+		+
robertiana-171	+		
spinescens-1	+		−
tournefortii-162	+		−
tournefortii-165	+		
tournefortii-167	+		−
Diplotaxis			
assurgens-1	++	−	−
berthautii-1	++		−
catholica-5	+		+
erucoides-7	+	−	−
erucoides-9	+	−	−
harra-4	+		−
harra-6	+		−
harra			
subsp. *lagascana*-8	+		−
subsp. *lagascana*-9	+		−
muralis-3	+	−	−
muralis-101	+		−
pitardiana-1	+	−	
siifolia-1	++		−
siifolia-3	+		−
tenuifolia-7	+		−
tenuisiliqua-1	++	++	−
tenuisiliqua-5	+		
virgata-4	+		−
virgata-10	+		−
Eruca			
stativa-9	+		−
sativa-12	−		−
vesicaria-3	+	−	−
vesicaria-5	+		−
vesicaria-6	+		−
vesicaria-8	++		−
Erucastrum			
abyssinicum-1	+		−
abyssinicum-2	+		
cardaminoides-1	++	+	−
gallicum-1	+		−
laevigatum-2	+		−
leucanthum-1	+		−
nasturtiifolium-2	+		+
varium-2	+		−
virgatum-1	+		−
Sinapis			
alba-25	+		−
alba-28	+		−
arvensis-13	++		
arvensis-16	++		−
arvensis-18	++	++	−
arvensis-19	+		−
pubescens-1	+		+
turgida-1	++	++	
Sinapidendron			
angustifolium-1	+	−	−
Subtribe Raphaninae			
Raphanus maritimus-4	+		−
Crambe maritima-1	+		−
Subtribe Moricandiinae			
Moricandia arvensis-14	++	++	+
Tribe LEPIDIEAE			
Lepidium sativum-1	++	−	+
Tribe DRABEAE			
Armoracia rusticana-1	+	+	++
Tribe HESPERIDEAE			
Hesperis matronalis-1	+		+
Tribe SISYMBRIEAE			
Arabidopsis thaliana-1	++	++	

[a] species classification was referred to Schulz.[9]

[b] ++; grow vigorously, +; grow a little, −; no growth.

[c] ++; produce many shoots, +; produce at least one shoot, −; produce no shoot.

Ability of callus growth in a liquid medium was also examined for some species. After 2 months of the subculture on agar medium, calli (1 cm^3 in volume) were transferred to 40 ml of liquid D 1 medium in a 100 ml flask and agitated at 120 stroke/min under dark.

For examining shoot regeneration, the leaf segments were directly inoculated on MS medium[5] supplemented with 1 mg/*l* α-naphthaleneacetic acid, 1 mg/*l* 6-benzylaminopurine, 100 mg/*l* casamino acids and 0.8% agar (NB medium). Additionally, a piece of primary callus (0.1cm^3 in volume) on D 1 medium was transferred to NB medium. At least two test tubes were used in each case. The ability of shoot regeneration was evaluated by the number of leaf segments and/or calli that produced shoots after 2 months of culture.

Results and Discussion

The appearance and growth of callus were different among species. Many of the species produced brown colored calli and their subcultured calli were not vigorous in growth. On the other hand, *Brassica tournefortii* and *Diplotaxis harra* produced whitish-green colored and chewing gum-like calli. Whitish-yellow colored and friable calli were obtained in *B. deflexa*, *B. maurorum*, *B. nigra*, *D. assurgens*, *D. berthautii*, *D. siifolia*, *D. tenuisiliqua*, *Erucastrum cardaminoides*, *Sinapis arvensis*, *S. turgida*, *Moricandia arvensis*, *Lepidium sativum*, etc. These whitish-yellow calli were vigorous in growth (**Table 1**). The calli of *D. tenuisiliqua*, *S. arvensis*, *S. turgida*, *M. arvensis* and *L. sativum* were subcultured for more than four years without browning. In some cases, similar response was observed between species that were reported by Harberd[6] to have the same genomic constitution, i.e. between *D. berthautii* and *D. siifolia*, and between *S. arvensis* and *S. turgida*. In general, however, the responses were different among strains, even when they belonged to the same species.

In suspension cultures, the calli of many species formed large aggregates and turned brown. However, rapidly-growing and friable cell suspension was obtained in *D. tenuisiliqua*, *S. turgida*, *M. arvensis* and *Arabidopsis thaliana* (**Table 1**).

As regards shoot regeneration, *B. fruticulosa*, *M. arvensis* and *Armoracia rusticana* produced shoots when the primary calli on the D 1 medium were transferred to the NB medium (**Table 1**). When the leaf segments were directly inoculated on the NB medium, shoot formation was observed in *B. oleracea* (wild kale), *D. catholica*, *E. nasturtiiforium*, *S. pubescens*, *Hesperis matronalis* and *A. rusticana*. Especially, *A. rusticana* (Horse raddish) produced many plantlets from every leaf segments after one month of culture on the NB medium.

Since given culture conditions were limited in the present experiment, it is not necessarily true that the browned calli have low potential in its growth. However, it is clear that the vigorously-growing calli could be taken as experimental materials for tissue culture. It was shown that the calli of *S. turgida* and *M. arvensis* were successfully used for producing somatic hybrids with cultivars.[7,8] We believe that the exchange of information will help for the further development of cell manipulation and crop improvement in Brassicaceae.

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≪和文要約≫

アブラナ科野生種のカルス増殖と再分化能

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Brassica 属およびその近縁属の野生種 79 系統（およそ 50 種）について，カルス増殖能と植物体再分化能を調査した．はじめに，葉切片を 1 mg/*l* の 2,4-ジクロロフェノキシ酢酸を含む MS 培地に置床してカルスを形成させ，さらにいくつかの種については同組成の液体培地で振盪培養を試みた．ほとんどの種についてカルス増殖が悪かったが，いくつかの種は活発に増殖するカルスを形成した．次に，葉切片と 1 次カルスを 1 mg/*l* のナフタリン酢酸，1 mg/*l* のベンジルアミノプリン，および 100 mg/*l* のカザミノ酸を含む MS 培地に置床して芽形成能を調査した．その結果，いくつかの種は芽を形成した．ここでは，調査したすべての系統についての結果を表に示した．