without staking, unlike the diploid plants.

We expected the flower produced by the tetraploid to be larger and fuller than those produced by the diploid. However, the petal width and flower natural spread of the tetraploid was not significantly larger than the diploid. Not all tetraploid flowers are larger than their diploid counterparts. In fact, the tetraploid form of *Tagetes erecta* 'Guinea Gold' actually produced smaller flowers than its diploid counterpart (Emsweller and Ruttle, 1941).

The tetraploid plants exhibited multivalents during meiosis, which are typically associated with autotetraploidy. These multivalents produced bridges and laggards during meiotic anaphase I leading to an 80% reduction in pollen viability. In addition, seed set was reduced to 2% of the diploid. In *Portulaca grandiflora*, pollen viability was reduced 80% and seed set was "poor" in the induced autotetraploid forms (Singh, 1979). In *Impatiens* cultivars, pollen viability was reduced ≈40% and seed set ≈35% in the induced autotetraploid forms (Arisumi, 1973).

The tetraploid form of *Eustoma* 'Blue Poppy' was horticulturally superior to the diploid form. The tetraploid had thicker stems and a shorter plant that could better support the flowers. The only negative characteristics of the tetraploid form was its low fertility (2% seed set). This characteristic could be eliminated through further breeding and selection. In autotetraploid maize, seed set was increased from ≈57% to 68% after six generations of selection (Mastenbroek et al., 1982). After 22 generations of selection, the autotetraploid's seed set was that of the diploid control (85%). Similar selection could be used on the autotetraploid forms of *Eustoma*.

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Plant Regeneration from Hypocotyl Callus of Radish

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Additional index words. callus, auxin, cytokinin, *Raphanus sativus*

Abstract. **Callus initiation and growth and plantlet regeneration were studied using eight cultivars of *Raphanus sativus* L., including six Japanese radishes, one Chinese and one small 'Comet' radish. The basal medium was composed of Murashige and Skoog inorganic salts, 2.0 mg myo-inositol/liter, 0.5 mg each of nicotinic acid and pyridoxine·HCl/liter, and 0.1 mg thiamine·HCl/liter, 30 g sucrose and 2 g Gelrite/liter. High callus yields were obtained on basal medium containing (mg·liter^{-1}) 0.1 2,4-D and 1.0 BA for two Japanese radishes and 0.1 NAA and 1.0 kinetin for 'Comet' radish. Shoots were regenerated from callus by subculturing on basal medium containing 0.1 or 1.0 mg BA/liter and then transferring to basal medium. Rooting occurred on basal medium. Although callus was obtained in all eight cultivars, shoots and plantlets were regenerated only from 'Moriguchi', 'Nerima Shirinaga', and 'Comet'. Chemical names used: 2-(1-naphthyl) acetic acid (NAA); *N*-(phenylmethyl)-1*H*-purine-6-amine (BA); 2,4-dichlorophenoxy acetic acid (2,4-D); 6-(furfurylamino)purine (kinetin).**

Many members of the Cruciferae are hybrids between self-incompatible parent lines. *Raphanus sativus* L., the radish, is self-incompatible, and hybrid varieties between self-incompatible parent lines have been widely cultivated. Parental lines, however, must be maintained by labor-intensive bud pollination or CO_2 treatment (Nakanishi and Hinata, 1973). Tissue culture may be another useful method for the maintenance and proliferation of self-incompatible lines. Although some genera of the Cruciferae can be propagated in vitro from apices and organs, and from callus and protoplast cultures (Zee and Johnson, 1985), callus initiation and plant regeneration of radish have not been reported. In the present paper, we describe the conditions for callus initiation and regeneration of plantlets from radish cultivars.

Six Japanese radishes, 'Bansei Shogoin', 'Harumaki Minowase', 'Moriguchi', 'Minowase', 'Nerima Shirinaga', and 'Sakurajima', one Chinese radish, 'Chinese Aonaga', and one small radish, 'Comet', were used.

Callus formation. Surface-sterilized seeds of 'Harumaki Minowase' were sown on basal medium consisting of MS (Murashige and Skoog, 1962) salts plus (all in mg·liter^{-1}) 2.0 myo-inositol, 0.5 nicotinic acid and pyridoxine·HCl, 0.1 thiamine·HCl, 3% (w/v) sucrose and 0.2% Gelrite. The pH was adjusted to 5.7. Cotyledons and hypocotyls of 5-day-old seedlings, root cambium of edible roots, and ovaries of field-grown plants were cut into squares (3 × 3 mm) or strips (3 mm). These explants were planted on basal medium containing 2,4-D and BA. Concentrations of each hormone were 0, 0.1, 1.0, and 10.0 mg·liter^{-1}. Ten explants were planted on 30 ml of medium in a 100-ml flask, and four flasks were used for each treatment. Cultures were incubated at 25C with a 16-hr day length supplemented with 20 μmol·s^{-1}·m^{-2} of fluorescent light for 30 days.

Regeneration of shoots and plantlets. Hypocotyls of eight cultivars were cultured on four kinds of callus initiation medium for 30 days. These media consisted of the basal medium containing all combinations of (mg·liter^{-1}) 0.1 auxin, i.e., 2,4-D and NAA, and 1.0 cytokinin, i.e., BA and kinetin.

For the further study of regeneration, callus that formed on medium containing (mg·liter^{-1}) 0.1 2,4-D and 1.0 BA was subcultured on the following media: 0.1 2,4-D and 1.0 BA, or 0, 0.1 and 1.0 BA. Callus was subcultured on 30 ml of medium in 100-ml flasks, and three flasks were used for each treatment. After 30 days, each callus was transplanted onto medium with the same composition as used initially. After 30 more days, each callus was transplanted onto basal medium. All shoots that regenerated were transplanted onto basal medium for root initiation.

Callus formation. Higher callus yields were obtained on media containing 0 to 10 mg

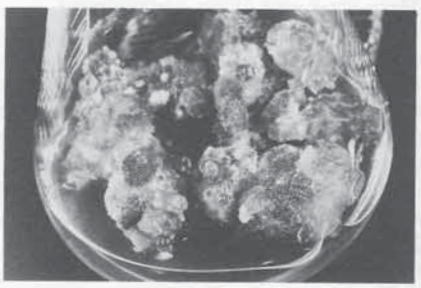

Fig. 1. Hypocotyl callus from 'Comet' cultured for 30 days on MS medium + 0.1 mg 2,4-D/liter and 1.0 mg BA/liter, followed by subculture for 30 days on MS + 1.0 mg BA/liter.

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Table 1. Effects of auxin and cytokinin on callus initiation and shoot and root regeneration of various radish cultivars.

Auxin 0.1 (mg·liter^{-1})	Cytokinin 1.0 (mg·liter^{-1})	Cultivar: Minowase	Nerima Shirinaga	Bansei Shogoin	Sakurajima	Moriguchi	Chinese Aonaga	Harumaki Minowase	Comet	Mean
		Explants forming callus (%)[z]								
2,4-D	BA	67 a–e	83 abc	100 a	60 b–f	100 a	77 abc	100 a	80 abc	83 a
2,4-D	K	90 ab	67 a–e	100 a	27 fgh	77 abc	50 c–h	70 a–d	90 ab	72 a
NAA	BA	17 h	20 gh	50 c–g	30 e–h	37 d–h	37 d–h	83 abc	57 b–g	41 b
NAA	K	17 h	20 gh	77 abc	27 fgh	37 d–h	60 b–f	70 a–d	80 abc	48 b
Mean		48 cd	48 cd	82 a	36 bc	60 bc	56 c	81 a	77 ab	
		Explants regenerating shoots (%)								
2,4-D	BA	0	0	0	0	0	0	0	0	
2,4-D	K	0	0	0	0	0	0	0	6.7(10)[y]	
NAA	BA	0	0	0	0	0	0	0	3.3(11)	
NAA	K	0	0	0	0	0	0	0	6.7(9)	
		Explants regenerating roots (%)[z]								
2,4-D	BA	0 d	0 d	40 b	0 d	0 d	0 d	0 d	0 d	5.0 c
2,4-D	K	0 d	0 d	0 d	0 d	0 d	0 d	0 d	0 d	0.0 d
NAA	BA	7 cd	7 cd	33 bc	56 ab	7 cd	0 d	10 cd	10 cd	16.3 b
NAA	K	17 cd	17 cd	70 a	77 a	27 c	10 cd	10 cd	0 d	28.3 d
Mean		5.8 b	5.8 b	35.8 a	33.3 a	8.3 b	2.5 b	5.0 b	2.5 b	

[z]Mean separation (in rows and columns) by Duncan's multiple range test, $P = 0.05$.
[y]Figures in parentheses indicate mean number of shoots per explant that regenerated shoots.

Table 2. Callus growth and number of explants that regenerated shoots and roots during initiation and subcultures of various radish cultivars.[z]

Cultivar	Fresh wt of callus (mg)	Explants that regenerated shoots (no.)	Explants that regenerated roots (no.)
Chinese Aonaga	163 b	0.0 b	0.0 b
Bansei Shogoin	201 ab	0.0 b	1.13 ab
Harumaki Minowase	170 b	0.0 b	0.60 b
Minowase	160 b	0.0 b	0.75 ab
Moriguchi	363 a	0.94 a	2.06 a
Nerima Shirinaga	133 b	0.44 a	0.56 ab
Sakurajima	245 ab	0.0 b	1.75 ab
Comet	167 b	0.0 b	0.5 ab
Analysis of variance			
Cultivars (8)			
Sum of squares (% of total)	16.84	15.84	8.80
Significance	**	**	**
Culture (4)			
Sum of squares (% of total)	2.17	4.04	4.50
Significance	**	**	**
Phytohormones			
Sum of squares (% of total)	23.35	1.67	1.31
Significance	**	**	**
Cultivars × cultures			
Sum of squares (% of total)	4.65	14.80	28.91
Significance	**	**	**
Cultivars × phytohormones			
Sum of squares (% of total)	24.49	23.71	18.83
Significance	**	**	**
Cultures × phytohormones			
Sum of squares (% of total)	1.18	2.86	2.30
Significance	NS	**	**
Cultivars × cultures × phytohormones			
Sum of squares (% of total)	7.09	32.31	20.02
Significance	*	**	**

[z]Initiation medium contained 2,4-D and BA at 0.1 and 1.0 mg·liter^{-1}, respectively.
[NS,*,**]Nonsignificant and significant at $P = 0.05$ and 0.01, respectively.

2,4-D and 0.1 mg BA/liter, irrespective of the source of explants. Although calli from cotyledons and ovaries were relatively large, these calli and root cambium callus tended to be brown after 30 days. Friable and white callus was formed from hypocotyls, but it was slow growing (Fig. 1).

Regeneration of shoots and plantlets. Callus initiation and growth and shoot regeneration depended on the initiation medium and cultivar (Table 1). At least 60% of explants on medium containing 2,4-D and BA in all the cultivars formed callus. On medium containing 2,4-D and kinetin, at least 50% of explants formed callus in seven cultivars; the exception was 'Sakurajima'. Fewer explants formed callus on media containing NAA and BA or NAA and kinetin. However, roots regenerated on these media. Fewer than 7% of 'Comet' explants regenerated shoots, except on media containing 2,4-D and BA.

Callus fresh weight and number of explants that regenerated shoots and roots on initiation medium containing 0.1 mg 2,4-D and 1.0 mg BA/liter and after subculture onto maintenance medium are shown in Table 2. Highest callus yields were obtained with 'Moriguchi' and the lowest with 'Nerima Shirinaga'. Callus fresh weight decreased with repeated subculture in all cultivars except 'Sakurajima' and 'Moriguchi'. Highest callus yield was obtained on maintenance medium with the same formulation as the initiation medium, but it was not significantly different on the other media. Shoot regeneration from callus occurred in 'Nerima Shirinaga' and 'Moriguchi' (Fig. 2). In 'Nerima Shirinaga', 10% of explants regenerated shoots on subculture medium containing 1.0 mg BA/liter, and each explant regenerated six shoots. After transfer to the basal medium, 60% of explants regenerated 11 shoots per explant. With 'Moriguchi', 10% to 50% of explants regenerated several shoots per explant after subculturing on maintenance medium in the presence or absence of BA. Root regeneration occurred directly from callus in all cultivars except 'Chinese Aonaga', depending on the medium. Root regeneration was not observed on medium containing 2,4-D and BA. All shoots rooted after transfer onto basal medium (Fig. 3).

Some species of self-incompatible plants belonging to the genus *Brassica* have been regenerated in vitro. Regeneration of radish from callus is reported for the first time in the present study, although it has been demonstrated for only three cultivars among eight that were tested. These three differ widely in morphological and physiological characters. A clear relation between these factors and regeneration among the cultivars tested was not observed.

Effective phytohormones for callus initiation and plant regeneration differ among plants. 2,4-D usually promotes callus initiation and growth, but it inhibits or is ineffective for regeneration. For regeneration of

Fig. 2. Plantlet regeneration from hypocotyl callus of 'Moriguchi' cultured for 30 days on initiation medium (MS + 0.1 2,4-D + 1.0 mg BA/liter), followed by two subcultures on MS + 1.0 mg BA/liter for another 60 days, and then transplanted onto MS medium.

Fig. 3. Regenerated plant of 'Moriguchi' radish transplanted to vermiculite.

plants, callus usually must be transplanted onto media without 2,4-D, with only cytokinin, or with other auxins, e.g., NAA or IAA, plus cytokinin. In radishes, shoots regenerated on subculture media without 2,4-D in 'Moriguchi' and 'Nerima Shirinaga', as reported for cauliflower (Walkey et al., 1974) and red cabbage (Bajaj and Nietsch, 1975). In the case of 'Comet' on medium containing 2,4-D and kinetin, shoots regenerated directly from hypocotyl cambium. Although many kinds of explants, such as flower organs, leaf and leaf-vine, stem and cotyledon, shoot tip and hypocotyl, have been used for tissue culture of cole crops, the hypocotyl has been optimal for radish callus. Hypocotyls have also been good for callus initiation of broccoli (Hui, 1980), red cabbage, and leaf-mustard (Bajaj and Nietsch, 1975). The number of regenerated plants has varied depending on media and cultivars tested. One explant cut into four sections at every subculture, on average, results in 70.4 'Nerima Shirinaga' and 16.6 'Moriguchi' plants after 4 months of culture.

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Seed and Embryo Growth in Pod Cultures of *Phaseolus vulgaris* and *P. vulgaris* x *P. acutifolius*

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Additional index words. common bean, interspecific hybridization, ovary culture, tepary bean

Abstract. **Interspecific hybrid embryos resulting from crosses between *Phaseolus* species generally fail to reach maturity, and embryo rescue techniques are required to recover plants. To determine if ovary (pod) culture could permit interspecific hybrid embryo development, pods of *P. vulgaris* L. x *P. acutifolius* A. Gray and *P. vulgaris* L. (control) were cultured upright, supported by glasswool, in modified Murashige-Skoog liquid medium. The weight of seeds and length of embryos in *P. vulgaris* pods increased significantly during culture. However, usually only one or occasionally two seeds located at the middle positions of the pods developed to maturity. The same pod culture procedure promoted precocious germination of early cotyledonary-stage *P. vulgaris* x *P. acutifolius* embryos without further maturation of the embryos. These findings confirm that developmental arrest of the interspecific hybrid embryos in vivo is due to intrinsic deficiencies.**

Phaseolus vulgaris (common bean) is susceptible to several viral, bacterial, and fungal diseases. Resistance has been identified in related species, *P. coccineus* Lam. (runner bean), *P. acutifolius* (tepary bean), and *P. lunatus* L. (lima bean). For instance, *P. acutifolius* contains resistance to common blight (Coyne et al., 1963; Yoshii et al., 1978) and golden mosaic virus (CIAT, 1973). In addition, this species is drought tolerant and adapted to dry-land culture. Transfer of desirable traits from related *Phaseolus* species to *P. vulgaris* could greatly improve agronomic characteristics of the common bean. Therefore, interspecific hybridization has been used to facilitate genetic exchange between *Phaseolus* species.

Although fertilization occurs and embryos are formed, interspecific hybrid embryos are generally limited in their developmental potential (Mok et al., 1986). The particular stage at which the developmental arrest occurs depends on the interspecific combination as well as the direction of the cross. The immature embryos, with the exception of those of *P. lunatus* x *P. vulgaris* that develop only to the four-celled stage, can be cultured in vitro, germinated, and grown to plants (Hucl and Scoles, 1985).

The development of a suitable pod culture system for *Phaseolus* may facilitate elucidation of factors influencing the ontogeny of interspecific embryos. Development of alternative or supplemental methods to embryo culture to optimize the recovery of interspecific hybrids would also be desirable. We report here the procedures and results of culturing immature pods of *Phaseolus*, beginning with selfed pods of *P. vulgaris* to establish the protocols and followed by pods

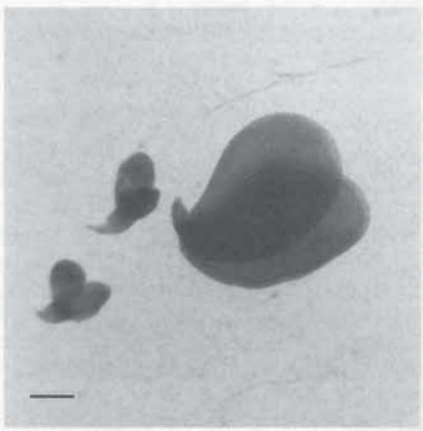

Fig. 1. Embryos of *P. vulgaris* GN (large) and *P. vulgaris* GN x *P. acutifolius* Ac2 (small) 14 days after pollination. Bar = 1 mm.

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