271998 (2.83) was considerably higher than in the generation means experiment (1.13), due probably to growing season differences. The plant material for the generation means analysis was planted in October, while for the analysis of tolerant by tolerant crosses, the plants were started in June. Fall- and winter-grown bean plants typically have had a lower EER in our laboratory.

Evaluation of field-grown germplasm. Additional germplasm was screened for EER. Ten *P. vulgaris* genotypes ['BBL 47', 'UI 111', PI 271998, 'Strike', 'Labrador', 'Red Kidney', 'GNUI 59', 'Hebei No. 1', G 4727 (Ancash 66), USDA 85-CT-4986-3] and a *P. acutifolius* (tepari bean) entry were field-grown 80 km from the laboratory on an irrigated Hubbard series soil (sandy, mixed Udorthentic Haploborolls) under standard cultural practice. 'BBL 47' and 'UI 111' (a 'Pinto' type) had been identified in earlier work as HT sensitive via conductivity, while all others had been identified as HT tolerant (unpublished data). Plants were spaced 0.1 m apart in 1.5-m-long unreplicated, single-row plots with 0.75 m between rows. Plants were evaluated the morning following a 33/27C day/night, which had been preceded by several diurnal cycles of cooler temperatures (30/25C day/night). In a protocol similar to that of the first experiment, leaf samples were collected for EER determination and prepared for incubation before transit to the laboratory. The transit time constituted their 2-hr incubation. Ten samples, each from a single plant, were taken from each plot. Although the bean genotypes were not replicated in the field planting, the samples were regarded as replications due to the small area of field and uniform environment involved.

A 4-fold range in mean EER (0.62 to 2.52 $\mu\text{L g}^{-1}\text{hr}^{-1}$) was found among the field-grown entries, and for most of the entries, the standard deviation was less than one-half of the mean (Table 4). The two previously identified HT-sensitive entries, 'BBL 47' and 'UI 111', showed the greatest EER among the 11 entries. Four of nine HT-tolerant entries, as determined by conductivity, had considerably lower EER than 'BBL 47' or 'UI 111', further suggesting that a relationship may exist between EER and heat tolerance. Results for nine of the 11 entries were compared to data obtained by Li et al. (1990) via electrolyte conductivity (Table 4). Both methods identified the HT-sensitive material and are correlated, having a Pearson correlation coefficient of -0.70 ($P \geq 0.05$). A weaker relationship was observed for comparisons within the HT-tolerant entries.

These results suggest that substantial variation exists for EER and that lower EER should be transferable from a donor parent by backcrossing. Evidence in the literature for association between higher EER and flower and flower bud abscission in response to heat stress is increasing. The screening of plants at an early vegetative stage for EER as a response to heat stress could be useful in breeding programs.

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Colchicine-induced Polyploidy in *Eustoma grandiflorum*

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Abstract. Tetraploid forms of lisianthus [*Eustoma grandiflorum* (Grisebach) Schinners] were produced by applying 0.5% (w/v) aqueous colchicine to seedling apices. The tetraploids had stronger stems and reduced plant stature, which provided better support to flowers than diploids.

Eustoma grandiflorum is native to the midwestern and southern prairies of the United States. Native plants have blue-purple flowers that are similar in form to the common tulip (Halevy and Kofranek, 1984; Roh and Lawson, 1984). Several Japanese seed companies have introduced commercial cultivars that have an extended color range of blue-purple, white, pink, and rose. In addition to new colors, these new cultivars have fuller flowers and improved growth characteristics

compared to the native plant. Lisianthus can be grown as pot plants and for cut flowers (Halevy and Kofranek, 1984; Roh and Lawson, 1984).

Many commercial cultivars have weak stems that cannot support the flowers. Therefore, we attempted to produce polyploids by colchicine treatment to develop plants with stronger stems and larger and fuller flowers.

Small, 2- to 3-cm-tall seedlings of the following cultivars were treated with colchicine: 'Blue Poppy' (Sahin Co., Alphen, Netherlands), 'Yodel Pink' (Sakata Seed Co., Morganhill, Calif.), four different USDA basally branched somaclonal variants (Griesbach and Semenik, 1987), 'Blue Poppy' x somaclonal variant, and 'Yodel Pink' x somaclonal variant. Treatments consisted of placing 1 drop of 0.05% aqueous colchicine on the apical meristem daily for 0, 3, or 5

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Fig. 1. Comparison of the diploid and tetraploid *Eustoma* 'Blue Poppy'. (A) Tetraploid plant; (B) diploid plant; (C) meiotic metaphase I of diploid ($1n = 1x = 18$); (D) meiotic metaphase I of tetraploid ($1n = 2x = 36$).

Table 1. Phenotypic characteristics of diploid and tetraploid *Eustoma grandiflorum* 'Blue Poppy'.

Characteristic	Type of plant ^a	
	Diploid	Tetraploid
Pollen diameter (μm)	34.8 ± 1.2	49.8 ± 4.3
Stomata diameter (μm)	31.0 ± 2.4	45.5 ± 6.1
Stem diameter (cm)	0.80 ± 0.20	1.20 ± 0.20
Leaf width (cm)	4.08 ± 0.90	6.96 ± 1.22
Petal width (cm)	5.20 ± 2.41	5.52 ± 2.82

^aMeasurements are reported as the mean of samples taken from 50 sister seedlings. Five samples were taken from each seedling. Values are reported as the mean $\pm$ SD $\times$ the *t* value at the 95% level of probability.

days. After the final treatment, the plants were washed free of any residual colchicine. There were nine plants per treatment.

Chromosomes were counted from young flower buds fixed at 23°C for 3 hr in acetic alcohol (1 part glacial acetic acid : 3 parts absolute ethanol) and stored in 70% ethanol at 5°C. Flower buds between 5 to 6 mm long contained meiotic figures. The anthers were removed and squashed in propiocarmine (1% carmine in 45% propionic acid). More than 200 intact, meiotic-metaphase I cells were counted.

The lower leaf epidermis was peeled and mounted in water and 100 stomata were measured at $\times 400$. Upon anthesis, 100 pollen grains were measured at $\times 100$.

Because Roh and Lawson (1984) reported

that high temperatures and low water can inhibit the growth and elongation of the central apical meristem, our plants were grown under nonstress, greenhouse conditions. We maintained the plants in a greenhouse below 30°C and above 20°C under natural daylengths and irradiance from September through November. Plants were potted in a mixture of 3 peat : 1 sand : 1 perlite (by volume) and never allowed to dry out.

After the 5-day colchicine treatment, apical meristems on all the treated plants ceased growth, and the lateral buds below the central, apical meristem initiated growth. When these lateral shoots were removed, the central meristem resumed growth in only three of the 72 plants within the 5-day treatment. One of these plants developed a tetraploid

shoot. In the 3-day treatment, the central apical meristem of all the treatment plants was not inhibited by the colchicine and in every case developed into a normal diploid shoot.

Of 216 treated plants, only one tetraploid was identified. This plant resulted from the 5-day treatment of 'Blue Poppy'.

Rork (1949) reported the chromosome number of sectioned root tips of a single plant of *E. grandiflorum* to be $2n = 2x = 72$. The chromosomes are very small ($1\mu\text{m}$ long) and sticky. We had considerable difficulty in obtaining an accurate count through root tip squashes and had to rely on meiotic metaphase I and II for accurate counts. In our study, meiotic counts clearly show the chromosome number to be $1n = 1x = 18$ or $2n = 2x = 36$ (Fig. 1C).

The tetraploid plant and the diploid parent were self-pollinated and a population of 50 seedlings each was grown. All the seedlings from the tetraploid parent were polyploid as determined by meiotic chromosome count. Table 1 and Fig. 1 describe and show the differences between the seedlings of the diploid and tetraploid 'Blue Poppy'.

The tetraploid form of 'Blue Poppy' had thicker stems than the diploid form. In addition, the tetraploid was shorter in stature. Because of these characteristics, the tetraploid plants were able to support the flowers

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 $\approx 40\%$ and seed set $\approx 35\%$ 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 $\approx 57\%$ 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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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 ($\text{mg}\cdot\text{liter}^{-1}$) 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)-1H-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 $\text{mg}\cdot\text{liter}^{-1}$) 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 $\text{mg}\cdot\text{liter}^{-1}$. 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 25°C with a 16-hr day length supplemented with $20 \mu\text{mol}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ 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 ($\text{mg}\cdot\text{liter}^{-1}$) 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 ($\text{mg}\cdot\text{liter}^{-1}$) 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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