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Development of Somatic Hybridization Systems Involving Cytoplasmic Traits

S. Izhar, K.C. Sink, R. Frankel, J. Beckmann

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C. Abstract

The goals of this projects were to improve cell culture systems in Solanaceae crop species for further somatic hybridization.

High plating efficiency of tomato protoplasts was reported by (Sink and Niedz, 1983) who also decribed the neccessary conditions for it. A simplified method for enucleation and obtainment of cytoplasts was described (Lesney et al 1983) and of evacuolation of protoplasts (Griesbach and Sink 1983) to obtain approximatly 100% viable protoplasts of Petunia.

High mitotic index reported (Guri et al) in Petunia cell suspension cultures by the sequential treatment with aphidicolin and colchicine.

(Griesbach et al, 1983) describe the induction of chromosome instability by the use of griseofulvin. The instability was expressed as loss of chromosomes as compared to the control. This experiment was carried out with somatic hybrid plants. Variegation in the chlorophyl was observed due perhaps to position effect.

The genus Capsicum which was found difficult to handle in cell and tissue culture showed species variation in regeneration shoots from calli. We recently were able to show development of callus from protoplasts and signs of shoot regeneration but this need further work (Neuman et al).

D. Body of report:

Following are some papers, proceeding of lectures and preprints in which the work done under this project is summerized.

Induction of high mitotic index in Petunia suspension cultures
by sequential treatment with aphidicolin and colchicine

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Abstract

Significantly higher than normal mitotic index (MI) values were induced in Petunia cell suspensions, following treatments with colchicine, aphidicolin, drastic medium replacement, or a sequential application of aphidicolin and colchicine. This last treatment yielded the highest MI values: cells incubated with 30 µg/ml aphidicolin for 18h, then cultured in drug-free medium for 8h and finally exposed to 0.1% colchicine for 8 additional hours exhibited MI of 52.8% and 65.7% respectively, for the two cell lines in study.

Abbreviations: MI, mitotic index; 2,4-D, 2,4-dichlorophenoxyacetic acid

INTRODUCTION

Recently Sala et al. (1982) reported that the antibiotic aphidicolin inhibits α -like DNA polymerase in tobacco. As a result of applying aphidicolin, cell division is stopped at the G1/S border (Pedrali-Noy et al. 1980) and when the drug is removed the cells start a synchronous wave of

Contribution from the Agricultural Research Organization, The Volcani Center, Bet Dagan 50-250, Israel. No. 1028-E, 1984 series.

DNA synthesis. Nagata et al. (1982) and Galli & Sala (1983) obtained high MI values in cell suspension cultures of tobacco and root-tip meristems of Haplopappus gracilis, respectively, following a transient exposure to aphidicolin.

In the present study we describe the effects of colchicine and aphidicolin and of their sequential application, on the MI exhibited by Petunia cell suspensions, and suggest a protocol which enables to generate cell populations with a very high proportion of cells in mitosis.

MATERIALS AND METHODS

Cell suspensions

Cell lines of Petunia hybrida (Hook) Vilm line 3704 and F₁ hybrid of P. parodii L.S.M. x P. hybrida line 4221 (Izhar et al., 1983) were grown as suspension cultures in MS medium (Murashige and Skoog, 1962) supplemented with 3% sucrose and 1 mg/l 2,4-D, unless stated otherwise. The cultures were maintained by regular subcultures at 4-day intervals.

Synchronization treatments

(i) Colchicine treatment: On the second day post-subculture, log-phase cells were incubated with various concentrations of colchicine (Sigma) for 8h. This treatment duration had been found to be optimal in preliminary experiments.

(ii) Sequential medium replacement and colchicine treatment: Log phase cells were centrifuged for 10 min and then resuspended in fresh medium. After 14 h 0.1% colchicine was added to the medium and cells were cultured for 8 additional hours.

(iii) Sequential aphidicolin and colchicine treatment: Log phase cells were incubated with aphidicolin (kindly provided by Imperial Chemical Industries, UK) for 18 h. The cells were centrifuged and washed with fresh medium four times and then cultured in a 1:1 mixture of conditioned medium and fresh medium. After 8h 0.1% colchicine was added and incubation continued for 8 additional hours.

Cell fixation, staining and MI evaluation: Following the various synchronization treatments, cells were immediately fixed in 3:1 ethanol:acetic acid. Feulgen staining was done according to Evans & Reed (1981). MI was determined by screening 4,500 cells (three replications of 1,500 cells each).

RESULTS AND DISCUSSION

Preliminary experiments showed that log-phase cell suspensions maintained in low auxin concentrations (0.1 mg/l 2,4-D), exhibited higher MI value (4.4%) than cells cultured at higher (0.5 or 1 mg/l) auxin levels (1.8%). However, low auxin cultures required almost daily transfers and contained large cell aggregates, thus hampering fixation and staining. Additionally, when low and high auxin cultures were subjected to synchronization treatments, the difference in the MI levels reached by both types was negligible. Therefore, a 2,4-D level of 1 mg/ml was adopted in all subsequent experiments.

Application of colchicine increased significantly the MI values as compared to controls (Table 1). However, at drug levels of 0.2% and above, a decline in MI values occurred. Subsequently, we attempted to improve synchronization by medium replacement followed by colchicine application. This sequential treatment yielded 30.2% MI for line 3704 and 27.5% MI for line 4221. These MI values were significantly higher than

those obtained previously following colchicine treatment (Table 1). On the other hand, if medium was replaced as described and cells were fixed before addition of colchicine, MI values of 11.4% and 9.8% were observed for lines 3704 and 4221, respectively.

The combined effects of colchicine (0.1%) and several aphidicolin concentrations on the MI of cell suspensions are shown in Table 2. The highest MI values were obtained by sequential treatments with 30 µg/ml aphidicolin and colchicine. Application of aphidicolin (5, 15, 30 µg/ml) under the same conditions but without subsequent colchicine treatment yielded MI values of 19.2%, 24.6% and 27.9% respectively, for line 3704 and of 20.3%, 26.3% and 31.5%, respectively for line 4221. The various synchronization protocols and the highest MI values achieved in every case are summarized in figure 1.

The high MI values obtained in our studies are similar to those described by Nagata et al. (1982) for tobacco cell suspensions treated with 5 µg/ml aphidicolin.

Undoubtedly this technique can contribute to the isolation of metaphase chromosomes in high yields, thus facilitating studies of chromosome-mediated gene transfer (Szabados et al. 1981; Griesbach et al. 1982, Mathews, 1983).

Acknowledgments

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Table 1. The effect of colchicine on the mitotic index (%) ^{*} of Petunia suspension cultures

Petunia lines	Colchicine concentration (%)									
	0	0.025	0.05	0.10	0.15	0.20	0.30	0.40	0.50	
3704	1.83 e	11.06 cd	17.92 ab	18.93 a	17.84 ab	12.22 c	8.73 d	2.71 e	0.63 e	
4221	1.80 e	12.11 bc	15.03 bc	19.04 a	20.16 a	11.86 cd	8.63 d	2.45 e	0.71 e	

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* Each value is an average of three replications

a-e Mean separation by Duncan's Multiple Range Test, at $\bar{P} = 0.05$ ($Sx = 0.98$).

Table 2. Effect of aphidicolin on the mitotic index(%) of Petunia suspension cultures, pretreated with colchicine (0.1%)

Petunia		Aphidicolin (ug/ml)									
lines	0	5	10	15	20	30	40	50	60	70	100
3704	19.18 fg	25.62 e	33.14 d	56.07 b	54.23 b	62.80 a	54.06 b	44.10 c	14.21 g	3.66 h	0.32 h
4221	20.38 ef	30.10 de	33.00 d	52.16 b	53.42 b	65.74 a	52.33 b	42.03 c	17.11 fd	13.06 h	0.20 h

* Each value is an average of three replications

a-h Mean separation by Duncan's Multiple Range Rest, at $P = 0.05$ ($S\bar{x} = 1.97$)

Figure 1. Schematic demonstration of the synchronization treatments.
COL, addition of colchicine; MR, medium replacement;
APH, addition of aphidicolin.

Induction of callus development and shoot regeneration in explants of three
Capsicum species*

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Abstract

The in vitro morphogenetic ability of three pepper species (Capsicum spp.) was studied. Hypocotyl or cotyledon explants obtained from 20 day-old axenic seedlings were exposed to various growth regulators and light regimes, in order to optimize callus formation and plant regeneration.

Two cultivars of C. annuum (cv. California Wonder, cv. Maor) produced abundant tissue of high friability, when exposed to 0.1-1 mg/l of 2,4-D, while callus with lower friability was formed in the presence of other growth regulators. Plantlet regeneration was efficient in media containing BAP+IAA; no such response was observed if NAA replaced the IAA.

C. pendulum was quite refractory to callus induction, unless explants were incubated in media containing a high auxin concentration (2 mg/l 2,4-D) for prolonged periods. On the other hand, this species exhibited the highest plant regeneration capabilities among all tested genotypes.

The callus formation ability of C. chinense was intermediate between the two other species. No plantlet regeneration was observed in C. chinense explants in any of the tested incubation treatments.

No conditions can be defined yet for reliable shoot regeneration in established callus cultures of the three Capsicum species studied.

Key words: Capsicum, callus induction, shoot regeneration, growth regulators.

① Introduction

The availability of efficient in vitro plantlet regeneration is an important prerequisite for the application of modern technologies to the genetic manipulation and rapid propagation of crop species [6, 7]. Pepper (Capsicum spp.), a major target for such studies in our laboratory, is an important solanaceous crop for which only limited data were reported in the literature on those aspects. The modulation of shoot, root and callus morphogenesis in Capsicum explants by the presence of several growth regulators was reported [2], and the effect of growth regulators and seedling age, as well as the position of explants in source-seedlings, on the morphogenetic response of Capsicum hypocotyl segments, was assessed [1]. Additionally, it was reported that Capsicum mesophyll protoplasts underwent sustained cell division and regenerated flowering plants [4].

We report here our results of an in-depth study on the induction of callus development and shoot regeneration in three different Capsicum species (C. annuum, C. chinense and C. pendulum), as well as our attempts to regenerate shoots from established callus cultures of these species.

1

Materials and Methods

2

Plant material

The Capsicum species included in this work were: C. annuum (cv. California Wonder, cv. Maor), C. pendulum and C. chinense. Seeds were kindly provided by Dr. Chen Shifriss (ARO).

2

Growth of seedlings and culture of explants

Seeds were surface-sterilized for 20 minutes with a 20% solution of commercial bleach, rinsed thoroughly, and placed for germination in Murashige & Skoog (MS) medium [3] supplemented with 3% sucrose and 0.9% agar (Sigma). Seedlings were grown in a culture room at 26°C and 16 h photo-period, with a light intensity of 2000 lux. Twenty-day-old seedlings were used as a source for root, cotyledon and hypocotyl explants, while the apex was discarded. Unless stated otherwise, explants were cultured under the above mentioned environmental conditions, in 200 ml capacity screw-cap jars containing 25-30 ml of MS medium, supplemented with 3% sucrose, 0.9% agar and appropriate growth regulators.

Treatments were assayed in duplicate jars, containing five identical explants each, or as stated otherwise.

1

Results and Discussion

Preliminary observations on in vitro behaviour of different explants indicated that, except for occasional development of limited amounts of callus tissue, roots had a substantially lower morphogenetic potential than other explant types (results not shown). Therefore, the present study was

concentrated on the response exhibited by hypocotyl and cotyledon explants when cultured in the presence of several growth regulators under different environmental conditions. An overall view of the observed morphogenetic responses of the four genotypes included in this work, is summarized in Table 1. C. pendulum exhibited the greatest shoot regeneration ability. Shoots were also generated by both C. annuum cultivars, although at a narrower range of the tested growth regulators. On the other hand, C. chinense did not form any shoot under any of the assayed conditions. In most cases, no substantial differences were found between the morphogenetic response of cotyledon and hypocotyl explants. Light conditions affected in several instances the degree of callus development and its color.

Since we were interested in producing large masses of callus tissue for further experiments, additional combinations of growth regulators were applied to primary explants in order to increase callus formation. The results obtained are described in Table 2. C. annuum cv. California Wonder and C. chinense were most responsive. The highest degree of callus induction was achieved in both cases in media containing 2,4-dichlorophenoxyacetic acid (2,4-D) as the only growth regulator. On the other hand, when induction of green callus was preferred (so as to be used in subsequent shoot regeneration experiments), the best response was achieved in C. annuum by using cytokinin-containing media, such as 6-benzylaminopurine (BAP) or zeatin, while a combination of a cytokinin and an auxin was required in C. chinense in order to elicit a comparable response. In all the tested genotypes, the induced callus tissue exhibited generally intermediate friability, except for explants grown in media containing 2,4-D as the only growth regulator, which generated large amounts of white and highly friable callus.

Explants from the species C. pendulum were extremely refractory to the inductive conditions described in Table 2; only limited amounts of callus tissue were detected in some of the explants. Therefore, combinations of a cytokinin (zeatin) and an auxin (2,4-D) were tested further at several concentrations, and the incubation period was prolonged, in order to define conditions under which manageable callus amount could be generated

able 3 (Table 3). No substantial differences were detected between the response exhibited by hypocotyl and cotyledon explants. Higher concentrations of auxin stimulated markedly the development of callus tissue, while zeatin concentrations higher than 1 mg/l seemed to reduce the amount of callus formed by the cultured explants. Callus tissue generated in relatively lower auxin concentrations (up to 1 mg/l) was greenish, while that formed at higher concentrations was white. The overall friability of C. pendulum calli was much lower than that of other species included in this study. The data in Table 3 suggest that, if incubation time is prolonged and medium composition is adjusted carefully, even recalcitrant species like C. pendulum can be induced to produce abundant callus tissue for further experiments. In recent experiments, further improvement in the amount and friability of callus produced by this species was obtained by the use of our "culture medium No. 9", which is similar to the "Callus and Cell Culture Medium" used for tobacco [5], but is supplemented with 0.25 mg/l kinetin, 1.5 mg/l 2,4-D, and 0.5 mg/l naphthaleneacetic acid (NAA), instead of the originally formulated growth regulators (results not shown).

Fig 1 In order to optimize the shoot regeneration response of Capsicum spp. different combinations of growth regulators at several levels were tested further. For C. pendulum cotyledons, the use of high concentrations of BAP (5 mg/l) combined with different levels of NAA, seems particularly

efficient (Fig. 1), while a weaker response was obtained when hypocotyl segments were subjected to similar conditions. When indoleacetic acid (IAA) was employed as the auxin (Fig. 1, C and D), no dramatic differences were observed between both explant types. At high NAA/BAP ratios, shoot formation was severely reduced, while no such effect was observed with IAA. Additionally, we observed that shoots formed in the presence of IAA grew faster than with NAA (data not shown).

Explants from C. annuum were subjected to similar experimental conditions, except that a wider range of growth-regulator concentrations was utilized. No shoot formation was observed when NAA was utilized in the regeneration medium, except for a sporadic response to extremely high (15 mg/l) BAP concentrations were also included (results not shown). This study confirms the previously reported lack of response to NAA+BAP by C. annuum explants [2].

fig 2
On the other hand, IAA supplemented in combination with BAP elicited a high number of shoots (Fig. 2). In most cases, BAP concentrations higher than 5 mg/l did not further enhance the shooting response of explants. Cotyledons seem to respond somewhat better than hypocotyls, based on the observed number of regenerated shoots.

Shoot regeneration was also attempted in serially subcultured calli from the three species under study. For this purpose, complex mixtures of IAA with several cytokinins (kinetin, zeatin, zeatin-riboside, BAP, BAP-riboside, 6-dimethylallylaminopurine, 6-benzyl-9-tetrahydropyran-adenine), gibberellic acid, and coconut milk were evaluated. No conditions were yet found which permit the reliable obtention of shoots in subcultured callus tissue.

In conclusion, this study enabled us to define appropriate conditions for induction of callus growth and shoot differentiation in several Capsicum genotypes. The data gathered will contribute to the design of future experiments on the in vitro genetic manipulation of this crop.

1) Literature

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LEGENDS

Fig. 1.

Effect of growth regulators on degree of shoot differentiation in
Capsicum pendulum explants

Explants were obtained and cultured as described in Table 1. Cotyledon (A,C) or hypocotyl (B,D) cultures were supplemented with BAP at the indicated concentrations plus NAA (A,B) or IAA (C,D) at 0.1 (○), 0.5 (△), 1 (□) or 2 (☆) mg/l. The number of adventitious shoots was visually determined, with the aid of a magnifying lens following 30 days of incubation.

Fig. 2

Effect of growth regulators on degree of shoot differentiation in explants
of Capsicum annuum cv. California Wonder

Explants were obtained and cultured as described in Table 1. Cotyledon (A) or hypocotyl (B) cultures were supplemented with BAP at the indicated concentrations plus IAA at 0.5 (○), 1 (△), 2 (□) or 4 (☆) mg/l. For further details see fig. 1.

Table 1

Hypocotyl (Hyp.) or cotyledon (Cot.) explants from 20-day-old axenically grown seedlings of Capsicum annuum cv. California Wonder (AN-CW), C. annuum cv. Maor (AN-M), C. pendulum (PEND) and C. chinense (CHIN) were cultured in Murashige and Skoog's medium [3], supplemented with 3% sucrose, 0.9% agar and the indicated growth regulators. Every treatment consisted of triplicate bottles containing four explants each. Explants were maintained at 26°C, either at a 16 h photoperiod or in complete darkness.

Morphogenetic responses were scored following 30 days of incubation (C = callus development, Sh = multiple shoot regeneration, R = root formation, Sh* = single shoot-regeneration on explant, N.G. = no growth was detected). The degree of callus development (1 = slight, 2 = intermediate, 3 = profuse) and its color (g = green, y = yellowish, w = white) were visually estimated.

Table 2

Explants were obtained and cultured as described in Table 1, except that all experiments were performed under 16 h photoperiod conditions, and that every treatment consisted of duplicate bottles containing five explants each. For further details, see Table 1.

Table 3

Explants were obtained and cultured as described in Table 1, except that all bottles were incubated for 54 days under 16 h photoperiod conditions. For further details, see Table 1.

Table I. Morphogenetic responses of Capsicum spp. explants to various growth regulators.

a. 16 hours photoperiod

growth regulators (mg/l)	organ	An-CW	AN-M	PEND	CHIN
kinetin(1)	Hyp. Cot.	C. (2,g)+R C. (1,y)	C. (1,g) C. (1,g)	Sh* N.G.	C. (1,g) N.G.
2,4-D(1)	Hyp. Cot.	C. (3,w) C. (3,w)	C. (3,w) C. (2,w)	C. (1,w) C. (1,w)	C. (2,w) C. (1,w)
BAP (1)	Hyp. Cot.	C. (2,g) C. (2,g)	C. (3,w) C. (3,g)	Sh* C. (0-1,g)	C. (1,g) C. (1,g)
BAP (2)	Hyp. Cot.	C. (2,g) C. (2,g)	C. (3,g) C. (2,g)	C. (2,g)+Sh. C. (2,g)+Sh.	C. (1,g) C. (1,g)
BAP(2)+ IAA(0.5)	Hyp. Cot.	C. (2,g)+Sh. C. (2,g)+Sh.	C. (2,g)+Sh. C. (2,g)+Sh.	C. (2,g)+Sh. C. (2,g)+Sh.	C. (2,g) C. (2,g)
BAP(2)+ IAA(1)	Hyp. Cot.	C. (2,g)+Sh. C. (2,g)+Sh.	C. (2,g)+Sh. C. (2,g)+Sh.	C. (2,g)+Sh. C. (2,g)+Sh.	C. (2,g) C. (2,g)

b. dark incubation

Kinetin	Hyp. Cot.	C. (1,y)+R C. (1,w)	C. (1,g) C. (1,y)	C. (1,g)+Sh* N.G.	C. (1,g) C. (1,w)
2,4-D(1)	Hyp. Cot.	C. (2,w) C. (2,w)	C. (2,w) C. (2,w)	C. (1,w) C. (1,w)	C. (1,g) C. (1,w)
BAP (1)	Hyp. Cot.	C. (2,y) C. (2,y)	C. (2,y)+R C. (2,y)+R.	Sh* C. (1,g)	C. (1,g) C. (1,g)
BAP(2)	Hyp. Cot.	C. (2,y) C. (2,y)	C. (2,y) C. (2,y)	C. (2,g)+Sh. C. (2,g)+Sh.	C. (2,g) C. (1,g)
BAP(2)+ IAA(0.5)	Hyp. Cot.	C. (2,g)+Sh. C. (2,g)+Sh.	C. (2,g)+Sh. C. (2,g)+Sh.	C. (2,g)+Sh. C. (2,g)+Sh.	C. (2,g) C. (2,g)
BAP(2)+ IAA(1)	Hyp. Cot.	C. (2,g)+Sh. C. (2,g)+Sh.	C. (2,g)+Sh. C. (1,g)+Sh.	C. (2,g)+Sh. C. (2,g)+Sh.	C. (2,g) C. (2,g)

Table 2. Induction of callus growth in Capsicum spp. explants

Growth regulators (mg/l)	Organ	AN-CW			PEND			CHIN		
		color	growth	fria-bility	color	growth	fria-bility	color	growth	fria-bility
2,4-D(0.1)	Hyp.	white	3	high	white	0-1	high	white	3	high
	Cot.	white	3	high	white	0-1	medium	white	3	high
Zeatin(1)	Hyp.	green	2	medium		0		green	1	medium
	Cot.	green	3	medium		0		green	1	medium
Zeatin(1)+ 2,4-D(0.1)	Hyp.	green	2	medium	pale green	0-1	medium	green	2	medium
	Cot.	green	2	medium	pale green	0-1	medium	green	2	medium
BAP(1)	Hyp.	green	2	medium		0		green	1	medium
	Cot.	green	2	medium		0		green	1	medium
BAP(1)+ 2,4-D(0.1)	Hyp.	green	2	medium	green	0-1	medium	green	2	medium
	Cot.	green	2	medium	white	1	medium	green	2	medium

Table 3. Effect of 2,4-D and zeatin concentrations on degree of callus development in Capsicum pendulum explants.

2,4-D (mg/l)	Organ	Zeatin (mg/l)		
		1	2	5
0.1	Hyp.	1	1	1
	Cot.	1	1	1
0.2	Hyp.	1	1	1
	Cot.	1	1	1
0.5	Hyp.	2	1	1
	Cot.	2	1	1
1	Hyp	2	1	1
	Cot.	2	1	1
2	Hyp.	3	2	2
	Cot.	3	3	2

Figure 1

Figure 2

E. Description of co-operation

As mentioned in progress reports 1 & 2, the co-operating institutions continued to collaborate by exchanging valuable information and genetic stocks of Petunia and Lycopersicon. More recently, S. Izhar has provided a cytoplasmic controlled chlorophyll-deficient line of P. hybrida (CMS line 7208) which will be very valuable in CMS transfer studies, heterokaryon identification and somatic hybridization experiments. In addition, S. Izhar has provided the cell suspension culture protocol for Petunia parodii CMS which has enabled K. Sink to immediately have this genotype available as a protoplasts system for the enucleation study. The principal and co-operating investigators met November 1981 and in July, 1982 to discuss different aspects of protoplasts isolation, fusion, selection and regeneration and to plan joint experiment to be executed both in MSU and ARO. K.C. Sink, visited ARO during the January 1984 to further discuss experiments and to teach the protocol of enucleation and handling tomato protoplasts.

A continuation of this BARD project will be proposed during 1984.

F. Benefit to Agriculture

The basic information about efficient growth of tomato protoplasts culture as well as of other Solanaceae crop species together with the regeneration of shoots from different cultures improve the chance to obtain efficient protoplast to plant systems in several species. The enucleation study will contribute much toward future transfer and exchange of plasmons between Solanaceae crop species.

G. List of Publications

1. Sink, K.C. and R.P. Niedz (1982). Factors controlling plating efficiency in tomato. Proc. 5th Intl. Cong. Plant Tissue & Cell Culture.
2. Lesney, M.S., P.W. Callow and K.C. Sink (1983), simplified method for bulk production of cytoplasts from suspension culture derived protoplasts of Solanum nigrum (L). Protoplast 1983, Poster Proc. Eds Potrykus/et al, Basel, Birkhauser pp:116-117.
3. Griesbach, R.J. and K.C. Sink (1983). Evacuolation of mesophyll protoplasts. Plant Sci. Lett. 30:297-301.
4. Griesbach, R.J., L.S. Schnabelrauch and K.C. Sink (1983). Griseofulvin - induced chromosome instability and reduction in a Petunia somatic hybrid. Jour. Amer. Soc. Hort. Sci. 108(5): 714-716.
5. Passiatore, I.E. and K.C. Sink (1981). Plant regeneration from leaf mesophyll protoplasts of selected ornamental Nicotiana tobacco species. Jour. Amer. Hort. Sci. 106 (6): 799-803.
6. Guri A., A. Zelcer and S. Izhar. Induction of high mitotic index in Petunia suspension culture by sequential treatments with aphidicolin and colchicine. Submitted to Plant Cell Reports.
7. Neumann, S., A. Zelcer and S. Izhar. Induction of callus development and shoot regeneration in explants of three Capsicum species.
(Submitted) Plant Cell Tissue and Organ Culture.
8. Zelcer, A., Ofra Soferman and S. Izhar (1983) Shoot regeneration in root cultures of several Solanaceae species. Pl. Cell Reports 2:252-254.

9. Schnabelrauch, L.S., F. Kloc-Bauchan and K.C. Sink, 1984. Expression of nuclear-cytoplasmic genome incompatibility in interspecific Petunia somatic hybrids. (In preparation) Theo. App. Gen.
10. Schnabelrauch, L.S., F. Kloc-Bauchan and K.C. Sink. 1984. Genetics of stable and unstable traits in Petunia somatic hybrids. (In preparation) Genetics.
11. Niedz, R.P., S.M. Rutter, L.W. Handley and K.C. Sink. 1984. Plant regeneration from leaf protoplasts of four tomato cultivars. (In preparation) Plant Sci. Lett.
12. Izhar, S. and A. Zelcer (1984). Cell tissue and organ culture in Petunia Springer Verlag (in press).