THE DIFFERENCES BETWEEN PRODUCTS OF GENE EXPRESSION IN MALE, FEMALE AND HERMAPHRODITE CUCUMBER FLORAL BUDS (*CUCUMIS SATIVUS* L.)

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INTRODUCTION

Cucumber is a species in which sex expression has been extensively studied. Sexual differentiation is controlled by genotypic and environmental factors. The main genes responsible for sex determination have been described but the mechanism of their action remains unexplained [1]. In this study we attempted to find cDNA clones which can be connected with sex differentiation and flower development in cucumber.

RESULTS

Two pairs of nearly isogenic lines: GY-3 (gynoecious – *FFMMGG*) and HGY-3 (hermaphrodite - *FFmmGG*), B10 (monoecious - *ffMMGG*) and 2gg (gynoecious - *FFMMgg*) were used to search for differences in gene expression in young (1 – 2mm) cucumber floral buds.
In order to obtain differentially expressed cDNA clones the differential screening and the differential subtraction chain (DSC) [2] methods were used. Altogether above 900 cDNA clones were isolated and part of them were randomly chosen and sequenced (tab. 1 and 2).
To observe the expression patterns of isolated cDNA clones in developing flowers at different developmental stages, we performed *in situ* RT-PCR [3]. Here we present the results for two cDNA clones designed as 216GY3 and 35GY3.

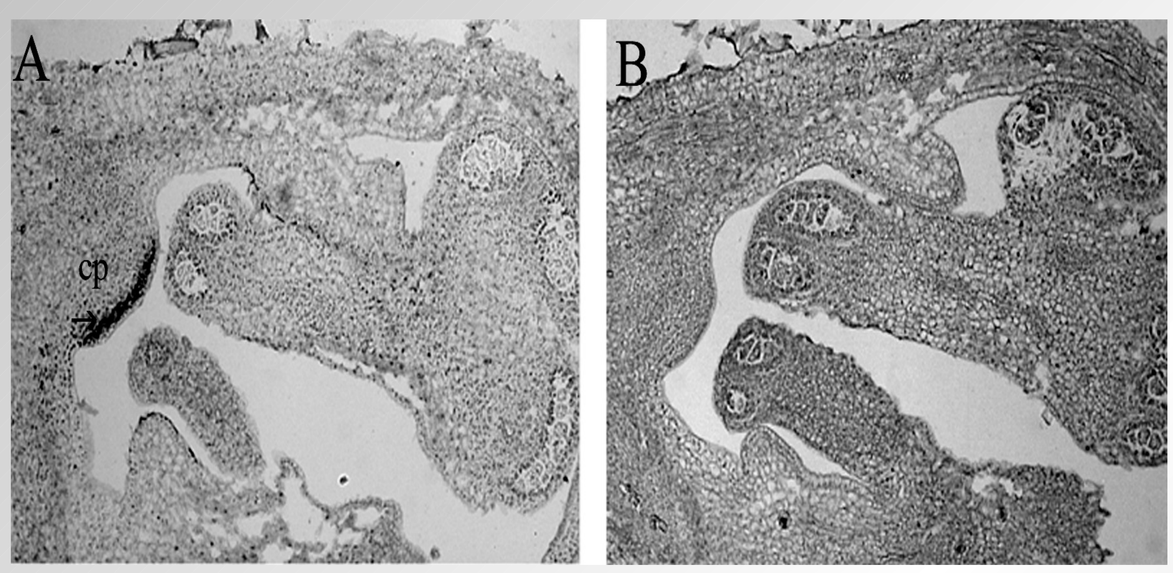

Fig1 *In situ RT-PCR* of 216GY3 clone analyzed in cross-sectioned 2 mm male buds of *Cucumis sativus* L.A. - reaction with specific 216GY3 primers; localization signals of transcripts in carpel primordium (cp); B. - control reaction without specific 216GY3 primers in the RT-PCR mix.
The pattern of weak expression for clone 216GY3 in 1-2 mm buds of the GY3 line is visible in pistil primordia, stamens primordia, corolla sepals. In older buds the signal of clone 216GY3 was located in stamen primordia, ovules and corolla sepals. In HGY3 buds transcripts were distributed in pistil, corolla sepals, anther sacs. In 2 mm male flower buds of the B10 line a specific expression in the pistil primordium was noted. In older buds no signals were observed. In contrast female flowers line B10 and 2gg showed expression in larger buds. The signals were observed in stamen primordia, ovules, corolla. The accumulation of large amounts of the transcripts of this clone in primordia of male flower pistils which will thus not develop is interesting.

Clone	ACC. No[a]	Description[b]	E-Value[c]	ACC. No[d]	Length compared
11/B10	BU791027	No significant similarity found			70
21/B10	BU791028	expressed protein, *A. thaliana*	7e-32	NP563718	446
39/B10	BU791029	testa pericarp cDNA clone, *Hordeum vulgare*	0.024	BG416265	125
45/B10	BU791030	putative glycine and proline rich protein, *SP stapfianus*	2e-06	CAB61840	480
49/B10	BU791031	No significant similarity found			60
64/B10	BU791032	No significant similarity found			86
73/B10	BU791033	expressed protein, *A. thaliana*	4e-27	NP565087	569
75/B10	BU791034	putative protein, *A. thaliana*	4e-25	NP567908	650
79/B10	BU791035	Drought medicago cDNA, *Medicago trunculata*	e-118	BF636273	650
81/B10	BU791036	nucleolar protein, *Cicer arietinum*	3e-53	CAA10127	480
83/B10	BU791037	Hypothetical protein, *A. thaliana*	6.6	TO4562	217
84/B10	BU791038	Irradiated *Medicago trunculata* cDNA , *M. trunculata*	1e-71	BI269381	481
85/B10	BU791039	Tomato shoot meristem cDNA, *Lycopersicon esculentum*	8e-36	BG643739	264
94/B10	BU791040	hypotethical protein, *A.Thaliana*	4.3	T51787	97
95/B10	BU791041	Brassinosteroid biosynthetic protein, *Pisum sativum*	2e-37	AF325121	552
97/B10	BU791042	Post infection cDNA clone, *Brugia malayi*	0.33	AA841573	140
100/B10	BU791043	expressed protein, *A. thaliana*	2e-58	NP563718	401
105/B10	BU791044	putative protein, *A. thaliana*	2e-12	NP195958	501
106/B10	BU791045	ebiP7741 cDNA, *Anophelas gambiae*	0.36	EAA00905	389
122/B10	BU791046	putative glycine and proline rich protein, *Sporobolus stapfinus*	4e-06	CAB61840	400
128/B10	BU791047	brain and reproductive organ-expressed protein, *A. thaliana*	2e-23	NP199062	568
133/B10	BU791048	chaperonin beta subunit protein, *Pisum sativum*	1e-28	PO8927	489
138/B10	BU791049	bZIP transcription factor protein, *A.thaliana*	2e-23	CAC40022	467
157/B10	BU791050	chaperonin beta subunit protein, *Pisum sativum*	3e-25	PO8927	588
163/B10	BU791051	hypothetical protein, *A. thaliana*	1e-09	NP 174322	355
166/B10	BU791052	flower bud cDNA clone, *A. thaliana*	0.82	AV534491	199
449/B10	BU791053	hypotethical protein, *A. thaliana*	1e-22	NP180382	600
460B10	BU791054	delta cop protein, *Zea mays*	1e-20	AF216852	297

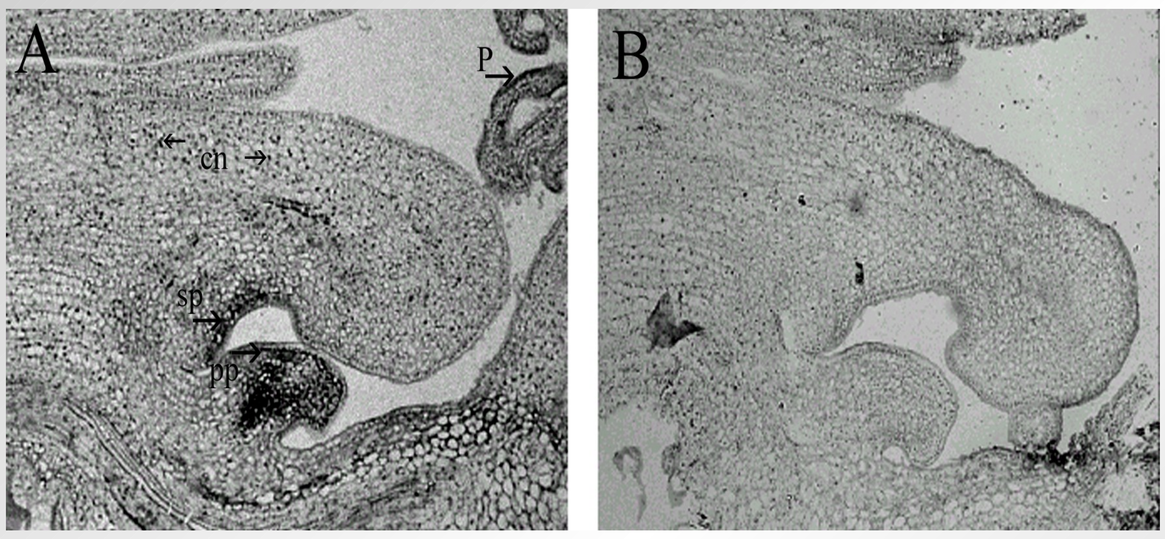

Fig.2 *In situ* RT-PCR of 35GY3 clone analyzed in cross-sectioned 4mm female buds of *Cucumis sativus* L. A. - reaction with specific 35GY3 primers; localization signals of transcripts in stamen primordium (sp), petal primordium (pp), petals (p) and cell nuclei (cn) in the whole bud; B. - control reaction without specific 35GY3 primers in the RT-PCR mix. In 2 mm flower buds of the GY3 line a signal was observed in the layer of ovule. In 4 mm buds a strong signal occurred in stamen primordia, petals and cell nuclei. In buds over 4 mm in size a signal was observed in stamen primordia, ovules and cell nuclei. The patterns of expression in the female buds of line B10 and 2gg were similar. There was no expression in male and hermaphrodite cucumber floral buds. Signals of expression of clone 35GY3 were thus observed only in female cucumber flower buds and were the strongest in a site where as it seems only the development of stamens should be inhibited.

Clone	ACC. No[a]	Description[b]	E-Value	ACC. No[c]	Length compared[d]
15/Gy3	BU791055	Dev./immature green fruit cDNA clone, *L. esculentum*	2e-79	BF051149	390
27/Gy3	BU791056	CC-1690 cDNA clone *C. reinhardtii*	2e-99	BG858504	568
28/GY3	BU791057	Irradieted *Medicago trunculata* cDNA, *M. trunculata*	6e-90	BQ157564	523
34/GY3	BU791058	Irradieted *Medicago trunculata* cDNA, *M. trunculata*	6e-75	BQ155709	402
35/Gy3	BU791059	Hypothetical protein, *A. thaliana*	4e-08	NP192387	482
37/Gy3	BU791060	Drought *Medicago trunculata* MT cDNA, *M. trunculata*	2e-27	BG451667	273
40/Gy3	BU791061	Rice callus cDNA clone AD425, *O. sativa*	4e-07	D43028	162
47/Gy3	BU791062	2-oxoglutarate dehydrogenase protein, *A. thaliana*	6e-34	NP201376	380
77/Gy3	BU791063	MT pSPORT cDNA clone, *Aedas aegypti*	2.3	AI629981	81
79/GY3	BU791064	developing caryopsis cDNA clone, *Hordeum vulgare*	e-104	AL507226	571
80/GY3	BU791065	Irradieted *Medicago trunculata* cDNA, *M. trunculata*	e-104	BQ153011	614
81/GY3	BU791066	Phosphate starved leaf cDNA, *Medicago trunculata*	e-100	BQ158032	585
87/Gy3	BU791067	ADPglucose pyrophosphorylase protein, *E. coli*	6e-87	AAB26162	510
91/GY3	BU791068	TAMU callus cDNA clone, *Lycopersicon esculentum*	4e-91	AW033645	442
97/GY3	BU791069	Irradiated *Medicago trunculata* cDNA, *M. trunculata*	2e-81	BQ156151	413
104/GY3	BU791070	phosphate starf leave cDNA, *Medicago trunculata*	1e-55	BG457817	442
112/GY3	BU791071	Drought *Medicago trunculata* cDNA, *M. trunculata*	1e-73	BQ144018	530
139/Gy3	BU791072	cDNA clone – leaves, *C. japonica*	9e-03	AU036908	365
141/GY3	BU791073	Peppermint glandular trichome, *M. peperita*	2e-25	AW255034	254
145/GY3	BU791074	developing leaf cDNA, *Medicago trunculata*	7e-22	BQ150528	470
148/GY3	BU791075	Drought *Medicago trunculata* cDNA, *M. trunculata*	8e-84	BG451745	505
153/GY3	BU791076	Irradiated *Medicago trunculata* cDNA, *M. trunculata*	e-110	BQ154263	585
154/GY3	BU791077	pheromone gland Shuko x Ryu cDNA clone, *B. mori*	e-118	AV404071	572
190/Gy3	BU791078	Putative protein, *A. thaliana*	2e-33	NP200140	490
192/GY3	BU791079	tassel primordium cDNA clone, *Zea mays*	0.12	BE123402	500
216/Gy3	BU791080	Chaperonin 60 beta chain precursor protein, *S. tuberosum*	1e-07	TO7733	407
218/GY3	BU791081	ATP-binding component of 3rd arginine transport, *E. coli*	7e-74	NP286633	498
222/Gy3	BU791082	putative protein, *A. thaliana*	1e-35	NP194916	500
223/GY3	BU791083	Irradiated *Medicago trunculata* cDNA, *M. trunculata*	1e-77	BQ152215	442
286/GY3	BU791084	Irradiated *Medicago trunculata* cDNA, *M. trunculata*	e-122	BQ 154333	690
305/GY3	BU791085	Putative transferase, *E. coli*	e-105	NP415971	665
315/Gy3	BU791086	Irradiated *Medicago trunculata* cDNA, *M. trunculata*	3e-48	BI269734	570
420/GY3	BU791087	E2 ubiquitin- conjugating enzyme protein, *A. thaliana*	2e-81	NP566563	640

Tab 1 and 2 Sequence similarity of the cDNA clones to the corresponding known genes in the database
[a]Accesion number of cDNA clone
[b]A description of the best data match is given together with data base accession number of homologous genes[d]
[c]Sequence similarity

SUMMARY & CONCLUSIONS

1. **The most of isolated and identified clones (tab.1 and 2) are involved in same part of a light or hormone signaling cascade and they may participate in the "cascade of sex expression" in cucumber.**
2. **Signals of expression of clone 35GY3 were observed only in female cucumber flower buds and were the strongest in a site where as it seems only the development of stamens should be inhibited.**
3. **The accumulation of large amounts of the transcripts of 216 GY3 clone in primordia of male flower pistils which will thus not develop is interesting.**

References

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