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On the front cover: Bitter melon, *Momordica charantia*. From *Flore des serres et des jardins de l'Europe*, 1845 – 1888.

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Research Reports

Cucumber (*Cucumis sativus*)

Cucurbita spp.

In Vitro Culture of Cucumber Microspores

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Introduction

Cucumber (*Cucumis sativus* L.) microspores possess relatively few, large mitochondria and these mitochondria are paternally transmitted to progeny (Havey et al. 2002). Our aim was to develop a protocol for embryogenesis of cucumber microspores as possible targets for mitochondrial transformation. Very little work has been conducted on isolated microspore cultures in *Cucurbitaceae*. Trials on isolated microspore cultures in cucumber were reported by Suprunova and Shmykova (2008). The authors used NLN liquid medium (Lichter 1982) with 10% sucrose and 2,0 mg/l 2,4-D. Callus formation from vegetative cell of pollen was reported at low frequency; however, plants were not regenerated.

Materials and Methods

Cucumber seeds were obtained from inbred line B (Burza and Malepszy 1995) and a Chinese hybrid (CH) 'Ningjia No. 1' previously reported to produce haploid plants from anther culture (Song et al. 2007). Seeds were soaked in tap water for 1–2 h, surface-disinfested in 70% (v/v) ethanol for 5 min, 10% (w/v) solution of Chloramine T (Sigma, St Louis, MO) in water for 15 min, and rinsed three times in sterile water for 5 min each. Seeds were then placed in 300-ml Magenta boxes (Magenta Corp., Chicago, IL) containing 60 ml of basal MS medium. Basal MS medium was prepared using powdered MS salt mixture including vitamins (Phyto Technology Laboratories, Shawnee Mission, KS, USA). Microspores at uninucleate stage were isolated from greenhouse-grown plants. Different conditions were tested for surface sterilization of male flower buds, but it was not possible to obtain sterile cultures. However sterile plants were produced *in vitro* from seed (Kielkowska and Havey, 2011). Uninucleate microspores from *in vitro* cultured plants were isolated according to standard protocols (Custers, 2003). Microspores were cultured on eight liquid media based on MS (Murashige and Skoog, 1962), B5 (Gambrog et al., 1968), NLN (Lichter, 1982), TM (Bal and Touraev, 2009), AT3 (Hoefer et al. 1999)] and supplemented with 10 or 13% sucrose and various plant growth regulators. Prior to induction of sporophytic development of the microspores, whole tissue cultured plants were subjected to cold stress (15 ºC) during flower bud formation, or after isolation microspores were placed for 1 or 2 days in 35 ºC. Viability of microspores was tested with use of fluorescein diacetate (FDA). A stock solution was prepared by dissolving 3 mg of FDA (Sigma, St Louis, MO) in 1 ml of acetone. Staining solution was prepared by mixing 0.5 ml of stock solution with 2 ml 10% sucrose solution. Approximately 100 µl of staining solution was applied to the Petri dishes with 4 ml of medium and samples were incubated in the dark for 15 min in room temperature. The fluorochromatic reaction was excited using epi-illumiantion under blue light excitation at 510 nm with 525 nm barrier filter, observations were made using Axiovert S 135 (Carl Zeiss, Göttingen, Germany). Viable microspores had yellow-green fluorescence, nonviable cells were not visible.

Results and Discussion

Protocols for successful isolation and viability testing of cultured microspores were identified. Mean percentage of viable microspores from *in vitro* cultured plants was low and reached about 18% (Table 1) in the second day after isolation. During the first 7 days of culture we observed swelling of the microspores and displacement of the nucleus to a peripheral position. Applied heat and cold stress did not induced microspore divisions. Although sterile cultures of uninucleate microspores were produced (Havey and Kiełkowska, 2011), no *in vitro* embryogenesis or maturation of microspores was observed due to meiotic abnormalities and decreased viability.

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Table 1. Effect of accession, culture density and medium on viability of cucumber microspores 24 hours after isolation from *in vitro* cultured plants.

Factor	% microspore viability	
Accession:		
Line B	17.8±2.0	ns
Chinese hybrid	17.9±1.8	ns
Culture density/ ml:		
20 000	6.8±1.7	c
40 000	9.3±1.9	bc
60 000	21.6±2.0	a
80 000	18.5±2.6	ab
10 0000	17.3±4.0	ab
Culture medium:		
NLN13	17.2±3.7	ab
NLN13+1µM BA+2µM 2,4-D	19.7±1.3	ab
MS 10+ 4,5 µM 2,4-D+9,3 µM BA	13.5±1.7	a
MS 10+ NAA 0.5µM +BA 13.3µM	20.2±3.4	ab
B5 10+ 4,5 µM 2,4-D+9,3 µM BA	12.0±4.5	b
B5 10+1µM BA+2µM 2,4-D	15.2±3.5	ab
TM	26.6±2.2	a
AT3	17.1±3.7	ab
Mean	17.9±1.9	

Values in column followed by the same letter were not significantly different ($p \leq 0.05$, LSD); ns – no significant difference

2014 Public Sector Cucumber Research Priority Global Survey

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Summary

In 2014, the author conducted a global survey for public sector cucumber research priorities among major stakeholder groups. Data from 38 respondents were analyzed. Several critical issues common to major market classes of cucumbers were identified including resistances for downy mildew (DM), cucurbit yellow stunting disorder virus (CYSDV) or cucumber green mottle mosaic virus (CGMMV), higher fruit yield and better pre-harvest fruit quality, increase of genetic diversity in cultivated cucumber, as well as development of molecular tools to expedite cucumber breeding. Priority issues for specific market classes or geographic regions of cucumber production were also identified. For North American pickling and slicing cucumbers, additional research priorities included resistances to *Phytophthora* fruit rot (PFR) and angular leaf spot (ALS), improvement of post-harvest fruit quality, and development of parthenocarpic varieties.

Introduction and survey methods

The mission of the Cucumber Improvement Program in the Vegetable Crops Research Unit (VCRU) of USDA-RS, Madison is to understand the genetic base of traits important for growers and consumers and to develop enhanced germplasm using classical and biotechnological approaches. For researchers in a public institution, it is important to survey the clientele and prioritize the research to address critical issues in crop production. In 2008, the author conducted a national wide survey in the U. S. to identify priorities for pickling cucumber research (Weng Y, 2009, Cucurbit Genetics Cooperative Report 31-32:1-4). The 2014 survey carried out by the author was expanded to include other major market classes and geographic regions of cucumber production. The 2014 survey design is provided as an appendix at the end of this report. The questions in the 2014 survey were grouped in six categories: 1) Fungal/bacterial resistances, 2) Viral resistances, 3) Insect resistances, 4) Abiotic stress tolerance, 5) Fruit yield and quality, and 6) Development of biotechnological tools. In each category, the respondents were asked to identify and rank in the order of importance the research priorities. Write-in space was provided to allow the respondents to add additional important issues.

The survey was sent to members of the Pickle Packer International (PPI) and Midwest Pickle Association (MWPA) which include cucumber growers, processors, green shipper and salters in the U. S. Feedbacks were solicited from cucumber breeders and R&D leaders in international seed companies, cucumber researchers in public institutions (mainly university research and extension faculty) from home and abroad. The survey was also distributed to participants of the 2014 Cucurbitaceae meeting (Bay Harbor, MI). Thirty-eight feedbacks from 12 countries (Canada, China, India, Israel, Japan, Jordan, the Netherlands, Philippines, Poland, Thailand, Turkey, and the United States) were received. While the responses from some companies were based on individual breeder's personal judgment, those from some other companies were the result of group discussions within the company.

Due to the very diverse environments in which cucumber grows, different market requirements and consumer preferences, it is understandable that research priorities may vary in different geographic regions or market classes. There may also be different perceptions on priorities between private and public researchers. As such, the data were compiled in four groups:

1) International seed companies targeting on global markets (total 8);
2) US Pickling and slicing cucumbers which was further divided into two sub-groups: private (9 respondents) and public (7 respondents);
3) Asian type cucumbers focusing on breeding for fresh market cucumbers (North China, South China types, Japanese Long, and mini cucumbers) (total 10 respondents);
4) Middle East fresh market cucumbers (mainly beit alpha or mini cucumber) (4 respondents).

Priorities in each category among different stakeholder groups and market classes

We first looked into ranking of traits under each of the six categories. In the questionnaire, each respondent was asked to rank the top five priority traits using 1 (top priority) to 5 (low priority). Some respondents only listed the top priorities; others ordered from 1 to 10. To make the data comparable, only traits ranked between 1 and 5 were used; traits not ranked or ranked above 5 were assigned 6, and means of ranking for each trait were calculated. The final ranking was based on the means with the lowest mean having the highest priority.

The results for the four stakeholder groups are summarized in **Table 1**. The issues in each category were arranged according to the overall ranking of their importance among all respondents. For cucumber fungal diseases, it seems that downy mildew (DM) had the highest priority. The other four most important diseases were, in order of importance, powdery mildew (PM), *Phytophthora* fruit rot (PFR), *Fusarium* wilt (FW), and angular leaf spot (ALS) or anthracnose (AR). For viral pathogens, the top five are CYSDV, CMV, WMV, ZYMV and CGMMV. The whiteflies, heat tolerance, high fruit yield, and development of molecular markers for important traits were, respectively, the top priorities in the remaining four categories.

From Table 1, DM was consistently ranked as the top priority by all four groups for fungal or bacterial pathogens, but the ranking of other traits within the top five varied among different stakeholder groups. Meanwhile, some traits that were not among the top five using mean rankings were listed as the top or high priorities by individual groups. These traits are listed in **Table 2**. For example, one respondent in North American pickling group listed the machine harvest and post-harvest damages of cucumbers as the top priority. Resistances to ToLCNDV, MYSV, and CVYV, or tolerance to cold temperature or waterlogging were listed as top issues by different respondents from international companies or Asian fresh marker cucumber researchers.

Overall priorities among different stakeholder groups and market classes

In addition to ranking top five priorities in each of the six categories, the survey also asked the respondents to list and rank 10 top priorities across all traits. The details of rankings are listed in **Table 3** (for international seed companies), **Table 4** (for North American pickling and slicing cucumbers), **Table 5** (for Asian fresh market cucumbers), and **Table 6** (for Middle East cucumbers), respectively. For convenience, the top five priority traits in the four groups are highlighted in **Table 7**.

As shown in Table 7, higher fruit yield was listed among the top five traits by all four stakeholder groups or market classes, whereas DM, pre-harvest fruit quality were in the top-5 list of three groups. Among disease resistances, FPR was of top priority next to DM for North American pickling cucumbers (ranked in the same order by private and public respondents), whereas CYSDV, PM and CMV were important for Middle East cucumber productions. For international seed companies, CGMMV resistance, and development of molecular markers are important (among top 5).

Conclusions

Although application of rigorous statistical methods was not possible to analyze the data, this survey provided useful information for prioritizing public research to address critical issues in cucumber production. Data from this survey indicated that, while critical issues in cucumber production may vary depending on the markets and geographic distributions, several issues are shared among different market class cucumbers including resistances for DM and CYSDV, higher fruit yield and better pre-harvest fruit quality, and development of molecular tools as community resources to expedite cucumber breeding, which may include development of a better cucumber draft genome assembly, molecular markers for horticulturally important traits, and other applied genomic resources. Increase of genetic diversity in cultivated cucumber is also an important issue. For North American pickling and slicing cucumbers (open field production with once-over machine harvest), additional priority issues to address may include resistances to *Phytophthora* fruit rot (PFR) and angular leaf spot (ALS), improvement of post-harvest fruit quality, as well as development of parthenocarpic varieties.

On the other hand, to gain more from this kind of survey, the design of the survey may be improved in several aspects. The survey should be conducted in additional representative cucumber market classes, and sampling size for each market class need to be balanced. Several traits listed in the present survey, such as pre-harvest, post-harvest, fruit yield and fruit quality need to be better defined, or their component traits should be listed explicitly.

Acknowledgements

The author thanks all cucurbit research colleagues in both public and private sectors for taking time to participate in this survey.

Table 1. Ranking of research priorities of traits in six categories among different stakeholder groups. Each group ranked top five traits with 1 (high priority) to 5 (low priority). All traits not in top 5 were assigned 6 for calculating the mean ranking. Final ranking of top 5 traits (last column) was based on average ratings of respondents within each group.

Category	Traits	International	Middle East	US Pickles	US Public	Asia (China/Japan)	Overall Ranking across Stakeholder Groups (Weighted)			
		n = 8	n = 4	n = 9	n = 7	n = 10				
Fungal/bacterial disease resistances							Sum	Count*	Mean	Rank within group
3.1	DM	**1**	**1**	**1**	**1**	**1**	5	5	1.0	**1**
3.2	PM	**2**	**2**	6	**3**	**2**	15	4	3.0	**2**
3.8	GSB	**3**	**5**	6	6	6	26	2	5.2	
3.5	FW	**4**	**3**	6	6	**3**	22	3	4.4	**4**
3.3	ALS	**5**	6	**3**	**5**	6	25	3	5.0	**5**
3.4	TLS	6	6	6	6	**4**	28	1	5.6	
3.6	FPR	6	6	**2**	**2**	**5**	21	3	4.2	**3**
3.9	AN	6	**4**	**5**	**4**	6	25	3	5.0	**5**
3.10	Belly Rot	6	6	**4**	6	6	28	1	5.6	
Virus resistances										
4.2	CYSDV	**1**	**1**	**3**	**1**	**3**	9	5	1.8	**1**
4.1	CMV	**2**	**4**	**1**	**4**	**1**	12	5	2.4	**2**
4.6	CGMMV	**3**	**3**	6	6	6	24	2	4.8	**5**
4.5	ZYMV	**4**	**2**	**2**	**3**	**2**	13	5	2.6	**3**
4.3	PRSV	**5**	6	**5**	**5**	**4**	25	3	5.0	
4.4	WMV	6	**5**	**4**	**2**	**5**	22	4	4.4	**4**
Insect resistances										
5.2	Whiteflies	**1**	**1**	**4**	6	**1**	13	4	2.6	**1**
5.6	Thrips	**2**	**2**	6	**3**	**3**	16	4	3.2	**2**
5.7	Aphids	**3**	**3**	**3**	**5**	**2**	16	5	3.2	**2**
5.3	Spider mites	**4**	**4**	6	**4**	**5**	23	4	4.6	**5**
5.4	Leaf miners	**5**	**5**	**5**	6	6	27	3	5.4	
5.1	Beetles	6	6	**1**	**1**	**4**	18	3	3.6	**3**
5.5	Pickleworm	6	6	**2**	**2**	6	22	2	4.4	**4**
Abiotic stress tolerances										
6.4	Heat	**1**	**4**	**4**	**2**	**2**	13	5	2.6	**1**
6.1	Chilling damage	**2**	**1**	**5**	**5**	**1**	14	5	2.8	**2**
6.3	Drought	**3**	**5**	**3**	**4**	**3**	18	5	3.6	**4**
6.5	Salt	**4**	**2**	6	**1**	**4**	17	4	3.4	**3**
6.2	Cold germination	**5**	**3**	**1**	**3**	**5**	17	5	3.4	**3**
6.6	Herbicide	6	6	**2**	6	6	26	1	5.2	**5**
Fruit yield/quality										
7.1	High fruit yield	**1**	**1**	**1**	**1**	**2**	6	5	1.2	**1**
7.3	Pre-harvest	**2**	**4**	**2**	**2**	**1**	11	5	2.2	**2**
7.4	Post-harvest	**3**	**2**	**3**	**3**	**3**	14	5	2.8	**3**
7.2	Parthenocarpy	**4**	**3**	**4**	**4**	**4**	19	5	3.8	**4**
Molecular tools for breeding										
8.2	Molecular markers	**1**	**1**	**1**	**2**	**2**	7	5	1.4	**1**
8.3	Genomic resources	**2**	**3**	**2**	**4**	**4**	15	5	3.0	**3**
8.1	Draft genome	**3**	**2**	**5**	**5**	**5**	20	5	4.0	**4**
8.5	Genetic diversity	**4**	**4**	**4**	**1**	**1**	14	5	2.8	**2**
8.4	Transformation	**5**	**5**	**3**	**3**	**3**	19	5	3.8	**5**

*** Number of respondents who listed this trait in top-5 priorities.**

Table 2. Traits with regional importance but that were not listed among top 5 priorities in ranking based on means. Number in each cell is the original ranking by individual respondents (R in short, in parentheses).

Category	Traits	International	Middle East	US Pickles	US Public	Asia (China/Japan)
3.11	**Scab**		2 (R1)		5 (R2, R7)	5 (R4, R8)
3.12	**Botrytis/Pythium**	5 (R3)				
3.13	**Fusarium rot**		5 (R3)			
3.14	**Phytophthora**					1 (R3)
4.7	**ToLCNDV**	3 (R1, R6)				1 (R5)
4.8	**TSV**	3 (R1)				
4.9	**MYSV**					1 or 2 (R8, R9, R10)
4.10	**CVYV**					2 (R8)
5.9	**Seed corn maggot**			3 (R2, R4)	2 (R7)	
6.7	**Cold tolerance**	1 (R4)				
6.8	**Waterlogging**					1 (R3)
7.6	**Machine harvest and postharvest damage**			1 (R4)		
8.7	**Software for drone use in phenotyping**				2 (R3)	
8.6	**VIGS**	5 (R1)				

Table 3. Overall ranking of research priorities by international seed companies (C in short, C1 to C8). Individual ranking is based on 1 (high priority) to 6 (low priority). All traits not in top 6 were assigned 7 (shaded) for calculating the mean ranking. Last column shows top-10 list.

Category	Traits	C1	C2	C3	C4	C5	C6	C7	C8	Counts	Mean	Ranking
3.1	**DM**	**3**	**5**	**1**	**5**	**1**	**1**	**3**	**2**	8	2.6	**1**
7.1	**High fruit yield**	**5**	**2**	**2**	**2**	7	7	7	**1**	5	4.1	**2**
8.2	**Molecular markers**	7	**1**	7	**1**	**2**	**3**	7	7	4	4.4	**3**
4.6	**CGMMV**	7	7	7	7	7	**2**	**1**	**6**	3	5.5	**4**
7.3	**Pre-harvest**	7	**3**	7	7	**4**	**5**	7	7	3	5.9	**5**
4.2	**CYSDV**	7	7	**5**	7	7	**6**	7	**3**	3	6.1	**6**
8.5	**Genetic diversity**	**4**	7	**3**	7	7	7	7	7	2	6.1	**6**
8.3	**Genomic resources**	7	7	7	7	**3**	**4**	7	7	2	6.1	**6**
8.1	**Draft genome**	**1**	7	7	7	7	7	7	7	1	6.3	**7**
3.8	**GSB**	**2**	7	7	7	7	7	7	7	1	6.4	**8**
3.6	**FPR**	7	7	7	7	7	7	**2**	7	1	6.4	**8**
3.12	Botrytis/Pythium	7	**4**	7	7	7	7	7	7	1	6.6	**9**
6.5	Salt tolerance	7	7	**4**	7	7	7	7	7	1	6.6	**9**
4.11	CVYV	7	7	7	**4**	7	7	7	7	1	6.6	**9**
6.2	Cold germination	7	7	7	7	7	7	7	**4**	1	6.6	**9**
3.10	Belly Rot	7	7	7	7	7	7	**4**	7	1	6.6	**9**
7.4	Post-harvest	7	7	7	7	**5**	7	7	7	1	6.8	**10**
6.3	Drought tolerance	7	7	7	7	7	7	7	**5**	1	6.8	**10**
5.2	Whiteflies	7	7	7	7	7	7	**5**	7	1	6.8	**10**
4.1	CMV	**6**	7	7	7	7	7	7	7	1	6.9	
4.3	PRSV	7	**6**	7	7	7	7	7	7	1	6.9	
5.6	Thrips	7	7	**6**	7	7	7	7	7	1	6.9	
3.2	PM	7	7	7	**6**	7	7	7	7	1	6.9	
6.1	Chilling damage	7	7	7	7	**6**	7	7	7	1	6.9	
6.4	Heat tolerance	7	7	7	7	7	7	**6**	7	1	6.9	

Table 4. Overall ranking of research priorities for North American pickling and slicing cucumbers. Ranking is based on nine private and 7 public respondents. Each respondent ranked the priority with 1 (high) to 6 (low). Traits not in top 6 were assigned 7 (shaded) for calculating the mean ranking. Final ranking of top-10 list is shown in the last column of each sub-group.

		Private												Public									
Category	Traits	R1	R2	R3	R4	R5	R6	R7	R8	R9	Count	Mean	Rank	R1	R2	R3	R4	R5	R6	R7	Count	Mean	Rank
3.1	**DM**	1	6	1	3	1	1	1	4	1	9	2.7	**1**	2	1	2	1	1	1	2	7	1.4	**1**
3.6	**PFR**	2	5	4	2	2	2	2	6	5	8	3.9	**2**	4	2	7	7	7	7	1	3	5.0	**2**
7.3	**Pre-harvest**	4	2	7	5	7	4	7	2	3	7	4.4	**3**	7	7	1	7	7	4	7	3	5.7	**4**
7.1	**High fruit yield**	7	1	7	7	3	3	7	1	2	6	4.8	**4**	7	7	7	3	2	2	7	3	5.0	**3**
7.4	**Post-harvest**	5	3	7	7	7	5	7	3	4	5	6.1	**5**	7	7	7	7	7	3	3	3	5.9	**5**
3.3	**ALS**	7	7	2	4	7	7	3	7	7	3	6.1	**6**	7	7	7	7	7	7	7	1	7.0	
6.6	**Herbicide**	7	7	7	7	7	7	5	7	7	4	6.2	**7**	7	7	7	7	7	7	7	0	7.0	
7.2	**Parthenocarpy**	3	7	7	7	4	7	7	7	7	3	6.3	**8**	7	7	7	7	7	5	4	2	6.3	**8**
6.4	**Heat**	7	7	7	7	7	7	6	7	7	3	6.6	**9**	7	7	7	7	7	7	7	0	7.0	
8.2	**Molecular markers**	7	4	7	7	7	6	7	5	7	3	6.7	**10**	7	7	7	2	7	7	7	1	6.3	**8**
6.3	**Drought**	7	7	7	7	7	7	7	7	7	1	6.7	**10**	7	7	7	7	7	6	7	1	6.9	
7.6	**Machine harvest and postharvest damage**	7	7	7	1	7	7	7	7	7	1	6.7	**10**	7	7	7	7	7	7	7	0	7.0	
5.5	**Pickleworm**	7	7	5	7	6	7	7	7	6	3	6.7	**10**	6	7	5	7	7	7	7	2	6.6	**10**
4.2	**CYSDV**	7	7	7	7	7	7	7	7	7	0	7.0		5	7	6	7	3	7	7	3	6.0	**6**
3.2	**PM**	7	7	7	7	7	7	7	7	7	0	7.0		1	7	7	7	7	7	7	2	6.1	**7**
8.5	**Genetic diversity**	7	7	7	7	7	7	7	7	7	1	7.0		7	7	3	7	6	7	7	2	6.3	**8**
6.5	**Salt**	7	7	7	7	7	7	7	7	7	0	7.0		7	7	4	7	5	7	7	3	6.3	**8**
3.5	**FW**	7	7	7	7	7	7	7	7	7	1	7.0		3	7	7	7	7	7	7	1	6.4	**9**
5.2	**Whiteflies**	7	7	7	7	7	7	7	7	7	0	7.0		7	7	7	7	4	7	7	1	6.6	**10**
3.7	**BW**	7	7	7	7	7	7	7	7	7	1	6.8		7	7	7	7	7	7	7	0	7.0	
3.9	**AN**	7	7	3	7	7	7	7	7	7	1	6.8		7	7	7	7	7	7	7	0	7.0	
3.10	**Belly Rot**	7	7	7	7	7	7	7	7	7	2	6.8		7	7	7	7	7	7	7	0	7.0	
4.1	**CMV**	7	7	7	7	7	7	4	7	7	2	6.8		7	7	7	7	7	7	7	0	7.0	
5.1	**Beetles**	7	7	6	7	7	7	7	7	7	1	6.8		7	7	7	7	7	7	7	1	7.0	
5.7	**Aphids**	7	7	7	7	5	7	7	7	7	1	6.8		7	7	7	7	7	7	7	1	7.0	
4.5	**ZYMV**	6	7	7	7	7	7	7	7	7	1	6.9		7	7	7	7	7	7	7	0	7.0	
4.4	**WMV**	7	7	7	7	7	7	7	7	7	1	6.9		7	7	7	7	7	7	7	0	7.0	
6.1	**Chilling damage**	7	7	7	6	7	7	7	7	7	1	6.9		7	7	7	7	7	7	7	0	7.0	
6.2	**Cold germination**	7	7	7	7	7	7	7	7	7	2	7.0		7	7	7	7	7	7	7	0	7.0	
8.4	**Transformation**	7	7	7	7	7	7	7	7	7	2	7.0		7	7	7	7	7	7	7	0	7.0	
4.3	**PRSV**	7	7	7	7	7	7	7	7	7	1	7.0		7	7	7	7	7	7	7	0	7.0	
8.1	**Draft genome**	7	7	7	7	7	7	7	7	7	1	7.0		7	7	7	7	7	7	7	1	7.0	

Table 5. Overall ranking of research priorities for Asian fresh market cucumbers (mainly in China and Japan). Ranking is based on 10 respondents, and each respondent ranked the priority with 1 (high) to 10 (low). Traits not in top 10 were assigned 11 for calculating the mean rankings. Final top-10 list is shown in the last column.

Category	Traits	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	Count	Mean	Rank
8.2	**Molecular markers**	11	1	4	3	3	11	2	2	2	5	8	4.4	**1**
7.1	**High fruit yield**	3	7	5	1	2	1	5	10	11	4	9	4.9	**2**
8.1	**Draft genome**	11	11	11	11	1	8	4	1	4	7	6	6.9	**3**
7.3	**Pre-harvest**	11	3	1	10	11	3	1	9	11	11	6	7.1	**4**
8.3	**Genomic resources**	11	2	11	4	11	11	11	3	3	6	5	7.3	**5**
3.1	**DM**	11	11	11	2	6	2	11	4	6	11	5	7.5	**6**
3.2	**PM**	6	5	11	7	10	9	6	5	7	11	8	7.7	**7**
6.1	**Chilling damage**	5	11	8	9	4	6	8	11	9	9	8	8.0	**8**
8.4	**Transformation**	7	11	3	11	11	11	3	11	5	11	4	8.4	**9**
4.9	**MYSV**	11	11	11	11	11	11	11	8	1	1	3	8.7	**10**
4.1	**CMV**	11	11	11	11	11	5	9	7	11	2	4	8.9	
3.14	**Phytophthora melonis**	2	11	2	8	11	11	11	11	11	11	3	8.9	
7.2	**Parthenocarpy**	11	4	11	5	11	11	11	11	11	3	3	8.9	
6.4	**Heat**	1	11	6	11	11	11	10	11	10	10	5	9.2	
6.3	**Drought**	4	11	11	11	11	7	11	11	11	11	2	9.9	
5.2	**Whiteflies**	10	9	11	11	9	10	7	11	11	11	5	10.0	
8.5	**Genetic diversity**	8	11	7	11	11	11	11	11	11	11	2	10.3	
7.4	**Post-harvest**	11	11	11	11	11	4	11	11	11	11	1	10.3	
5.6	**Thrips**	11	11	11	11	11	11	11	11	8	8	2	10.4	
6.2	**Cold germination**	11	11	11	11	5	11	11	11	11	11	1	10.4	
5.7	**Aphids**	11	8	9	11	11	11	11	11	11	11	2	10.5	
3.3	**ALS**	11	11	11	11	11	11	11	6	11	11	1	10.5	
3.6	**PFR**	11	11	11	6	11	11	11	11	11	11	1	10.5	
6.8	**Waterlogging**	11	6	11	11	11	11	11	11	11	11	1	10.5	
4.2	**CYSDV**	11	11	11	11	7	11	11	11	11	11	1	10.6	
6.5	**Salt**	9	10	11	11	11	11	11	11	11	11	2	10.7	
4.10	**CVYV**	11	11	11	11	11	11	11	8	11	11	1	10.7	
5.1	**Beetles**	11	11	11	11	8	11	11	11	11	11	1	10.7	
3.5	**FW**	11	11	10	11	11	11	11	11	11	11	1	10.9	

Table 6. Research priorities for Middle East fresh market cucumbers. Ranking is based on three respondents, and each respondent ranked the priority with 1 (high) to 6 (low). Traits not in top 6 were assigned 7 for calculating the mean rankings. Final top-5 list is shown in the last column.

#	Category	Traits	R1	R2	R3	Count	Mean	Rank
1	7.1	**High fruit yield**	1	1	7	2	3.0	**1**
2	3.1	**DM**	2	4	7	2	4.3	**2**
3	4.2	**CYSDV**	3	5	7	2	5.0	**3**
4	3.2	**PM**	7	7	1	1	5.0	**4**
5	4.1	**CMV**	7	7	2	1	5.3	**5**
6	6.1	**Chilling damage**	7	2	7	1	5.3	**5**
7	5.3	Spider mites	7	7	3	1	5.7	
8	8.5	Genetic diversity	7	3	7	1	5.7	
9	6.2	Cold germination	4	7	7	1	6.0	
10	6.4	Heat	7	7	4	1	6.0	
11	6.3	Drought	5	7	7	1	6.3	
12	7.3	Pre-harvest	7	7	5	1	6.3	
13	4.6	CGMMV	6	7	7	1	6.7	
14	8.1	Draft genome	7	6	7	1	6.7	
15	8.2	Molecular markers	7	7	6	1	6.7	

Table 7. Top five priority traits for public cucumber research in four stakeholder groups (1 indicates highest priority). Same traits are shaded with the same color.

Ranking	International	N. American pickles	Asian fresh market	Middle East
1	DM	DM	Molecular markers	High fruit yield
2	High fruit yield	FPR	High fruit yield	DM
3	Molecular markers	Pre-harvest	Draft genome	CYSDV
4	CGMMV	High fruit yield	Pre-harvest	PM
5	Pre-harvest	Post-harvest	Genomic resources	CMV/Chilling

Appendix

2014 Public Cucumber Research Priority Survey

1. I am a (check all that apply)

_____ Grower _____ Processor _____ Salter, green shipper
_____ Public researcher _____ Private researcher _____ State extension specialist
_____ Others. Please specify _____

2. My work focuses primarily on

_____ Fresh market cucumber ____ Processing cucumber _____ Both
Target market of my work is (geographic region) _____

3. Fungal/bacterial diseases (rank top 10 priorities of research, 1 = highest priority)

3.1 _____ Downy mildew (DM)
3.2 _____ Powdery mildew (PM)
3.3 _____ Angular leaf spot (ALS)
3.4 _____ Target leaf spot (TLS)
3.5 _____ Fusarium wilt (FW)
3.6 _____ Phytophthora fruit rot (FPR)
3.7 _____ Bacterial wilt (BW)
3.8 _____ Gummy stem blight (GSB)
3.9 _____ Anthracnose (AR)
3.10 _____ Belly rot
3.11 _____ Scab
3.12 _____ *Botrytis*/*Pythium*
3.13 _____ *Fusarium* rot
3.14 _____ *Phytophthor melonis*
3.15 _____ Other diseases. Please specify_____

4. Virus pathogens (rank top 5 priorities of research, 1 = highest priority)

4.1 _____ Cucumber mosaic virus (CMV)
4.2 _____ Cucurbit yellow stunting disorder virus (CYSDV)
4.3 _____ Papaya ring spot virus (PRSV)
4.4 _____ Watermelon mosaic virus (WMV)
4.5 _____ Zucchini yellow mosaic virus (ZYMV)
4.6 _____ Cucumber green mottle mosaic virus (CGMMV)
4.7 _____ Tomato leaf curl New Delhi virus (ToLCNDV)
4.8 _____ Tobacco streak virus (TSV)
4.9 _____ Mellon yellow spot virus (MYSV)
4.10 _____ Cucurbit chlorotic yellows virus (CCYV)
4.11 _____ Cucumber vein yellowing virus (CVYV)
4.12 _____ Other viruses. Please specify_____

5. Insect pests (**rank top 5 priorities of research**, **1 = highest priority**)

5.1 _____ Cucumber beetles
5.2 _____ Whiteflies
5.3 _____ Spider mites
5.4 _____ Leaf miners
5.5 _____ Pickleworm
5.6 _____ Thrips
5.7 _____ Aphids
5.8 _____ Others. Please specify _____

6. Abiotic stresses (rank top 5 priorities, 1 = highest priority)

6.1 _____ Chilling damage
6.2 _____ Cold germination
6.3 _____ Drought stress
6.4 _____ Heat damage
6.5 _____ Salt stress

6.6 _____ Herbicide damage
6.7 _____ Others. Please specify _____

7. Fruit yield and quality (rank top 4 priorities, 1 = highest priority)

7.1 _____ High fruit yield
7.2 _____ Parthenocarpic pickling cucumber
7.3 _____ Pre-harvest fruit quality: fruit shape, color, taste/flavor, internal defect etc.
7.4 _____ Postharvest fruit quality: brining quality, shelf-life etc.
7.5 _____ Others. Please specify _____

8. Molecular/biotechnological tool development (rank top 5 priorities, 1 = highest priority)

8.1 _____ Improve cucumber draft genome assembly and genome annotations
8.2 _____ Develop molecular markers for important traits for marker-assisted selection
8.3 _____ Develop more applied genomic resources (maps, genome sequencing etc.)
8.4 _____ Develop genetic transformation techniques for cucumber
8.5 _____ Broaden cucumber genetic diversity through exploring other *Cucumis* resources
8.6 _____ Others. Please specify _____

9. Now, from Categories 3 to 8 above, please list the overall TOP 10 priorities (# 1= highest priority; #10 = lowest priority). You can put the item number (for example 3.1) in the blanks.

#1 _____ #6 _____
#2 _____ #7 _____
#3 _____ #8 _____
#4 _____ #9 _____
#5 _____ #10 _____

10. Additional comments related to public research needs.

11. If you wish to receive the survey results, please provide your email/mail address or other methods of communication.

An Easily Created Tri-Specific Squash Hybrid [(*Cucurbita argyrosperma* × *C. moschata*) × *C. maxima*]

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Cucurbita argyrosperma Duchesne and *C. moschata* Huber are known to hybridize easily, mostly with *C. argryrosperma* as the maternal parent (2,3,4,6,7,9), though the reciprocal is also possible (1). *Cucurbita maxima* Duchesne and *C. moschata* also have a high degree of inter-compatibility (5,9), high enough that it is commercially viable to sell F_1 seeds as cultivars (5). *Cucurbita maxima* × *C. moschata* F_1 hybrids are popular in Japan and Brazil (5), represented by cultivars such as 'Tetsukabuto', 'Triunfo', 'Supremo' 'Greenstone' and others. In the United Kingdom there has been the recent release of an interspecific *C. maxima* × *C. moschata* cultivar called 'Squashkin'. *Cucurbita maxima* × *C. moschata* F_1 hybrids have a high degree of sterility and must be planted with one of the parental species as a pollinator to produce fruit. Additionally, hybrids of *C. argyrosperma* and *C. maxima* have been created without much difficulty (3,9). To the authors knowledge a trispecific hybrid between all three species has not been previously reported.

In the summer of 2009 in Mansfield Center, Connecticut USA an interspecific F1 hybrid of 'Green Striped Cushaw' *C. argyrosperma* × 'Butterbush' *C. moschata* was grown in the same field as the *C. maxima* cutivar 'Bush Buttercup'. Both squash types flowered simultaneously. Two female flowers of the *C. argyrosperma* × *C. moschata* were hand pollinated with 3 male flowers each from the *C. maxima* cultivar using the standard masking tape pollinator exclusion method (8). One of the two female flowers formed a mature fruit with 14 plump seeds with fully developed embryos. In the spring of 2010, six seeds of the trispecific cross were planted, and four germinated. The resulting plants were bush to semi-bush in habit. The female flowers formed well, but male flowers aborted. The female flowers were allowed to be open pollinated with *C. maxima* and *C. moschata* growing in the same plot. Six fruits matured. Fruits were smooth, green, disc to pyriform in shape, and weighed about 1-2 kg. The fruits had good storage qualities lasting until March of 2011. The flesh was medium orange, slightly lighter in color than 'Waltham butternut' *C. moschata* and had about the same culinary qualities. The trispecific hybrids did not produce any viable seeds.

This short report communicates that trispecific hybrids appear to be easy to obtain combining the genomes of *C. argyrosperma*, *C. moschata*, and *C. maxima*. The immediate utility of the trispecific hybrid is not readily apparent, but they may be useful in future cucurbit breeding programs. The author believes that these hybrids are generally sterile but hypothesize that if grown in larger numbers some fertile seed could be produced. Unique fruit color combinations and patterns could be developed from such crosses. These hybrids could also be used as bridges between the species, allowing transfer of traits from one species to another.

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