Final Scientific Report

Cover Page

BARD Project Number: IS-4321-10

Date of Submission of the report: November, 2013

Project Title: A Three Crops Test for the ODO Breeding Method

Investigators

Institutions

Principal Investigator (PI): Yaakov Tadmor, Agricultural Research Organization (ARO)

Co-Principal Investigator (Co-PI): Zachary Lippman, Cold Spring Harbor Laboratory

Collaborating Investigators:

David Jackson,

Cold Spring Harbor Laboratory

Dani Zamir,

Hebrew University

Keywords heterosis, mutant, over-dominance, maize, tomato, melon.

Abbreviations commonly used in the report, in alphabetical order:

HPLC

Budget: IS: \$184,000

US: \$126,000

Total: \$310,000

Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

Final Scientific Report

Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted) BARD support acknowledged				
Submitted, in review, in preparation				
Invited review papers				
Book chapters				
Books				
Master theses			1	
Ph.D. theses				
Abstracts				
Not refereed (proceedings, reports, etc.)				

Postdoctoral Training: List the names and social security/identity numbers of all postdocs who received more than 50% of their funding by the grant. NONE

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings	1	1		
Longer Visits (Sabbaticals)				

Details of cooperation

Cooperation among the participants was excellent: The participating researchers met several times to discuss and coordinate the project: Y. Tadmor visited CSH in the summer of 2011. Z. Lippman visited Tadmor and Zamir at 2012. The frequent meetings and discussions affected the successful completion of this project.

ABSTRACT – Final report IS-4312-10

Hybrid vigor is the leading concept that rules crops breeding for almost a century. Yet, the exact mechanism that underlies heterosis is not clear. Over dominance interaction between alleles is one of the possible explanations. Our preliminary results indicated that severe developmental mutations at the heterozygous state have significant potential to improve plant performance. This led us to propose the 'ODO breeding method' that is based on replacing a parental line of a successful hybrid with its mutated form to improve hybrid performance. Our BARD research challenged this method in three crop systems: maize, tomato and melon.

In maize we could not detect any effect of mutant heterozygosity on yield or yield components when hybrids were tested however when we analyzed the effect of heterozygosity in mutant genes at B73 genetic background we could detect ODO in yield components using certain mutants. Our results indicate that the potential of utilizing the ODO Breeding Method for maize remains unclear.

In tomato we overcame technical problems we faced in creating an EMS mutation library in indeterminate glasshouse tomatoes and now we have in our hands advanced material to study the putative ODO hybrids. We transferred some of the promising ODO mutations from M82 to indeterminate glasshouse tomatoes and putative ODO hybrids are ready to be evaluated this winter.

In melon we compared putative 'ODO hybrids' with their isogenic hybrids lacking the mutant allele and our results indicated a potential for the ODO breeding method to improve yield, fruit number per plant, and carotenoids content. Additional experiments are required to estimate better the expected success percentage of the ODO breeding method in melon so that it will become a recommended practice for improving hybrid performance.

Based on our results we can't yet recommend the 'ODO breeding method' as a general tool to improve hybrid performance and more efforts are necessary to evaluate the percent of success of this method. The increased carotenoid content we found in association with CRTISO heterozygosity is promising and additional experiments are currently being performed to characterize this finding.

Achievements

Our collaborative research brings up more hints that the ODO breeding method could have a practical effect on crop breeding. Details of our results appear in our appendix. A major finding is the effect of heterozygosity in CRTISO gene on total carotenoids in both tomato and melon. We created advanced material to test this finding.

Description of the resulting publications

At this stage we did not have any official publication coming out of our scientific activity

Details of cooperation

Cooperation among the participants was excellent: The participating researchers corresponded routinely through e-mails and met several times to discuss and coordinate the project: YT visited CSH in 2011 and ZL visited Israel in 2012

Final report BARD project IS-4321-10

Appendix

Table of contents

ODO in maize	2
ODO in Indeterminate tomatoes	5
ODO carotenoids of tomato CRTISO mutant.....	10
ODO in melon.....	13
GENERAL CONCLUSIONS	23

Publications

Thus far no publications came out of this project

ODO in maize

Over the last three years, the Jackson and Lippman laboratories at CSHL collaborated to evaluate the 'ODO Breeding Method' in maize. Several classical and recently discovered mutants studied by Jackson that affect meristem transitions and size were selected and assessed for yield component traits in isogenic mutant heterozygotes generated by crossing homozygous mutants with their wild type progenitors. Due to limits of only two generations of maize genetics per year, heterozygous seeds were generated during each year's winter nursery in Puerto Rico or Mexico, which were then tested in summer agricultural fields in Riverhead, New York. A focus was placed on meristem mutants, given their direct impact on reproductive traits, especially in the developing ears. In the homozygous mutants *fasciated ear 2 (fea2)*, *fasciated ear 3 (fea3)*, *fasciated ear 4 (fea4)*, and *compact plant 2 (ct2)* ears show dramatically increased, but disorganized, kernel row number as a result of meristem over-proliferation at early stages of inflorescence meristem development. In *ramosa 1 (ra1)* and *ramosa (ra3)* mutants, ears show increased branch number and length based on prolonged activity of basal branch meristem identity prior to transitioning to spikelet meristems – the precursors to spikelets (flowers). The *Dwarf3 (D3)* gene encodes a gene involved in the early step of GA biosynthesis, resulting a dwarf phenotype in homozygous mutants. In all mutants, yield, as determined by kernel row number and kernel number/weight, is reduced due to disorganized meristem and ear growth. However, based on the model that altering dosage from heterozygous mutations can trigger semi-dominant phenotypic effects that can translate to improved yield components, each mutation provided an opportunity to test the ODO Breeding Method. For example, we hypothesized that single mutant heterozygotes of *fea* class of mutants might cause a subtle increase in meristem size through weak semi-dominant effects that increases row number, generating an improved hybrid performance.

To test mutant heterozygote yield performance, all homozygous mutants in the standard B73 background were crossed to both B73 and Mo17 in both directions (using mutants as males and females), creating F1 hybrids in the isogenic background (B73) and in full genome heterozygous conditions (B73 X Mo17 F1). In three years of field experiments, eight replicates for all genotypes including controls, with ten plants in each replicate, were planted in a randomized block design. From each replicate, ten ears were harvested and the best eight ears were subjected to detailed phenotypic measurements. To evaluate yield components, we

measured ear length, circumference, kernel line number (number of kernels along the length of the ear), and kernel row number, and compared the mutant hybrids with B73, Mo17, and B73 X Mo17 F1 hybrids. For each trait, normal distributions of the data were first tested, and then all traits were subjected to one-way ANOVA within each genotype to identify and eliminate potential outliers of replicates, which were confirmed using images taken for each replicate group of ears. The cleaned data set was then subjected to one-way ANOVA for each trait across genotypes, and all pairwise comparisons between genotypes were made using Tukey's HSD test.

Not surprisingly, strong mutant heterozygote effects were detected in all traits when comparing full genome F1 hybrids (between mutants in the B73 background and Mo17) to both B73 and Mo17, consistent with the known strong heterosis resulting from whole genome hybridization between B73 and Mo17. Unfortunately, mutant heterozygous effects were not detected in the background of full genome B73 x Mo17 heterozygosity, suggesting that strong heterosis of the classical hybrid likely masks any potential single gene dosage effects. Therefore, we focused on comparisons within the B73 background, and indeed, isogenic mutant heterozygotes revealed more encouraging results. In one year, *fea3* showed significantly increased kernel row number in the heterozygous condition, but this effect was not reproducible in subsequent years. Specifically, in the last field season, only one comparison between *fea3*/+ mutant heterozygotes with B73 controls showed increased circumference, kernel line number and kernel row number, but similar significant differences were not detected for other independent *fea3* mutant heterozygote comparisons. One possibility is that additional backcrosses beyond three are needed to eliminate residual full genome heterozygosity, which could be contributing to improved yield effects not due to *fea3*/+ heterozygosity per se. Future experiments would require further backcrosses, and testing ODO effects in segregating families coming from progeny seed of isogenic *fea3*/+ heterozygotes. Last year, encouraging results showed several *fea4*/+ isogenic heterozygotes with significant differences in ear length, circumference, and kernel line number, but, surprisingly, these effects were not consistent with respect to direction of effect: in some comparisons, *fea4*/+ heterozygotes showed increased values, while in others values decreased. We do not have an explanation for these inconsistencies, but *fea4* dosage may be worth exploring further, again, focusing on additional backcrosses and segregating isogenic populations. Finally, throughout this project, additional mutants, particularly of the fasciated class, were screened from EMS mutagenesis that could be tested in future years, and the

Jackson and Lippman Laboratories hope to evaluate these mutants in the heterozygous conditions in coming years using independent funds.

Based on these experiments, consistent or reproducible ODO effects within and between years from maize mutant heterozygotes were not observed, indicating the potential of utilizing the ODO Breeding Method for maize remains unclear. However, to fully evaluate the ODO hybrid Breeding, the experiment could be expanded using more mutants beyond those tested that cause primarily meristem size defects. Importantly, during this project, the Jackson lab reported that weak alleles of the *fea2* mutation cause a subtle increase in meristem size that translates to an increase of two kernel rows per ear (Bommert, Nature Genetics, 2013). Thus, the potential to use weak alleles of fasciated mutants may supplant the absence of dosage effects when strong alleles are in the heterozygous condition. Thus, identification of very weak alleles of *fea3*, *fea4*, or *ct2*, for example, may provide valuable germplasm for breeding in the grasses.

ODO in Indeterminate tomatoes

Our objective in this project was to identify mutations which when heterozygous increase productivity of indeterminate glasshouse tomatoes. About 10 years ago we produced a large number of mutations in the determinate background of the processing tomato variety M82. We have tried to test hybrids of mutants in the determinate (sp/sp) M82 with indeterminate M82 (SP/SP) and with additional indeterminate genetic backgrounds however the genetic background of M82 is highly incompatible with glasshouse cultivation and its hybrids produce less than half the yield per plant as leading indeterminate hybrids. Therefore our objective in this proposal was to generate mutants in a genetic background suitable for glasshouse cultivation. For this we selected a *ripening inhibitor (rin)* inbred that was developed in our lab and had a good combining ability.

The first round of EMS treatment on the *rin/rin* background did not succeed; we grew in the greenhouse 300 M3 families each with 24 plants and found no mutations although the seed treatment was similar to that given to the M82 seed which was very effective in producing thousands of mutations. In a second round of seed treatment we doubled the concentration of EMS to 1.0% using the same 12 h treatment as well as a 24 h treatment. The M1 generation composed of 300 plants from each of the treatments was planted in the soil in a greenhouse in CHATZAV next to non-treated controls; no dominant mutations were observed. The treated *rin/rin* plants were self pollinated and selfed seed was collected from six-hundred M2 families and was phenotyped in the greenhouse.

One of the reasons for using the *rin/rin* line was to try beyond selecting for overdominant mutations to find mutations that abolish the *rin/rin* phenotype. Such a plant was uncovered in the M1 generation – its color turned to red. In the M2 the segregation of *rin* was 3:1 – the first explanation for this unexpected phenotype was outcrossing that occurred in the glasshouse. Indeed in the M2 we could unequivocally identify the classic *rin* sequence published by Dr. Jim Giovannoni. Beyond the disappointment of not having a suppressor of *rin* we realized that we have to be careful when selecting plants as contaminations could lead to noise in our science. Therefore, we planted again independent M3 mutants (Figure 1; Table1) from the same family (2-4, 4 plants each) to look at their homogeneity. Ten independent pure mutants were selected and will be sent for SNP CHIP analysis (10K) next to the *rin* mutant to verify their identity before large scale trials will be conducted in Winter 2014.

In addition we noted that several of unknown mutation (ODO-X), which were heterotic in determinate tomatoes, seemed to have an effect in indeterminate background. However, to determine their value in high yielding genetic backgrounds they needed to be backcrossed to

glasshouse background. Based on observations in the backcross populations it seem that at least one of the mutation is clearly effective in that background. These will be tested in the Winter of 2014. In summary although critical examination of mutant heterosis in indeterminate genetic background was achieved yet due to unexpected delays in our timetable we expect to achieve results in the coming two years.

Figure 1: Selected mutants in the *rin* background.

41#	42#	gen.	description	Comments
4250		M3	rin-e18	
4251		M3	rin-e018m1	y small plant & fruit, single stem
4252		M3	rin-e63	disease response, necrotic spots
4253		M3	rin-e70m1	y yellow-small plant, single stem
4254		M3	rin-e70-2	small plant & leaves
4255		M3	rin-e70-3	small plant & leaves, small fruits
4256		M3	rin-e73	small plant, fruit without jelly
4257		M3	rin-e100m1	y yellow- small plant
4259		M2	rin-e002	
4261		M2	rin-e01	
4263	6894	M2	rin-e03m1	y yellow plant
4266		M2	rin-e06	
4267		M2	rin-e07	small purple
4269		M2	rin-e09	tiny
4271		M2	rin-e2	
4276		M2	rin-e7	dichotomous
4277		M2	rin-e8	
4279		M2	rin-e10	
4280		M2	rin-e11	
4282		M2	rin-e13	tiny
4283		M2	rin-e14	
4285		M2	rin-e16	tiny
4286		M2	rin-e17m1	n zebra
4287		M2	rin-e018m1	n yellow leaves
4288		M2	rin-e19	
4292		M2	rin-e23	
4293		M2	rin-e024m1	n plant is small green, yellow leaves, sterile
4296		M2	rin-e27	
4297		M2	rin-e28	
4298		M2	rin-e29	
4299		M2	rin-e30	
4300		M2	rin-e31	
4304		M2	rin-e35	
4305		M2	rin-e36	tiny
4306		M2	rin-e037m1	n odd meristematic leaves, leaflets are jagged and inside folded ?
4308		M2	rin-e39	
4310		M2	rin-e41	white, yellow- small plant
4311		M2	rin-e42	light green?
4312		M2	rin-e043m1	n light green leaves, small, sterile
4313		M2	rin-e44	
4316		M2	rin-e047m1	small plant
4318		M2	rin-e49	tiny+blind
4319		M2	rin-e50	
4321		M2	rin-e052m1	n yellow leaf, maybe Ty
4322		M2	rin-e53	?
4324		M2	rin-e055m1	n small, leaf shape, pale leaves sterile
		M2	rin-e055m2	n small
4327		M2	rin-e058m1	n white dots on leaves, debilis? , sterile
		M2	rin-e058m2	n small, dark green leaves
4333		M2	rin-e064m1	small, yellow complex leaves odd meristem
4334		M2	rin-e65	
4335		M2	rin-e66	
4336		M2	rin-e067m1	n small plant, disease response, small necrotic dots on leaves, sterile
		M2	rin-e067m2	n extreme mc (larger than control)
		M2	rin-e067m3	n seed lethality
4337		M2	rin-e68	
4338		M2	rin-e69	
4340		M2	rin-e71	
4341	6896	M2	rin-e072m1	y small plant thin stem strange inflos
		M2	rin-e072m2	yellow leaves
4344		M2	rin-e75	
4346		M2	rin-e77	
4347		M2	rin-e78	
4350		M2	rin-e081m1	white sectors on leaves
4354		M2	rin-e085m1	small, yellow leaves, strange phyllotaxis
4355		M2	rin-e86	
4356		M2	rin-e87	
4357		M2	rin-e088m1	small pale leaves, disease response, sterile
		M2	rin-e088m2	orange-small wilty
4359		M2	rin-e90	
4364		M2	rin-e95	tiny white- odd stem yellow- leaf texture variegated
4365		M2	rin-e96	
4366		M2	rin-e97	
4367		M2	rin-e98	
4368		M2	rin-e99	
4370		M2	rin-e101	
4371		M2	rin-e102	
4378		M2	rin-e109m1	small sterile
4382		M2	rin-e113m1	n disease response, small plant
		M2	rin-e113m2	n yellow leaflets shape, yellow edges sterile
4383		M2	rin-e114m1	n yellow leaves, wilty
4384		M2	rin-e115	

41#	42#	gen	Description	Comments
4250		M3	rin-e18	
4251		M3	rin-e019m1	y small plant & fruit, single stem
4252		M3	rin-e63	disease response, necrotic spots
4253		M3	rin-e70m1	y yellow-small plant, single stem
4254		M3	rin-e70-2	small plant & leaves
4255		M3	rin-e70-3	small plant & leaves, small fruits
4256		M3	rin-e73	small plant, fruit without jelly
4257		M3	rin-e100m1	y yellow- small plant
4259		M2	rin-e002	
4261		M2	rin-e01	
4263	6894	M2	rin-e03m1	y yellow plant
4265		M2	rin-e06	
4267		M2	rin-e07	small purple
4269		M2	rin-e09	tiny
4271		M2	rin-e2	
4276		M2	rin-e7	- dichotomous
4277		M2	rin-e8	
4279		M2	rin-e10	
4280		M2	rin-e11	
4282		M2	rin-e13	tiny
4283		M2	rin-e14	
4285		M2	rin-e16	tiny
4286		M2	rin-e17m1	n zebra
4287		M2	rin-e018m1	n yellow leaves
4288		M2	rin-e19	
4292		M2	rin-e23	
4293		M2	rin-e024m1	n plant is small green, yellow leaves, sterile
4296		M2	rin-e27	
4297		M2	rin-e28	
4298		M2	rin-e29	
4299		M2	rin-e30	
4300		M2	rin-e31	
4304		M2	rin-e35	
4305		M2	rin-e36	tiny
4306		M2	rin-e037m1	n odd meristematic leaves, leaflets are jagged and inside folded 7
4308		M2	rin-e39	
4310		M2	rin-e41	white, yellow- small plant
4311		M2	rin-e42	light green leaves, small, sterile
4312		M2	rin-e043m1	n
4313		M2	rin-e44	
4316		M2	rin-e047m1	small plant
4318		M2	rin-e49	tiny-blind
4319		M2	rin-e50	
4321		M2	rin-e052m1	n yellow leaf, maybe Ty
4322		M2	rin-e53	?
4324		M2	rin-e055m1	n small, leaf shape, pale leaves sterile
4327		M2	rin-e055m2	n small
4327		M2	rin-e058m1	n white dots on leaves, debilis?, sterile
4333		M2	rin-e058m2	n small, dark green leaves
4333		M2	rin-e064m1	n small, yellow complex leaves, odd meristem
4334		M2	rin-e65	
4335		M2	rin-e66	
4336		M2	rin-e067m1	n small plant, disease response, small necrotic dots on leaves, sterile
4336		M2	rin-e067m2	n extreme mc (larger than control)
4336		M2	rin-e067m3	n seed lethality
4337		M2	rin-e68	
4338		M2	rin-e69	
4340		M2	rin-e71	
4341	6896	M2	rin-e072m1	y small plant thin stem strange inflos
4341		M2	rin-e072m2	yellow leaves
4344		M2	rin-e75	
4346		M2	rin-e77	
4347		M2	rin-e78	
4350		M2	rin-e081m1	white sectors on leaves
4354		M2	rin-e085m1	small, yellow leaves, strange phyllotaxis
4355		M2	rin-e86	
4356		M2	rin-e87	
4357		M2	rin-e088m1	small pale leaves, disease response, sterile
4357		M2	rin-e088m2	orange-small wilty
4359		M2	rin-e90	
4364		M2	rin-e95	tiny white- odd stem yellow- leaf texture variegated
4365		M2	rin-e96	
4366		M2	rin-e97	
4367		M2	rin-e98	
4368		M2	rin-e99	
4370		M2	rin-e101	
4371		M2	rin-e102	
4378		M2	rin-e109m1	small sterile
4382		M2	rin-e113m1	n disease response, small plant
4382		M2	rin-e113m2	n yellow leaflets shape, yellow edges sterile
4383		M2	rin-e114m1	n yellow leaves, wilty
4384		M2	rin-e115	

11#	12#	gen	description	comments
4250		M3	rin-e18	
4251		M3	rin-e019m1	y small plant & fruit, single stem
4252		M3	rin-e63	disease response, necrotic spots
4253		M3	rin-e70m1	y yellow-small plant, single stem
4254		M3	rin-e70-2	small plant & leaves
4255		M3	rin-e70-3	small plant & leaves, small fruits
4256		M3	rin-e73	small plant, fruit without jelly
4257		M3	rin-e100m1	y yellow- small plant
4259		M2	rine-002	
4261		M2	rin-e01	
4263	6894	M2	rin-e03m1	y yellow plant
4266		M2	rin-e06	
4267		M2	rin-e07	small purple
4269		M2	rin-e09	tiny
4271		M2	rin-e2	
4276		M2	rin-e7	dichotomous
4277		M2	rin-e8	
4279		M2	rin-e10	
4280		M2	rin-e11	
4282		M2	rin-e13	tiny
4283		M2	rin-e14	
4285		M2	rin-e16	tiny
4286		M2	rin-e17m1	n zebra
4287		M2	rin-e018m1	n yellow leaves
4288		M2	rin-e19	
4292		M2	rin-e23	
4293		M2	rin-e024m1	n plant is small green, yellow leaves, sterile
4296		M2	rin-e27	
4297		M2	rin-e28	
4298		M2	rin-e29	
4299		M2	rin-e30	
4300		M2	rin-e31	
4304		M2	rin-e35	
4305		M2	rin-e36	tiny
4306		M2	rin-e037m1	n odd meristematic leaves, leaflets are jagged and inside folded ?
4308		M2	rin-e39	
4310		M2	rin-e41	white, yellow- small plant
4311		M2	rin-e42	(p2 rim?)
4312		M2	rin-e043m1	n light green leaves, small, sterile
4313		M2	rin-e44	
4316		M2	rin-e047m1	small plant
4318		M2	rin-e49	tiny+blind
4319		M2	rin-e50	
4321		M2	rin-e052m1	n yellow leaf, maybe Ty
4322		M2	rin-e53	?
4324		M2	rin-e055m1	n small, leaf shape, pale leaves sterile
			rin-e055m2	n small
4327		M2	rin-e058m1	n white dots on leaves, debilis? sterile
			rin-e058m2	n small, dark green leaves
4333		M2	rin-e064m1	small, yellow complex leaves, odd meristem
4334		M2	rin-e65	
4335		M2	rin-e66	
4336		M2	rin-e067m1	n small plant, disease response, small necrotic dots on leaves, sterile
			rin-e067m2	n extreme mc (larger than control)
			rin-e067m3	n seed lethality
4337		M2	rin-e68	
4338		M2	rin-e69	
4340		M2	rin-e71	
4341	6896	M2	rin-e072m1	y small plant thin stem strange inflos
			rin-e072m2	yellow leaves
4344		M2	rin-e75	
4346		M2	rin-e77	
4347		M2	rin-e78	
4350		M2	rin-e081m1	white sectors on leaves
4354		M2	rin-e085m1	small, yellow leaves, strange phyllotaxis
4355		M2	rin-e86	
4356		M2	rin-e87	
4357		M2	rin-e088m1	small pale leaves, disease response, sterile
			rin-e088m2	orange-small wilty
4359		M2	rin-e90	
4364		M2	rin-e95	tiny white- odd stem yellow- leaf texture variegated
4365		M2	rin-e96	
4366		M2	rin-e97	
4367		M2	rin-e98	
4368		M2	rin-e99	
4370		M2	rin-e101	
4371		M2	rin-e102	
4378		M2	rin-e109m1	small sterile
4382		M2	rin-e113m1	n disease response, small plant
			rin-e113m2	n yellow leaflets shape, yellow edges sterile
4383		M2	rin-e114m1	n yellow leaves, wilty
4384		M2	rin-e115	

ODO carotenoids of tomato CRTISO mutant

Results of melon's CRTISO increasing effect on carotenoids content in ODO hybrids prompted us to test the effect of heterozygosity in CRTISO mutation in tomato (Fig 1).

Plant and fruit yield per plant was significantly reduced however there was no significant difference in plant (vegetative) and fruit per plant weight between CRTISO heterozygote and homozygote wildtype (Fig 2)

We compared average fruit weight and total soluble solids (TSS, as indicator to fruit sugars content) and did not detect any significant difference between heterozygote and homozygote wild type (Fig. 3).

Figure 3 – Average fruit weight (left) and total soluble solids (TSS; right) of homozygotes and heterozygote CRTISO mutant (WT = M82, mutant = E3406); Different letters indicate significant difference (Tukey–Kramer; $P < 0.05$)

Figure 4 – Average carotenoids content and composition of homozygotes and heterozygote CRTISO mutant (WT = M82, mutant = E3406) grown in the field of 2012; Different letters indicate significant difference (Tukey–Kramer; $P < 0.05$)

Interestingly, in tomato too (see melon results Figs 14 & 15), under intense light conditions the wild type M82 had less carotenoid than the CRTISO heterozygote (Fig. 4). We repeated this experiment in the greenhouse under sub-optimal light conditions and the heterozygote had significantly less carotenoids than the wild type (Fig. 5). We will continue studying this phenomenon in tomato and in watermelon for which we also have a pro-lycopene accumulating fruit.

Figure 5 – Average carotenoids content and composition of homozygotes and heterozygote CRTISO mutant (WT = M82, mutant = E3406) grown in the greenhouse 2012/3; Different letters indicate significant difference (Tukey–Kramer; $P < 0.05$)

ODO in melon

Melon (*Cucumis melo*), a tropical old-world species, is among the most important fleshy fruits for fresh consumption that is cultivated across wide areas of the world. We used Ethyl Methane Sulfonate (EMS) on melon seeds to develop two mutation libraries; the first one is based on the inbred line 'Noy Yizre'el' - one of the famous 'Galia' hybrid parents, while the second mutation library was developed from the inbred line 'CEZ' - an orange flesh 'Charantais' type melon. The two libraries contain thousands of different mutated M₂ families, most of them recessive, part of them dramatically change plant morphology, reproduction or fruit morphology. During our efforts to isolate and stabilize mutant lines we had difficulties in stabilizing the mutants and they continued to segregate within segregants deriving from

self-pollination of a mutant. This phenomenon is not clear to us. It could derive from choosing polygenic characters, epigenetic phenotype or cross pollinations. Additional research is necessary to understand better this phenomenon.

We crossed stabilized homozygous mutants of 'Noy Yizre'el with 'Krimka', to produce a potential ODO 'Galia' hybrids and used the isogenic hybrid 'Galia', as our control (Fig. 1). In a previous study a 'CEZ' carotenoid isomerase (CRTISO) gene mutant, which

accumulates prolycopene instead of β -carotene was identified. This mutant was crossed with six inbred lines from different genetic backgrounds that were also crossed with 'CEZ' as controls (Fig. 2). Additionally, the effect of CRTISO mutation heterozygosity was tested in tomato. The ODO hybrids and their control hybrids were tested in the field of Newe Yaar (in the summer) and in a greenhouse (in the winter) for flowering, plant development, fruit yield and total soluble solids sugars (TSS). The 'CEZ' and CRTISO hybrids were also analyzed for the effect of heterozygosity at the CRTISO locus on carotenoids content and composition. A total of 17 isogenic hybrid pairs were analyzed; 11 were 'Galia' type ('Noy Yizre'el' mutants x 'Krymka') and six were with a parental line which originated from 'CEZ'. All hybrids were tested during three growing seasons; twice in the field (summers of 2011 and 2012) and once in the greenhouse (winter of 2012-3). Collecting data of flowering and growth parameters was aimed to identify phenotypic characters that contribute to a possible yield increase. With early growth parameters and flowering parameters – timing of first female flowers, number of female flowers and fruit set - the 'Galia' hybrid outperformed or was similar to the tested ODO hybrids (Fig. 3 & 4).

Figure 3 – Comparison between 'Galia' type potential ODO hybrids and control in growth parameters: leaves and stems dry weight 31 (A) and 42 (B) days after planting, length of stems (C) and number of fruit sets (D) 31 and 42 days after planting.

Figure 4 – Comparison between potential ODO hybrids and control in days for first female flower (A), average number of female flowers during the first month and percentage of fruit setting from first female flowers.

Fruit maturation of melon is a continuous process however in practice only the first peak of fruit maturation is harvested. To make our study most similar to commercial practice we harvested fruit till 105 days after planting in 2011 and 95 days after planting but included only the first peak (till day 80) in yield calculations (Fig. 5).

Figure 5 – Distribution of fruit harvest along the harvest time in the field of 2011 (A) and 2012 (B)

We analyzed fruit yield component such as fruit number per plant (Fig. 6), average fruit length and width and TSS (Fig. 7) and average fruit weight (Fig. 8) to understand better any yield change in 'Galia' type putative ODO hybrids.

Figure 6 – Average fruit number per plant of 'Galia' type putative ODO hybrids in the field of 2011 (A) and 2012 (B)

Figure 7 – Average fruit length (A), width (B) and TSS (C) per plant of 'Galia' type putative ODO hybrids in the field of 2011 (A)

Figure 8 – Average fruit weight (Kg) comparison between 'Galia' potential ODO hybrids grown in the field on 2011 (A) and on 2012 (B)

In 2011, out of the five putative ODO 'Galia' hybrids, one gave significantly more yield and in 2012 two out of six tested ODO hybrids significantly increased their yield compared to 'Galia' (Fig. 9 and Table 1).

Table 1 – mean yield per plant of 'Galia' type putative ODO hybrids grown in the field during the summer of 2011 and 2012. 'Galia' control is highlighted in yellow. 'Galia' parental lines, NY and KRY, were analyzed in 2012.

	Hybrid	Significance				Mean Yield/plant
2011	KRY X NYM1265	A				2.5873333
	KRY X NYM1358	A	B			2.2660667
	GALIA	A	B			2.1548
	KRY X NYM152	A	B			2.0463333
	KRY X NYM1200	A	B			2.0278
	KRY X NYM776		B			1.9042
2012	NYM3502 x KRY	A				3.955056
	NYM3369 x KRY	A				3.785
	NYM3114 x KRY		B			2.924792
	KRY		B			2.8455
	GALIA		B	C		2.675083
	NYM3896 x KRY		B	C		2.548833
	NYM3140 x KRY			C		2.226646
	NYM3512 x KRY			C		2.224229
	NY				D	1.589375

In the year of 2012 we grew also the 'Galia' parental lines, NY and KRY to test for heterosis. Interestingly 'Galia' do now show heterosis when it is compared to KRY (Table 1). This is most probably due to the fact that melon hybrids are mostly selected for fruit quality and yield is only secondary. Still NYM 1265, NYM 3502 and NYM 3369 when crossed with KRY to create a putative ODO hybrid significantly increased yield of 20%, 41% and 48%

respectively (Table 1) without decreasing fruit quality. This increase resulted most probably from the significant increase in fruit number (Fig. 6) and no reduction (NYM 1265) and significant increase in fruit weight for NYM 3502 and NYM 3369 (Fig. 8). These results are based on six replications with five plants harvested in each replication.

Putative ODO hybrids of CEM 3285 that is mutated in CRTISO gene were tested for yield and carotenoids in the field of 2011 and for carotenoids in the field of 2012 and in the greenhouse in the winter of 2012-2013. As with the 'Galia' ODOs we harvested all fruit till day 105 after planting (Fig. 10) but to make it similar to commercial practice we included in yield analysis only fruit harvested during the first wave of harvesting from day 60 to day 85 after planting.

Two putative ODO hybrids of CEM 3285 had significantly more fruit per plant in the field of 2011 (Fig. 11) however their fruit weight was smaller (Fig. 12) and no yield improvement

had been observed with the CRTISO mutant (CEM 3285) as a parental line (Fig. 13 and table 2).

Figure 11 – Average fruit number of putative CEZ ODO hybrids in the field of 2011

Figure 12 – Average fruit weight of putative CEZ ODO hybrids in the field of 2011; and 2012 and in the greenhouse of 2012/3

Table 10 – Average yield per plant of CRTISO ODO hybrids grown in the field of 2011. # indicates the pairs of hybrids to be statistically compared (the ODO and its control). Different letters indicate significant difference ($P < 5\%$)

#	Hybrid			Mean Yield/plant
a	CEZ x NUN5410	A		2.7349333
be	CEZ x NY	A	B	2.5470667
a	CEM3285 x NUN5410	A	B	2.5252
c	CEZ x X357	A	B	2.3956667
d	CEM3285 x KRY	A	B	2.3672
d	CEZ x KRY		B C	2.2092667
c	CEM3285 x X357		B C	2.1731333
b	CEM3285 x NY		B C	2.1297333
e	NY x CEM5243		B C	2.0952667
f	CEM3285 x MAJ		C	1.8580667
g	CEZ x NUN6131		C	1.8516
g	CEM3285 x NUN6131		C	1.8188667
f	CEZ x MAJ		C	1.7607333

No significant effect on yield among CRTISO ODO hybrids and their controls have been observed in other growing seasons (data not shown). Interestingly, when we analyzed the

carotenoid content of CRTISO ODO hybrids we could detect significant increase in carotenoid content even though CRTISO is considered as a recessive mutation. CRTISO mutant is incapable of isomerization *cis*-lycopene into all-*trans*-lycopene that serves as the precursor to lycopene β -cyclase that makes β -carotene from lycopene by the creation of two β -rings at the two ends of the linear lycopene. A CRTISO melon recessive mutant that we discovered accumulates pro-lycopene (tetra-*cis*-lycopene) as its major fruit carotenoid unlike the wild type orange melon that accumulates β -carotene. The effect of heterozygosity in recessive mutation on carotenoid content and composition was analyzed in six hybrid combinations. Surprisingly, four out of six hybrids showed significant increase in total carotenoid content as revealed by HPLC analysis (14A). This increase resulted mainly due to the significant increase in the major carotenoid in orange melon fruit, β -carotene (Fig 14B), however other carotenoids, such as lutein, also increased (Fig 14C). Interestingly, vitamin E content (both α - and γ -tocopherol) was also increased in hybrids that showed increased carotenoids content (not shown).

Figure 14
Comparison of carotenoid content in six CRTISO ODO hybrids and their wildtype controls. A – total carotenoid content; B – β -carotene content and C – lutein content in μg to gram fresh weight ($\mu\text{g/g FW}$). * indicates a significant difference ($p < 0.05$).

We repeated these experiments under three growing conditions, results are summarized in Fig 15. These results indicate the potential of melon CRTISO mutation at the heterozygote state to increase carotenoid content in certain genetic backgrounds. Next we analyzed the effect of CRTISO mutation in tomato (see tomato section) and we prepared a set of 50 new putative

ODO hybrids with melon's CRTISO mutant. Ten of these new putative ODO hybrids have been tested during the summer and results are being analyzed at these moments.

Figure 15 - carotenoid content and composition in CRTISO putative ODO hybrids grown in the field of 2011 (two left columns in each graph), greenhouse of 2012/3 (middle) and in the field of 2012 (right). Second parental line appears in the top of each graph.

GENERAL CONCLUSIONS

Our results indicate a potential for the ODO breeding method to improve quantity and quality characters in hybrids. Comparison of ODO hybrids with their isogenic hybrid lacking the mutant allele showed that several characters can be improved including: fruit (row) number per plant (ear), early fruit ripening in melon and carotenoids content in both melon and tomato. Nevertheless, only a relatively small number of hybrids were tested and additional experiments are required to estimate expected success percentage of the ODO breeding method in melon.

In tomato we created a new and unique EMS mutation library that will be further studies during the coming years