BARD

FINAL REPORT

PROJECT NO. US-2443-94

Molecular Marker Mapping of Genes Enhancing Tocol and Carotenoid Composition of Maize Grain

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2000

BARD Project Number: US-2443R

50909

Title: Molecular marker mapping of genes enhancing tocol and carotenoid composition of maize grain.

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Date of Submission of Final Report: June 29, 2000

Actual Start Date of Project: December, 1995

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הספריה המרכזית
למדעי החקלאות
בית דגן

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Abstract

Title: Molecular marker mapping of genes enhancing tocol and carotenoid composition of maize grain. The overall objective of this research was to identify chromosomal regions and candidate genes associated with control of concentration and forms of carotenoids (includes pro-Vitamin A) and tocopherols (Vitamin E), which are both antioxidants and are associated with health advantages. Vitamin A and E are included in animal feeding supplements and the eventual goal is to increase levels of these compounds in maize grain so that the cost of these supplements can be reduced or eliminated. Moreover, both compounds are antioxidants that protect unsaturated fatty acids from oxidation and thus maintaining maize oil quality for longer periods. We identified three SSR markers that are associated with 38% of the variation for total carotenoids and three SSR markers associated with 44% of the variation for total tocopherols in the cross W64a x A632. We identified two candidate genes associated with levels of carotenoids: *phytoene synthase* and *zeta carotene desaturase*. Evaluation of (Illinois High Oil x B73) B73 BC1S1 population for tocopherols detected additional chromosomal regions influencing the level of total tocopherols, and detected a common region on chromosome 5 associated with ratio of the more desirable alpha form to the gamma form of tocopherol. The results suggest molecular marker assisted selection for higher levels of these antioxidants in corn grain should be feasible.

Objectives of the original research proposal.

1. Molecular marker mapping of chromosomal regions associated with control of concentration and form of tocols primarily and also carotenoids in corn grain. Perform and compare molecular marker mapping analyses in two different mapping populations, with particular attention to possible genetic background effects on modification for tocol and carotenoid composition.
2. Relate the molecular mapping information to basic knowledge on the biochemical pathways of tocols and carotenoids. Where possible, use clones of known genes or new cDNA clones of biological relevance as probes in molecular marker mapping studies to facilitate this process.
3. Identify tightly linked molecular markers which flank chromosomal regions associated with control of desirable levels and forms of tocols and carotenoids in maize grain.
4. Evaluate testcross progeny of the first mapping population, perform chromosome mapping analyses on this data and compare to results from initial mapping analyses.
5. Develop the appropriate backcross progenies for subsequent marker assisted backcrossing programs.

We pursued all of the objectives outlined in our proposal and did not deviate from this research plan. We refer back to these five objectives by their number in the evaluation of research achievements section.

Relevant data, methodology, results and discussion

While evaluating and probing the molecular marker mapping population, we assessed genetic variation for tocopherols and carotenoids in a group of ten inbreds crossed in a diallel experiment. The 45 hybrids were grown in 1997 to obtain estimates of general and specific combining ability. (Cem Egesel Ph.D. Thesis). This study generated background information to provide some context and perspective for the molecular marker mapping studies. This work also served to optimize the procedures for HPLC analyses for tocopherols and carotenoids and provide a range of possible expected values for the mapping population. Table 1. below gives the mean values and standard deviation plus the minimum and maximum values for the 45 hybrids. The results in Table 1. revealed there is good variation for several of the carotenoid and tocopherol compounds. In addition, we screened several high oil and normal maize inbred lines for both tocopherol, carotenoids, oil and protein content. These analyses helped us to study the relationships among these quality components. In the normal maize inbred lines we found a correlation between oil content and vitamin E. In contrast in High Oil lines in general had more vitamin E than the normal lines but its amount was not associated with oil concentration. This indicates that tocopherols, which are oil soluble, are associated with oil concentration only to a limited degree.

Table 1. Means for 45 hybrids for several carotenoid and tocopherol compounds.

	Lutein	Zea xanthin	α-carotene	β-carotene	γ-tocopherol	α-tocopherol
Mean	13.6	5.6	0.23	0.6	69.8	23.8
S.D.	4.7	1.8	0.08	0.2	26.7	8.6
Min.	4.0	2.2	0.10	0.3	21.9	7.5
Max.	24.8	10.3	0.46	1.3	142.3	47.4

The estimates for general combining ability (GCA) for these traits are provided in Table 2. These results indicate considerable genetic variation exists among the hybrids evaluated. The GCA estimates also indicate selection for these traits should be effective if the inbreds with the highest GCA effects were utilized in a synthetic variety. These data indicate natural variation in maize could be used to improve the overall levels and specific forms of carotenoids and tocopherols.

Table 2

1997 Diallel Study- Vitamin Data *GCA and SCA estimates*

GCA Estimates for 10 inbreds

Parent (qi)	Pedigree	d-toc	g-toc	a-toc	total-toc	lutein	zeax.
1	W64a	-0.179 **	5.611 *	-10.192 **	-4.696	-0.617	0.267
2	A632	-0.779	-13.922 **	-2.081	-16.784 **	-3.983 **	1.006 **
3	AEC335	0.008	17.177 **	6.435 **	23.621 **	-0.314	-1.074 **
4	UHO416	0.162	20.108 **	8.143 **	28.413 **	0.576	-1.135 **
5	RSC 1/99	0.461 **	-5.731	-2.946	-8.217	3.556 **	0.474
6	RSC 91/9	-0.882 **	-37.444 **	2.079	-36.246 **	1.816 **	0.208
7	A619	0.334 *	16.755 **	1.772	18.873 **	3.9 **	1.933 **
8	B73	0.225	-6.227	3.149 *	-2.854	0.769	-0.263
9	Mo17	-0.212	-18.112 **	-2.078	-20.402 **	-0.169	0.803 **
10	R84	0.851 **	21.787 **	-4.345 **	18.292 **	-5.534 **	-2.217 **

Parent (qi)	Pedigree	t-pigm.	b-crypto.	a-carot.	b-carot.	t.crtns	t.cartnds
1	W64a	-0.265	0.068 *	-0.005	-0.177 **	-0.092	-0.359
2	A632	-2.893 **	0.26 **	-0.07 **	0.063 **	0.275 **	-2.617 **
3	AEC335	-1.48 *	0.045	0.019 **	-0.035	0.022	-1.459 *
4	UHO416	-0.899	-0.186 **	-0.004	-0.28	-0.169 **	-1.068
5	RSC 1/99	4.115 **	-0.204 **	0.028 **	-0.021	-0.236 **	3.879 **
6	RSC 91/9	2.112 **	-0.229 **	0.006	-0.164 **	-0.426 **	1.686 *
7	A619	5.917 **	0.204 **	0.046 **	0.404 **	0.634 **	6.593 **
8	B73	0.589	-0.042	0.024 **	-0.134 **	-0.132 *	0.459
9	Mo17	0.72	0.171 **	-0.01	-0.059 **	0.122 *	0.841
10	R84	-7.915 **	-0.087 **	-0.032 **	0.095 **	-0.04	-7.953 **

The results for the primary objective of this project, molecular marker mapping of genes controlling levels and forms of carotenoids and tocopherols in a cross of W64a x A632 are provided in the following pages. Much of this work was summarized in a M.S. thesis by Jeff Wong. We provide abstracts, tables and summary pages from his thesis which efficiently summarizes the main results. The results from development of HPLC procedures in Israel and the identification of QTL for tocopherols in the (IHO x B73)B73 population performed primarily in Israel then follows. We then briefly discuss the highlights and relationships of the two studies, which are also summarized in the next section - evaluation of research achievements.

Abstract

Vitamin A and vitamin E are two general terms that describe carotenoids and tocopherols, respectively. Both carotenoids and tocopherols have antioxidant activity, which refers to their ability to quench electronically excited molecules or free radicals. Both carotenoids and tocopherols are found naturally within the kernel of dent corn, with variation in levels of various forms. Altering levels of these compounds may provide added value, including health and economic benefits to the corn kernel.

A mapping population of 200 F3 families was analyzed for carotenoids including two carotenes, alpha (α) and beta (β) carotene, and three xanthophylls, lutein, zeaxanthin, and beta (β) cryptoxanthin. Three isomers of tocopherols were analyzed alpha (α), delta (δ), and gamma (γ) tocopherol. Carotenoid and tocopherol assays were done by high performance liquid chromatography (HPLC). To locate possible quantitative trait loci (QTL) for each compound, the population was assayed for forty microsatellite (SSR) markers.

Multiple regions were detected throughout the genome for individual carotenoids and tocopherols as well as the combined carotenoids and tocopherols. Multiple regression models for each trait explained a variable level of variation. Two chromosomal regions were associated with control of levels of the carotenoids, one in bin 6.00 and the other in bin 7.02, are of great interest because of possible relationships with two important biosynthetic enzymes. Bin 6.00 is very close to the *yellow1* (*y1*) mutant, which was has been shown to encode phytoene synthase, an important enzyme that effects all carotenoid compounds further down the biosynthetic pathway. Bin 7.02 is very close to two mutants, *yellow8* (*y8*), a known endosperm color mutant, and *viviparous9* (*vp9*) which has been associated with control of zetacarotene desaturase, another enzyme which, like phytoene synthase, is early in the carotenoid pathway. For tocopherols, bin 1.09, appears to have effects for each of the tocopherols, implying its relevance to some process in the biosynthetic pathway upstream from the tocopherols studied here. Another region of interest is in bin 5.04, which appears to affects the levels of γ-tocopherol and α-tocopherol.

Table 3. Markers significantly associated with combined carotene, combined xanthophyll, and combined carotenoids studied for the 200 F3 families of the cross A632 x W64a combined over 1996 and 1997.

Marker	Bin[1]	Significance Level	Additive Effects ($\mu g\ g^{-1}$)
Combined Carotenes			
BNGL100	1.09	.0010	-0.10*
BNGL166	2.04	.0484	n.s.
PHI075	6.00	.0224	-0.11**
PHI126	6.00	.0017	-0.14***
PHI034	7.02	.0001	-0.19***
PHI065	9.03	.0369	n.s.
Combined Carotenoids Studied			
BNGL100	1.09	.0026	-2.33**
BNGL166	2.04	.0039	n.s.
PHI074	4.04	.0138	-1.76**
PHI075	6.00	.0040	-2.02**
PHI126	6.00	.0007	-2.45***
PHI034	7.02	.0001	-4.09***

Marker	Bin[1]	Significance Level	Additive Effects ($\mu g\ g^{-1}$)
Combined Xanthophyll			
BNGL100	1.09	.0036	-2.15**
BNGL166	2.04	.0044	n.s.
PHI074	4.04	.0103	-1.68**
PHI075	6.00	.0040	-1.80**
PHI126	6.00	.0010	-2.18***
PHI034	7.02	.0001	-3.74***

[1] Bin numbers taken from MaizeDB.
*, **, *** denotes significance of 0.05, 0.01, and 0.001, respectively.
n.s. = Not Significant

Table 4. Markers significantly associated with combined tocopherols, and combined carotenoids and tocopherols studied for the 200 F3 families of the cross A632 x W64a combined over 1996 and 1997.

Marker	Bin	Significance Level	Additive Effects ($\mu g\ g^{-1}$)
Combined Tocopherols			
BNGL176	1.02	.0292	6.98*
BNGL439	1.03	.0297	6.35*
PHI001	1.03	.0439	5.86*
PHI037	1.08	.0001	19.38***
BNGL100	1.09	.0001	n.s.
PHI074	4.04	.0433	-5.62*
DUPSSR10	5.04	.0001	-10.60***
PHI034	7.02	.0023	-8.70***
DUPSSR26	n.m.	.0168	6.37*
DUPSSR05	n.m.	.0441	n.s.

Marker	Bin	Significance Level	Additive Effects ($\mu g\ g^{-1}$)
Combined Carotenoids and Tocopherols Studied			
PHI037	1.08	.0001	18.50***
BNGL100	1.09	.0001	n.s.
PHI074	4.04	.0082	-7.28**
DUPSSR10	5.04	.0025	-9.26***
PHI034	7.02	.0001	-12.73***
PHI065	n.m.	.0246	n.s.

[1] Bin numbers taken from MaizeDB.
*, **, *** denotes significance of 0.05, 0.01, and 0.001, respectively.
n.m. = not mapped n.s. = Not Significant

Summary

The study detected QTL throughout the genome for individual carotenoids and tocopherols as well as the combined carotenoids and tocopherols. Based on multiple regression models, markers explained differing amounts of variation. Very little of the variation in carotenoids was explained, with α-carotene having the most variation (28%) explained. Two of the tocopherols, α (20%) and δ-tocopherol (17%), also had a limited percentage of the variation explained. γ-Tocopherol did however have a substantial amount of variation explained (47%).

Two regions associated with control of levels of the carotenoids are noteworthy because of possible relationships with two important biosynthetic enzymes. One marker is near the *y1* mutant locus, which was shown to encode phytoene synthase, an important enzyme which has effects on all carotenoid compounds further down the biosynthetic pathway. One region significant for the carotenoids is near two mutant loci, *y8* a known color mutant and *vp9* which has been associated with control of zetacarotene desaturase, another enzyme early in the carotenoid pathway. Another noteworthy region for the carotenoids is associated with bin 8.04, which might have an effect on one of the lycopene cyclase enzymes, which controls the production of α-carotene versus β-carotene.

For the tocopherols, bin 5.04 is noteworthy because of its similarity to the action of γ-tocopherol methyltransferase. If the marker associated with 5.04 does have some control on γ-tocopherol methyltransferase, increasing levels of α-tocopherol may be possible. Another region of interest is bin 1.09, as it appears to have effects for each of the tocopherols, implying its importance to influencing some point in the biosynthetic pathway upstream for the tocopherols studied.

Table 5. W64 x A632 population, significant markers for total carotene, total xanthophyll, total carotenoid, total tocopherol, and total vitamin. Data presented is based on a total of 76 markers.

Total Carotene

Marker	Map	P_VALUE*
zag100	1.09	0.0008
phi126	6.00	0.0013
zct161	6.00	0.0061
zag249	6.01	0.0001
umc1178	6.01	0.0015
y1ssr	6.02	0.0003
phi034	7.02	0.0001
bmc1176	8.05	0.0040

Total Xanthophyll

Marker	Map	P_VALUE*
zag100	1.09	0.0035
bmc1144	3.03	0.0007
umc1288	4.02	0.0006
phi074	4.04	0.0046
phi126	6.00	0.0006
phi075	6.00	0.0046
zct161	6.00	0.0050
umc1178	6.01	0.0001
zag249	6.01	0.0001
y1ssr	6.02	0.0001
phi034	7.02	0.0001

Total Carotenoid Studied

Marker	Map	P_VALUE*
zag100	1.09	0.0025
bmc1144	3.03	0.0011
umc1288	4.02	0.0010
phi074	4.04	0.0064
phi126	6.00	0.0004
zct161	6.00	0.0035
phi075	6.00	0.0045
umc1178	6.01	0.0001
zag249	6.01	0.0001
y1ssr	6.02	0.0001
phi034	7.02	0.0001

Total Tocopherol

Marker	Map	P_VALUE*
phi037	1.08	0.0001
zag100	1.09	0.0001
dupo010	5.04	0.0001
umc1221	5.05	0.0007
umc1155	5.05	0.0035
mmc0081	5.05	0.0057
phi034	7.02	0.0022

Total Vitamin

Marker	Map	P_VALUE*
phi037	1.08	0.0001
zag100	1.09	0.0001
phi074	4.04	0.0039
dupo010	5.04	0.0010
umc1155	5.05	0.0026
umc1221	5.05	0.0032
phi034	7.02	0.0001

*Marker significant at 0.01 determined by single factor analysis of variance.

Table 6. W64 x A632 population, 76 markers analyzed by multiple regression for total carotene, total xanthophyll, total carotenoid, total tocopherol, and total vitamin.

Total Carotene

Marker	Map	Partial R^2	P_Value*
PHI034	7.02	0.1232	0.0001
ZAG249	6.01	0.1311	0.0001
BMC1176	8.05	0.0462	0.0004
PHI118	10.00	0.0237	0.0100
		0.3242**	

Total Xanthophyll

Marker	Map	Partial R^2	P_Value*
PHI034	7.02	0.1784	0.0001
ZAG249	6.01	0.1479	0.0001
UMC1288	4.02	0.0477	0.0002
PHI115	8.03	0.0341	0.0010
ZAG108	2.04	0.0329	0.0009
		0.4409**	

Total Carotenoid Studied

Marker	Map	Partial R^2	P_Value*
PHI034	7.02	0.1834	0.0001
ZAG249	6.01	0.1563	0.0001
UMC1288	4.02	0.0438	0.0003
ZAG108	2.04	0.0274	0.0031
PHI115	8.03	0.0305	0.0014
		0.4414**	

Total Tocopherol

Marker	Map	Partial R^2	P_Value*
PHI037	1.08	0.3052	0.0001
DUPO010	5.04	0.0972	0.0001
PHI034	7.02	0.0399	0.0003
		0.4422**	

Total Vitamin

Marker	Map	Partial R^2	P_Value*
PHI037	1.08	0.2563	0.0001
PHI034	7.02	0.0864	0.0001
DUPO010	5.04	0.0748	0.0001
ZAG244	9.02	0.0248	0.0038
BMC1154	6.05	0.0238	0.0039
UMC1300	3.04	0.0186	0.0093
		0.4847**	

*Marker significant at 0.01 deterermined by multiple regression.

**Total r-square value of the model.

Work accomplished to date on the cross of W64A x A632 F2:3 families.

1) Carotenoid and Tocopherol Concentrations determined by HPLC for 1995, 1996, and 1997, with 2 reps and 200 entries each year. (note: due to a poor growing season in 1995, HPLC information was not used for subsequent analysis).

2) Phenotypic analysis has been run, including looking at genotype by environment interactions. Best linear unbiased predictors (BLUPs) were also calculated, and were used for all QTL analysis.

3) 76 SSR markers have been scored on the population, with several more markers that have been determined to be polymorphic and will be run on the population.

4) Statistical analysis has been run including Single Factor Analysis and multiple regression. Both analysis have shown several regions with effects on concentration. Some mapping using the program Joinmap has been completed, but 15 to 20 well placed markers have been identified and need to be added to the population before a complete map can be drawn.

Abstract. The importance of tocopherols in human and stock nutrition is a subject of increasing economical importance. Maize is one of the most important grains worldwide, and a major source of vitamin E. Breeding towards improved vitamin-E content maize kernels is dependent on fast and reliable quantification of tocopherols. Maize lines differ greatly in their composition and total seed content of tocopherols and carotenoids. Carotenoid components present in some of the extracts are not always fully separated from the tocopherols when using HPLC for their determination. We observed that the absorbency of certain carotenoids can affect tocopherol determinations although tocopherols and carotenoids differ greatly in their UV-visible spectra. We have developed a reversed-phase HPLC system that effectively separates α-, δ-, and γ- tocopherol and avoids interference caused by carotenoids present in the extracts. The method is based on the utilization of low methanol level (3 %) in an acetonitrile-dichloromethane gradient in the presence of triethylamine. This method provides a reliable and accurate approach to the determination of tocopherols in maize kernels.

Table 7. Single factor analysis for (IHO x B73)B73 and (W64a x A632) for total tocopherol and percent α-tocopherol

(IHO x B73)B73

Total Tocopherol**

Marker	Map	P_Value*
Bngl381	2.03	0.0003
N410	4.01	0.0001
P200608	4.09	0.0132
Umc103	8.02	0.0005
Umc114	9.03	0.0003
Umc81	9.03	0.0006
ACCase	9.0?	0.0001
Bngl210	10.03	0.0027
Phi050	10.03	0.0058
Cdpfe85	11.00	0.0034

Percent α–Tocopherol***

Marker	Map	P_Value*
Phi113	5.02	0.0001
P150018	5.03	0.0001
P200589	5.04	0.0002
P100014	5.04	0.0001
P200531	5.06	0.0001
Phi034	7.02	0.0009
CO7BO2C	5.??	0.0001
Phi048	5.07	0.0001

(W64a x A632)

Total Tocopherol**

Marker	Bin	P_Value*
Phi074	4.04	0.0010
Dupo010	5.04	0.0001
Mmc0081	5.05	0.0001
Umc1155	5.05	0.0001
Umc1221	5.05	0.0001
Bmc1306	5.07	0.0001
Bmc1346	5.07	0.0001
Bmc2305	5.07	0.0001
Dupo015	6.06	0.0038
Bmc1136	6.07	0.0005

Percent α-Tocopherol***

Marker	Bin	P_Value*
Phi037	1.08	0.0001
Zag100	1.09	0.0001
Dupo010	5.04	0.0001
Umc1221	5.05	0.0008
Phi034	7.02	0.0022

* Marker significant at 0.01 determined by single factor analysis
** alpha + gamma tocopherol
*** alpha-tocopherol/ total tocopherol

The results from the (IHO/B73)B73 mapping population identified a number of chromosome regions associated with total levels of tocopherols. The results from the diallel analysis and the molecular marker mapping suggest that phenotypic selection in combination with molecular marker selection to pyramid QTL we have detected the may be very effective in improving levels of tocopherols and carotenoids. The results from comparison of the two molecular marker mapping studies suggest that different QTL in different genetic backgrounds could potentially be pooled to enhance levels of tocopherols in maize. The results from the two studies identify the same region(s) on chromosome 5 associated with ratio of alpha (more desirable form) to gamma tocopherol.

Evaluation of the research achievements as it relates to the original research proposal and its objectives. (Numbered objectives described in earlier section).

Objective 1.

We performed molecular marker mapping throughout the genome to search for associations of chromosomal regions with concentrations and form of tocols and carotenoids. This was performed on the W64a x A632 F3 mapping population (76 markers thus far) and the (IHO/B73)B73 BC1S1 mapping population (103 markers). The carotenoid data from the IHO/B73 BC population was not suitable for QTL analysis since it was segregating for the *y1* allele which is a null mutant of *phytoene synthase*. Comparison of QTL for tocopherols between the two populations revealed that a number of different QTL were detected in the different populations. Importantly though, a common region on chromosome 5 for ratio of alpha to gamma tocopherol was detected in both studies.

Therefore we essentially performed and completed objective 1.

Objective 2.

We used the information on carotenoid mutants, biosynthetic genes, and map locations to examine for associations of QTL for carotenoids and known genes. We have identified two candidate genes for carotenoids. We used a microsatellite marker internal to *y1, phytoene synthase*, bin 6.02, and this is the most significant marker in this region. We also have a leading candidate gene in bin 7.02, *zeta carotene desaturase*. For tocopherols, there were no previous genetic mutant stocks and clones of maize to work with. We have identified a region on chromosome 5 associated with ratio of alpha to gamma tocopherol in both mapping populations. This region is a candidate for the action of *gamma*

tocopherol methyl transferase. We recently obtained a putative *gamma tocopherol methyl transferase* gene of maize. If this gene does not map to 5, it is possible that chromosome 5 contains a regulatory region that controls the expression of *gamma tocopherol methyl transferase* gene.

Therefore we accomplished our goal for objective 2.

Objective 3.

We have identified some SSR markers in or near bins 6.02, 7.02 and 5.04 that appear tightly linked with QTL for carotenoids in A632 x W64a population. We have markers tightly linked with QTL for tocopherols in bins 1.08, 5.04, and 7.02 in the A632 x W64a population, and in bins 9.03 and 10.03 for the (IHO/B73)B73 BC1 S1 population. During the timeframe of this project our lab was in the process of switching from RFLPs to microsatellites (SSRs), and the marker coverage was very limited for SSRs in maize until very recently. However SSR markers are much better for marker assisted selection, so we decided to use SSRs rather than the older RFLP markers. Notably, the microsatellite in *y1*, is for a candidate gene *phytoene synthase*. If this candidate gene is confirmed, then this marker is as tightly linked as possible, since the marker is in the actual gene. Similarly if the two other candidates genes are confirmed, this provides the best kind of molecular marker, the gene itself, since there is no concern about recombination and linkage drag is minimized.

Therefore we accomplished to goals of this objective.

Objective 4.

We grew the testcross progeny of the first mapping population, (A632 x W64a F3s) x AEC335 in multiple locations and have saved seed in cold storage. We have performed

HPLC analysis for carotenoids and tocopherols on one environment of these materials. This is the only objective that we did not achieve fully. This is in part related to the HPLC equipment in Newe Ya'ar needed to be replaced, which was accomplished, but this took a couple years and then the system had to be optimized. We plan to run HPLC on one or more environments this fall at Newe Ya'ar and Illinois, after the new HPLC equipment just ordered comes to Illinois this summer (as the HPLC equipment we used at Illinois is in another lab is older and somewhat inefficient).

This is the only objective we did not fully accomplish during the time frame of the project. However we are very interested in the results and will perform these assays required to complete this objective with the improved and more expedient procedures developed during the course of this project.

Objective 5.

From the QTL analyses, we identified a series of W64a x A632 F3 progenies with higher overall levels of tocopherols and carotenoids and have one or more of the most favorable QTL for carotenoids and tocopherols. These families were backcrossed to a B73 type elite line (FR1064) to develop backcross progenies that are now available for marker assisted introgression. For carotenoids, we particularly wish to select QTL in bins 6.02 and 7.02. For tocopherols we wish to select QTL in bins 1.08 and 5.04.

Therefore we accomplished the goal of this objective.

Description of the Cooperation

There was one visit by Torbert Rocheford to Israel in February, 1997 and two visits by Kobi Tadmor to Illinois in September 1996, and October 1998. We regularly exchanged seed, materials, protocols and results between the locations. Illinois helped the Newe Ya'ar group with establishing a new HPLC procedures, sharing protocols and sending materials and checks to help in establishing this analytical capability. All HPLC analyses for the second population studied, IHO/B73 BC, was performed at Newe Ya'ar. Both locations grew genetic progenies and performed molecular marker assays. We are now integrating data sets and will be completing analyses this summer and fall. These results will be put into joint manuscripts with authors from both locations that will be submitted for publication. This was a totally new project, with initial progenies just created at the beginning of the project. We have progressed to develop routine HPLC and SSR marker expertise at both locations and will be shortly summarizing the fruits of these collaborative efforts. Some collaboration was hindered somewhat by Rocheford being on sabbatical in England for a one year period in 1997-1998. This resulted in the omission of Newe Ya'ar scientists on a couple abstracts due to the graduate student preparing the abstract and not being aware of the appropriate procedures. The papers we will submit and publish together soon (including HPLC paper accepted) will fully reflect our collaboration and will be co-authored.

We did not submit a renewal proposal due to the new BARD policy established in May 1999 that a scientist could not be involved on more than one BARD project at a time, since Kobi Tadmor and Nurit Katzir are on other BARD funded projects. Perhaps in

the future we will submit another proposal when allowable under this new policy, as we have interesting results that we wish to continue to collaborate on.

Conclusions

Overall the project was very successful in achieving its goals. We have identified molecular markers associated with large amounts of variation for overall levels and specific forms of tocopherols and carotenoids. Different QTL for tocopherols have been identified in maize grain in two mapping populations. We have found a considerable amount of variation for tocopherols and carotenoids in diallel analyses of 10 inbreds. Collectively, these results strongly suggest the levels of tocopherols and carotenoids could be increased in maize grain through conventional and molecular marker assisted selection. Two candidate genes have been identified for control of levels of carotenoids, phytoene synthase, and zeta carotene desaturase. Detection of these candidate genes suggests structural genes in the carotenoid biosynthetic pathway may have allelic variation that contributes to quantitative variation in levels of carotenoids. We have identified a QTL that affects the ratio of alpha (more desirable) to gamma tocopherol in both mapping population. A promising candidate gene responsible for this QTL may be *the gamma tocopherol methyl transferase* gene, or a regulatory gene that influences expression of *gamma tocopherol methyl transferase* gene or function of the gene product.

List of Publications

Y. Tadmor, J.Wong, O. Larkov, A. Meir, M. Minkhoff, E. Lastochkin, Y. Hershenhorn, M. Edelstein, S. Levin, T. Rocheford, and E.Lewinsohn. (2000) Reversed-phase HPLC determination of vitamin E components in maize kernels. Phytochemical Analysis. (In Press).

Y. Tadmor, E. Lewinsohn, O. Larkov, A. Meir, M. Minkhoff, E. Lastochkin, Y. Hershenhorn, M. Edelstein, S. Levin. Reversed-phase HPLC determination of vitamin E components in maize kernels. The annual meeting of the Israeli Analytical Chemistry Society. Tel-Aviv, Feb 2000.

C. O. Egesel, 1993 Inheritance and Genetic Variability in Carotenoids, Tocols, and Related Traits in Maize-University of Illinois, Urbana. M.S. Thesis.

J. C. Wong, 1999 Molecular Marker Mapping of Chromosomal Regions Associated with Levels of Carotenoids and Tocopherols in Maize Kernals-University of Illinois, Urbana. M.S. Thesis.

J.C. Wong, T.R. Rocheford, R.J. Lambert, and C.O. Egesel. Mapping Genes Associated with Levels and Forms of Carotenoids and Tocopherols in Zea Mays. Agronomy Society of America. October, 1997.

J. C. Wong, C. O. Egesel, A. C. Kurilich, T. R. Rocheford, R. J. Lambert and J. A. Juvik. Genetic Variation in Maize for Vitamin A and Vitamin E. 34th Corn Breeder School, Urbana, Illinois. March, 1998.

J.C. Wong, C.O. Egesel, A.C. Kurilich, R.J. Lambert, J.A. Juvik, and T.R. Rocheford. Mapping genes Associated with Levels and Forms of Carotenoids and Tocopherols in Maize. 40th Maize Genetics Conference Abstract. March, 1998.

Wong, J.C. , Lambert, R.J. , and Rocheford, T.R. Molecular Marker Mapping of Chromosomal Regions Associated with Carotenoids and Tocopherols in Maize. 42nd Maize Genetics Conference Abstract. March, 2000.

Report on any patents

No patents were filed during the period of this grant. We currently do not have plans to file patents in the near future based on the work performed in this project.