XA9846996

INCORPORATION OF CONVENTIONAL GENETIC MARKERS AND RAPD MARKERS INTO AN RFLP BASED MAP IN MAIZE

E.H. COE, Jr., M.D. McMULLEN, M. POLACCO
USDA-ARS, Plant Genetics Research Unit,
Columbia, Missouri, United States of America

G.L. DAVIS
USDA-ARS, Corn Host Plant Resistance Research Unit,
Mississippi State University, Mississippi, United States of America

S. CHAO
Institute of Botany,
Academia Sinica,
Nankang, Taipei, Taiwan

Abstract

Integration of classical genetic markers, in particular mutants, onto the maize Restriction Fragment Length Polymorphism (RFLP) map will provide the tools necessary to further our understanding of plant development and of complex traits. Initially integration was accomplished by visual alignment of common markers and sometimes involved the use of information from several different molecular maps to determine the relative placement of a single mutant. The maize core marker set was designed to provide a common set of markers which could be used for integration of map data. We have completed the mapping of 56 mutants on chromosome one relative to the core marker set. Phenotypes included whole plant, seedling, and kernel effects and represented a variety of biological processes. Since these mutants were previously located to chromosome arm, mapping required the use of only seven markers per mutant to define the correct bin location. Two mistakes in marker order relative to the classical map were identified, as well as, six groups of mutants which require allelism testing. Placement of mutants and cDNAs into bins using the core markers provides a necessary resource for identification of gene function in maize.

1. INTRODUCTION

Genetic and molecular maps in maize have been available for 61 and 11 years, respectively [1, 2]. Integration of the two will help us towards the goal of understanding complex traits. Establishment of a set of "core" markers which evenly divide the genome has provided the necessary bridge to accomplish integration of them [3, 4]. Through these markers any mutant mapped using publicly available clones can be placed on the maize bin map (http:\\www.teosinte.agron.missouri.edu). Individual bins comprise approximately 20 cM intervals along the chromosome. In addition to Restriction Fragment Length Polymorphism (RFLP) markers and mutants, Quantitative Trait Loci (QTLs) can also be assigned to bins. The most informative RFLP markers for studying complex traits are sequenced cDNAs (expressed sequence tags; ESTs) because of their potential to mark sites of known function. Randomly Amplified Polymorphic DNA (RAPD) markers, while more economical, present a less desirable alternative to cDNAs because they provide no potential information on function at the site they mark.

Given the myriad of mutants which have been identified in maize and the current technologies available for large scale sequencing, we are focusing our efforts in two areas: mapping of mutants relative to molecular markers and mapping of ESTs, with the goal of providing a resource of molecularly or functionally defined genes catalogued by location. Our specific objectives were to define a set of publicly available core markers for use in integrating mapping data and to use these markers to map maize mutants onto the bin map.

2. MATERIALS AND METHODS

2.1. Core markers

Potential core markers were chosen initially based on simple fragment pattern and even distribution at approximately 20 cM intervals on the chromosomes. Markers not among the previous core marker set identified by Gardiner *et al.* [3] were screened against inbreds A619, A632, B73, Mo17, CO159, and Tx303 using *Eco*RI, *Hind*III, *Eco*RV, *Bam*HI, *Dra*I, *Xba*I, *Bgl*II, and *Sst*I and the polymorphism rate was assessed. A marker was designated acceptable if it was polymorphic with a minimum of three of the eight enzymes with a majority of the inbred lines. A number of substitutions were made compared to the previous core marker set due to low levels of polymorphism or high fragment pattern complexity. Final choices were made based on even spacing, simple fragment pattern, high degree of polymorphism, and public availability. Markers meeting these criteria that had insert sizes less than 1000 base pairs were given preference to facilitate single-pass sequencing.

Core markers were prepared for sequencing using the alkaline lysis method. DNA quality was assessed using 0.8% agarose gels containing cut and uncut DNA. Quantity was determined using a spectrophotometer. Sequencing was performed either by dideoxy-termination reactions labeled with ^{35}S α-dCTP according to manufacturer recommendations for the T7 Sequenase Kit (US Biochem) or by PCR incorporation of fluorescently labelled bases followed by sample processing on the ABI sequencer. Results of the manual sequencing were entered into DNA to determine overlap. BLASTX searching was conducted against the nonredundant database of GenBank. All matches were evaluated using the MOTIFS program to determine whether homology occurred in a conserved protein domain.

2.2. Mutant mapping

Mutants used for this study were previously mapped to chromosome arm by genetic experiments involving other mutants or by cytogenetic tests. The mutants used represent whole plant, seedling and kernel defects and include a variety of biological processes. A list of mutants and phenotypes is provided in Table I. More detailed phenotypic descriptions are presented for the mutants which have been given gene designations.

One to four F_2 mapping populations were derived for each individual mutant where the nonmutant parent was one of the following inbreds, A619, A632, B73, or Mo17. Attempts were made to produce four segregating populations for each mutant to maximize the chance of obtaining polymorphisms for each of the core markers evaluated and to allow future evaluation of potential modifiers of trait expression.

Forty kernels per F_2 family were planted in the field. For mutants expressed in the seedling or adult plants, families were scored for presence of the mutant and individuals self-pollinated. Kernel phenotypes were scored on F_2 ears prior to planting in the field and again on F_3 ears. In families with seedling phenotypes, forty F_3 seed from each ear were evaluated in the sandbench to determine the genotype of each F_2 individual. In families with adult plant phenotypes, forty F_3 seed from each ear were evaluated in the field the following season to determine genotypes for each F_2 individual.

Tissue for RFLP analysis was collected from either field grown individuals or sandbench bulked F_3 progeny rows. 100 mg. of lyophilized tissue was extracted for each individual using

an ammonium acetate extraction technique. Extractions were divided into two aliquots and digested with *Eco*RI and *Hin*dIII. Southern analysis of *Eco*RI and *Hin*dIII digested DNA for each F_2 family was performed according to standard laboratory procedures [5].

TABLE I. MUTANTS AND THEIR ASSOCIATED PHENOTYPES

Mutant[1]	Phenotype
ad*	adherent seedling
ad1	adherent 1--seedling leaves, tassel branches, and occasionally top leaves adhere
an1	anther ear--andromonoecious dwarf of intermediate stature
blh*	bleached seedling
Blh1	bleached 1--dominant plants have pale green midveins and base in the upper leaves of the plant
bnk*	brown kernel
br*	brachytic plant
cp*	collapsed kernel
ct2	compact plant 2--plant is semi-dwarfed with a club tassel
d*	dwarf
dcr*	defective crown
de*	defective kernel
dnt*	dent
et*	etched
hcf31	high chlorophyll fluorescence 31--yellow/green seedlings lacking chlorophyll a/b binding protein
l*	luteus
l16	luteus 16--yellow green leaves with patches that bleach to paler yellow
lls1	lethal leaf spot 1--chlorotic to necrotic lesions similar to those of *Helminthosporium carbonum* infected plants
nec*	necrotic seedling
pg*	pale green
pg15	pale green 15--light yellow green seedling with patches bleaching to white
rs2	rough sheath 2--short, zigzag plants with wart-like distorted leaves and sheaths
Smk*	small kernel
spc2	speckled 2--green seedlings containing light green speckles
spc*	speckled seedling
sr1	striate 1--leaves contain many white stripes
tlr*	tillered plant
ty*	tiny plant
v*	virescent leaf
Vg1	vestigial glume 1--glumes very small leaving cob and anthers exposed; upper leaves also
have	scant ligules
vp*	viviparous
vp5	viviparous 5--embryo fails to become dormant; white endosperm and seedling; encodes phytoene C-11,12 desaturase
vp8	viviparous 8--embryo doesn't become dormant; produces small, pointed-leaved seedlings
w*	white seedling
wl*	white luteus seedling
wlu5	white luteus 5--cream colored seedling
zb4	zebra crossbands 4--alternating bands of green, yellow-green, and white; more pronounced in cooler temperatures
zb7	zebra crossbands 7--alternating bands of green and pale green on glossy seedlings
zl1	zygotic lethal 1--homozygous recessive zygotes do not develop

[1] Mutant designations followed by an astericks have not yet been designated as distinct genes pending further information regarding map locations and allelism tests.

Hybridizations were carried out using ^{32}P α-dCTP labelled probes in bulk lots of 40 membranes corresponding to a chromosome arm using each of the individual core markers for that arm. The hybridization mixture was reused the following day for a second set of 40 membranes. Prehybridization and hybridization protocols followed the standard laboratory procedures [5]. Following washing, the radioactive membranes were exposed to Kodak X-OMAT film in the presence of a Cronex-type intensifying screen at -80 C. Marker *bnl8.29* was substituted for *umc161* as *umc161* was not polymorphic in these materials.

Mapscores were aligned in core marker order in a spreadsheet and mutants were tentatively placed to an interval by minimizing the number of double-crossover events. Map locations were confirmed using Mapmaker for MacIntosh version 2.0. A second confirmation based on concurrent map locations of the same mutant mapped in different F_2 backgrounds was also used as a check.

3. RESULTS

3.1. Core markers

A set of ninety maize core markers were identified that best met the selection criteria. Table II lists the core markers, their bin number, and homology information. The average distance between core markers is 18 cM. Information regarding polymorphisms for the inbreds and enzymes tested is to be made available through the Maize Genome Database. Core marker sets are available through the probe request form of the database or by contacting the lab directly. The set consists of 75 genomic and 15 cDNA markers. Homology to genes of known function was identified by BLASTX searching of the nonredundant Genbank database for 20 of the 90 core markers with functionality of an additional 4 clones identified by targeted cloning experiments. Twenty-four percent of the genomic cores and 20% of the cDNA cores derived by non-targetted cloning had homology to genes with known function.

3.2. Mutant mapping

56 mutants were mapped to bins on maize chromosome 1 (Table III). Prior to this study, 44 of the mutants were known only to be located to chromosome arm, 21 on the short arm and 23 on the long arm. No mutants were mapped in bin 1.06 which is believed to contain the centromere based on translocation studies. The map order of *v5* relative to *sr1* and *zb4* relative to *p1* was found to be reversed on the classical genetic map. Six groups of mutants with similar phenotype were identified based on bin assignment which are candidates for allelism testing. They are *zl1*, *et*-0634C*, *vp5*, and *cp*-1078A*; *ty*-0780A and *bnk*-1519C*; *wl*-1930* and *wl*-144*; *wlu5*, *w*-1890*, *wl*-47*, *wl*-56*, and *wl*-60*; *l*-129* and *l*-544*; and *spc*-370*, *spc2*, and *blh*-43*. One group, *ty*-0780A* and *bnk*-1519C*, was identified when it was noticed that some plants in the family segregating for brown kernel were also dwarf. Subsequent analysis revealed that these were the brown kernel individuals.

4. DISCUSSION

A fast, easy, cost-effective strategy for mapping a large number of mutants has been developed. Approximately 400 samples can be isolated by one individual in a day at a cost of one cent each. A maximum of seven probes are required to place a mutant into its correct bin. Three additional probes for a total of ten will place it in a five centiMorgan interval. By grouping the mutants into bins prior to testing for allelism, the number of crosses needed to determine allelic relationships is greatly reduced.

TABLE II. UNIVERSITY OF MISSOURI - COLUMBIA (UMC) MAIZE CORE MARKERS INCLUDING BIN ASSIGNMENTS AND HOMOLOGY INFORMATION

Marker name	Bin[1]	Homology information
p-tub1	1.01	β-tubulin
p-umc157	1.02	no match
p-umc76	1.03	no match
p-asg45	1.04	protein kinase; U26746, *Brassica napus*
p-csu3	1.05	no match
p-umc67	1.06	no match
p-asg62	1.07	no match
p-umc128	1.08	no match
p-csu164	1.09	no match
p-umc107	1.10	DNA-binding protein; transcription factor U39361 human
p-umc161	1.11	no match
p-bnl6.32	1.12	no match
p-bnl8.45	2.01	no match
p-umc53	2.02	no match
p-umc6	2.03	no match
p-umc34	2.04	no match
p-umc131	2.05	pistil-specific extensin Z14019 *Arabidopsis thaliana*
p-umc255	2.06	no match
p-umc5	2.07	no match
p-asg20	2.08	no match
p-umc49	2.09	no match
p-php20581	2.10	extensins Z46674 tomato
p-umc32	3.01	no match
p-csu32	3.02	no match
p-asg24	3.03	glutamyl-tRNA synthetase; Z91787 *Lupinus luteus*
p-asg48	3.04	no match
p-umc102	3.05	no match
p-bnl5.37	3.06	no match
p-bnl6.16	3.07	various different proteins
p-umc17	3.08	no match
p-umc63	3.09	no match
p-csu25	3.10	P450; U3907 rat
p-agr r115	4.01	not sequenced
p-php20725	4.02	no match
p-umc31	4.03	no match
p-npi386	4.04	various proteins containing polyproline runs
p-agr r37	4.05	no match
p-umc156	4.06	no match
p-umc66	4.07	plant transposon; X52387 potato
p-umc127	4.08	no match
p-umc52	4.09	extensin; X55681 tomato
p-php20608	4.10	no match
p-umc169	4.11	extensin; X55686 tomato
p-npi409	5.01	no match
p-umc90	5.02	no match
p-tub4	5.03	β-tubulin
p-bnl4.36	5.04	not sequenced
p-csu93	5.05	no match
p-umc126	5.06	no match
p-umc108	5.07	various different proteins
p-bnl5.24	5.08	no match
p-php10017	5.09	no match

TABLE II. (cont.)

Marker name	Bin[1]	Homology information
p-umc85	6.01	no match
p-umc59	6.02	no match
p-npi393	6.03	no match
p-umc65	6.04	no match
p-umc21	6.05	no match
p-umc38	6.06	no match
p-umc132	6.07	no match
p-asg7	6.08	no match
p-asg8	7.01	myb related proteins; X13294 human A-myb protein
p-asg34	7.02	methylmalonate-semialdehyde dehydrogenase; M84911, *Pseudomonas aeruginosa*
p-asg49	7.03	no match
p-umc254	7.04	no match
p-umc245	7.05	no match
p-umc168	7.06	no match
p-npi220	8.01	no match
p-bnl9.11	8.02	leucyl-tRNA synthetase; M88581, *Bacillus subtilis*
p-umc124	8.03	choline kinase active site; U62317 human
p-bnl7.08	8.04	no match
p-bnl2.369	8.05	not sequenced
p-csu31	8.06	no match
p-npi268	8.07	no match
p-npi414	8.08	no match
p-agr r21	8.09	not sequenced
p-umc109	9.01	Constans protein; U62317, *Arabidopsis thaliana*
p-umc192	9.02	bronze 1
p-umc25	9.03	waxy 1
p-csu147	9.04	no match
p-umc95	9.05	no match
p-csu61	9.06	no match
p-asg12	9.07	no match
p-csu54	9.08	no match
p-php20075	10.01	gibberellin (and auxin) stimulated proteins: X63093, tomato
p-npi285	10.02	various calcium channel proteins; L29346 mouse
p-umc130	10.03	no match
p-umc64	10.04	no match
p-umc259	10.05	no match
p-umc44	10.06	no match
p-bnl7.49	10.07	homeobox domain; U34743 orchid

[1]Bins are defined as the interval between two adjacent core markers starting with the first (upper) core marker and continuing until just before the second (lower) core marker.

TABLE III. BIN ASSIGNMENTS OF 56 MAIZE MUTANTS

Mutant	Bin assignement[1]
zl1	1.01
et-0634C*	1.01
vp5	1.01
Blh1	1.01
dcr-1176A*	1.01-1.02
cp-1078A*	1.01-1.02
nec-0495B*	1.01-1.02
pg15	1.02
sr1	1.02
Smk-845B*	1.02-1.03
vp-1136B*	1.02-1.04
ct2	1.03
hcf31	1.03
lls1	1.03
de-1345B*	1.03
pg-484B*	1.03
de-1081*	1.03-1.04
l16	1.04
ty-0780A*	1.04
zb4	1.04
bnk-1519C*	1.04
de-1390A*	1.04
wl-1930*	1.05
rs2	1.05
wl-144*	1.05
de-1057B*	1.05
wlu5	1.07
w-1890*	1.07
wl-47*	1.07
wl-56*	1.07
wl-60*	1.07
Vg1	1.07
an1	1.08
ad1	1.08
w-1802*	1.08-1.09
Mutant	Bin assignment[1]
cp-0948A*	1.08-1.09
*br**	1.08-1.09
de-1016A*	1.08-1.09
de-1420*	1.08-1.09
d-1352B*	1.09
l-129*	1.09
dnt-1185A*	1.09
l-544*	1.09
tlr-2245*	1.09-1.11
d-0998B*	1.10
spc-370*	1.10
v-55*	1.10
zb7	1.10
ad-582*	1.10
blh-43*	1.10
de-1061A*	1.10-1.11
spc2	1.10-1.11
pg-343*	1.10-1.11
v-245*	1.11
vp8	1.11

[1] Mutants listed with a range of bin assignments are the result of lack of polymorphism for the intervening core marker and could be assigned to an individual bin using additional markers.

The maize RFLP and genetic maps can be aligned based on the framework established by the core markers. The current UMC (University of Missouri, Columbia) Maize RFLP map contains 982 loci, 49% of which have sequence information and 29% of which correspond to genes of known function [4]. Additional ESTs from the Brookhaven and INRA maps can be assigned to bins enhancing the number of loci with information about function [6, 7]. The current bin map maintained and updated in the Maize Genome Database contains 3516 binned loci including RFLPs, mutants, and cytological events. Combination of sequence and function information either as ESTs or phenotypic mutants provides the resource necessary for gene discovery in maize. Cataloguing the loci into bins enables identification of potential associations of functions with phenotypes including quantitative trait loci which warrant further investigation.

REFERENCES

[1] EMERSON, R.A., BEADLE, G.W., FRASER, A.C., A summary of linkage studies in maize, Cornell Univ. Agric. Exp. Stn. Memoir 180 (1935) 1-83.

[2] HELENTJARIS, R., SLOCUM, M., WRIGHT, S., SCHAEFER, A., NEINHUIS, J., Construction of genetic linkage maps in maize and tomato using restriction fragment length polymorphisms. Theor. Appl. Genet. 72 (1986) 761-769.

[3] GARDINER, J.M., COE, E.H., Jr., MELIA-HANCOCK, S., HOISINGTON, D.A., CHAO, S., Development of a core RFLP map in maize using an immortalized F2 population, Genetics 134 (1993) 917-930.

[4] YERK-DAVIS, G.L., McMULLEN, M.D., GRANT, D., MUSKET, T., BAYSDORFER, C., STAEBELL, M., XU, G., POLACCO, M., KOSTER, L., HOUCHINS, K., CHAO, S., COE, E.H., Jr., An improved maize map standard: ESTs and sequenced core markers in a 982-point map. (in preparation for submission to Genetics).

[5] RFLP Procedures Manual. Maize RFLP Laboratory. University of Missouri-Columbia, 44p.

[6] MATZ, E.C., BURR, F.A., BURR, B., Molecular maps based on TxCM and CoxTx recombinant inbred families, MNL 69 (1995) 257-267.

[7] CAUSSE, M., SANTONI, S., DAMERVAL, C., MAURICE, A., CHARCOSSET, A., DEATRICK, J., DEVIENNE, D., A composite map of expressed sequences in maize, Genome 39 (1996) 418-432.