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**Mapping and Tagging by DNA Markers of Wild
Emmer Alleles that Improve Quantitative Traits
in Common Wheat**

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CASL = chromosome-arm substitution line, RSL = recombinant substitution line,
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Mapping and Tagging by DNA Markers of Wild Emmer Alleles That Improve Quantitative Traits in Common Wheat

Abstract

The general goal was to identify, map, and tag, with DNA markers, segments of chromosomes of a wild species (wild emmer wheat, the progenitor of cultivated wheat) determining the number, chromosomal locations, interactions, and effects of genes that control quantitative traits when transferred to a cultivated plant (bread wheat). Slight modifications were introduced and not all objectives could be completed within the human and financial resources available, as noted with the specific objectives listed below:

1. To identify the genetic contribution of each of the available wild emmer chromosome-arm substitution lines (CASLs) in the bread wheat cultivar Bethlehem for quantitative traits, including grain yield and its components and grain protein concentration and yield, and the effect of major loci affecting the quality of end-use products. [*The quality of end-use products was not analyzed.*]
2. To determine the extent and nature of genetic interactions (epistatic effects) between and within homoeologous groups 1 and 7 for the chromosome arms carrying "wild" and "cultivated" alleles as expressed in grain and protein yields and other quantitative traits. [*Two experiments were successful, grain protein concentration could not be measured; data are partially analyzed.*]
3. To derive recombinant substitution lines (RSLs) for the chromosome arms of homoeologous groups 1 and 7 that were found previously to promote grain and protein yields of cultivated wheat. [*The selection of groups 1 and 7 was based on grain yield in pot experiments. After project began, it was decided also to derive RSLs for the available arms of homoeologous group 4 (4AS and 4BL), based on the apparent importance of chromosome group 4, based on early field trials of the CASLs.*]
4. To characterize the RSLs for quantitative traits as in objective 1 and map and tag chromosome segments producing significant effects (quantitative trait loci, QTLs) by RFLP markers. [*Producing a large population of RSLs for each chromosome arm and mapping them proved more difficult than anticipated, low numbers of RSLs were obtained for two of the chromosome arms.*]
5. To construct recombination genetic maps of chromosomes of homoeologous groups 1 and 7 and to compare them to existing maps of wheat and other cereals. [*Genetic maps are not complete for homoeologous groups 4 and 7.*]

The rationale for this project is that wild species have characteristics that would be valuable if transferred to a crop plant. We demonstrated the sequence of chromosome manipulations and genetic tests needed to confirm this potential value and enhance transfer.

This research has shown that a wild tetraploid species harbors genetic variability for quantitative traits that is interactive and not simply additive when introduced into a common genetic background. Chromosomal segments from several chromosome arms improve yield and protein in wheat but their effect is presumably enhanced when combination of genes from several segments are integrated into a single genotype in order to achieve the benefits of genes from the wild species. The interaction between these genes and those in the recipient species must be accounted for. The results of this study provide a scientific basis for some of the disappointing results that have historically obtained when using wild species as donors for crop improvement and provide a strategy for further successes.

Achievements

The ambitious goal of identifying, mapping, and tagging segments of chromosomes of a wild species (emmer wheat) determining the number, chromosomal locations, interactions, and effects of genes that control quantitative traits when transferred to a cultivated plant (bread wheat) was largely achieved through intensive research to prepare the appropriate genetic stocks, develop molecular maps of the chromosome arms, and test the derived lines under agricultural conditions in the field.

Rationale for the project is given in Appendix Section 1, details of the methods used and the genetic stocks developed are given in Appendix Section 2, and rather detailed results are summarized in Appendix Section 3 of this report. Below the major results and interpretations are summarized.

Main scientific achievements and their significance

1. Wild emmer wheat, *Triticum turgidum* ssp. *dicoccoides*, (identified as TTD henceforth) was shown to contribute genes that modify the morphology and performance of Bethlehem, the hexaploid wheat substitution recipient.
2. Genetic effects with respect to eight traits were found to have been contributed from throughout the A and B genomes of TTD; virtually every chromosome arm contained genes having significant effects.
3. The recipient variety, Bethlehem, is a modern bread wheat with very acceptable performance in Israel and California; substitution of chromosome arms for their homologues from an agriculturally unmodified wild species generally reduced the grain yield of Bethlehem.
4. Heterosis due to heterozygosity in the hybrids of the chromosome arm substitution lines (CASLs) with Bethlehem and between CASLs was large for grain yield and other traits.
5. Reduction in grain yield was due to interactive expression of genes controlling the endpoint components of grain yield, including kernel number per spike and kernel weight.
6. Genetic effects of yield components were distributed on all of the TTD chromosome arms, with both positive and negative effects apparent.
7. Molecular mapping of the effects contributed by TTD was not completely successful, due primarily to the complications of epistasis (the interaction of the contributed segments and existing genes in Bethlehem), and requires further analysis and experimentation.

8. This research has provided evidence that latent genetic variability in the wild species can be expressed in a domesticated wheat background and further that direct observations on the wild species do not provide good guidance for what genetic variation can be expressed in another species.
9. The results of this research have validated the use of single-chromosome arm substitution lines for dissecting complexly inherited quantitative traits. This is the first instance of such effort being accomplished and evaluated on a polyploid crop species.

This research has shown that a wild tetraploid species harbors genetic variability for quantitative traits that is interactive and not simply additive when introduced into a common genetic background, such as Bethlehem used in this study. The gene effects were distributed throughout both of the genomes of the wild tetraploid species. While the contributions of individual chromosome arms of TTD were detrimental to fitness of the recipient wheat variety, evaluation of the fitness components, i. e., endpoint components of grain (seed) yield, showed that compensating genetic effects caused the reduction in fitness and that these effects were due to many different chromosome arms. Hence, the scientific message is that fitness is due to highly complex interactions of genes affecting numerous morphophysiological traits.

The agricultural implications are clear, but not easily exploited in usual plant breeding practice. Combinations of genes from several chromosome arms must be integrated into a single genotype in order to achieve the benefits of genes from the wild species. The results of this study provide a scientific basis for some of the disappointing results that have historically obtained when using wild species as donors for crop improvement. The results also concur with research on other crop species, such as tomato, where dissection of the genetic effects contributed by a wild species revealed latent genetic variability that could be exploited in plant breeding.

The genetic variability introduced to bread wheat can be used with the assistance of molecular markers as tags, but as yet, specific markers were not identified. However, in the meantime, breeding can proceed by combining desired genes by hybridization of lines carrying specific chromosome arms followed by selection desired phenotypes.

Details of Cooperation

This research was possible because of the long-term efforts at the Weizmann Institute in Israel to construct the genetic stocks before this project was initiated.

Most of the 28 possible single-chromosome arms of the wild tetraploid *Triticum turgidum* ssp. *dicoccoides* were substituted in the hexaploid bread wheat, Bethlehem. The resulting CASLs were shared with researchers at the University of California, Davis. Other genotypes (e.g., ditelosomic lines) required for the production of RSLs were also sent from Israel to the US. The two research teams shared equally the responsibility for evaluating the CASLs in field trials, in the development of RSLs, and the determination of recombination points by molecular mapping. At Davis, a two-year study of a diallel cross using the CASLs of chromosome groups 1 and 7 was conducted using inter-CASL hybrids and Bethlehem x CASL hybrids as a second approach to determining gene interactions (epistasis) in quantitative traits. The Israeli group did grain protein analyses of both the Israel- and US-generated material. Both groups have been involved in data analyses and interpretation.

Investigators have been in regular electronic mail contact over the course of the project. In addition, the scientists have met several times at international meetings beginning just before the project commenced to discuss the work and plan future steps (August 1996, Sydney, NSW Australia; June 1997, Clermont-Ferrand, France; August 1998, Saskatoon, SK Canada; and August 1999, Viterbo, Italy). US investigator Qualset visited the Weizmann Institute in April 1999.

List of publications

No publications have been issued to date. Several abstracts have appeared at scientific meetings.

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Section 1. Rationale and Background

Wild species have characteristics that would be valuable if transferred to a crop plant. For example, common wheat, like many other crops, exhibits a relatively narrow range of genetic variation (Chao et al. 1989). This narrow genetic basis not only increases vulnerability of wheat to environmental and biotic stresses but, more importantly, greatly limits the yielding capacity and yield quality of this basic crop. Wild emmer wheat, *Triticum turgidum* ssp. *dicoccoides* ($2n=4x=28$; genomes AABB; a progenitor of durum and common wheat) contains many alleles with the potential to broaden the genetic basis of cultivated wheats and improve agronomically important traits such as grain yield, grain protein, and tolerance to stresses (Feldman et al. 1994). Genetic tests must be done to confirm the potential value of wild species as genetic resources. For practical reasons, these genetic tests are generally applied sequentially as (1) hybridization of the wild species to the cultivated species, (2) production of single-chromosome addition lines in the target variety, (3) production of single-chromosome substitution lines, (4) production of single-chromosome-arm substitution lines, and (5) integration of segments of chromosomes from the wild species into the recipient species by recombination (crossing over) between the wild and cultivated chromosome arms. At each stage the resulting plants are evaluated for expression of the traits that were shown in the wild species itself. Positive expression provides evidence for genetic control of the traits. The specific chromosomes controlling the traits are identified in stages 2 and 3 and the specific segments in stage 4.

This process can be abbreviated for traits from the wild species that are controlled by single genes because a single chromosome is involved and can be identified relatively easily (Rong et al. 2000). There are numerous examples of successful gene transfers that have used a variety of cytogenetic procedures (Feldman 1988). This work, while challenging, has become a routine plant breeding procedure. Unfortunately, this is not the case for quantitatively expressed traits, such as tolerance to environmental stresses, grain yield, and end-use quality properties, that are controlled by several or many genes that are widely distributed throughout the genome. A further complication is that genes interact epistatically, meaning that individual effects of genes are not cumulative and the resulting phenotype may be more or less than expected based on single gene expression. Conventional plant breeding has not been successful in making such complex transfers into

agronomically suitable varieties. We desire efficient methodologies to identify and select segments of chromosomes containing genes controlling complex traits.

It is possible to resolve epistatic effects by studying chromosome segments in defined combinations in a common genetic background. This can be done by intercrossing substitution lines and studying the means and frequency distributions for traits in the F_1 and F_2 or later generations. Results from these studies provide guidance about which genes should be selected in combination for the best performance, i.e., favorable epistatic interactions.

Two procedures facilitate genetic mapping of quantitative trait loci (QTLs) and their tagging with DNA markers. The first is based on the capacity of producing a “genomic chromosome segment library” of a genome of one genotype in the background of another by the use of CASLs and RSLs of these arms (Feldman and Millet 1995). In these lines the contribution of one or a few segments of the donor genotype can be assessed with a minimal interference from the other segments of the donor.

The second is the use of DNA markers (RFLP) for genetic mapping of wheat chromosomes. Several hundred DNA probes have been produced in various laboratories and mapped to bread wheat chromosomes. In this way, a genetic map of wheat, consisting of a large number of markers, and in more than one genetic background, has been produced.

To identify alleles of wild emmer that improve quantitative traits in common wheat, it is necessary to reduce or even eliminate undesirable wild alleles with deleterious effect that may mask the expression of the desirable ones. Hence, it was imperative to dissect the wild genome into small segments and to introduce each segment separately into the background of common wheat, i.e., to produce a “genomic library” of the wild emmer genome in a cultivated wheat background. This ambitious goal was largely achieved through intensive research to prepare the appropriate genetic stocks, developing molecular maps of the chromosome arms, and testing the derived recombination lines under agricultural conditions in the field.

Section 2. Materials and Methods

Plant material

Parental stocks. Accession TTD140 of wild emmer wheat, *Triticum turgidum* ssp. *dicoccoides* (TTD) as donor genotype and the Israeli high-yielding modern wheat *T. aestivum* cv Bethlehem (BL) as recipient genotype were the starting plant materials. These genotypes facilitate molecular mapping of chromosome arms since a higher polymorphism is found between these two genotypes than is generally found between two genotypes of cultivated wheat.

Single chromosome arm substitution lines. The initial material was a series of chromosome arm substitution lines (CASLs) that were developed by the Israel collaborators by the method described in Feldman and Millet (1995). Twenty-two of the possible 28 CASLs were available for the current research (Table 2.1). In each CASL, a single desired pair of chromosome arms of BL was replaced by the homologous pair of chromosome arms from TTD.

Table 2.1. TTD chromosome arm substitution lines of Bethlehem wheat. (X indicates available line, O indicates that the line has not been produced.)

Chromosome group	Genome			
	A		B	
	Substituted arm		Substituted arm	
	Short	Long	Short	Long
1	X	X	X	X
2	X	O	X	X
3	X	X	X	X
4	X	O	O	X
5	X	O	X	O
6	X	X	O	X
7	X	X	X	X

Diallel cross. The eight CASLs of chromosome groups 1 and 7 were intercrossed in all possible one-way combinations and with BL to produce a 9-parent diallel cross. Reciprocal crosses were not made. The crosses were made in the greenhouse at Davis in 1996 for the 1997 field trial. Results from that trial indicated that a larger number of seeds were needed. These were produced over the next two greenhouse seasons in 1998 and 1999 for the 2000 field trial.

Fig. 1.1 Production of recombinant substitution lines (RSLs) containing chromosome segments of wild emmer in the background of a bread wheat cultivar (BL). Unshaded and shaded bars represent the BL and TTD chromosome segments, respectively.

Recombinant substitution lines (RSLs). RSLs for each TTD chromosome arms of groups 1, 4, and 7 were produced by the procedure described in Fig. 1.1. For example, for homoeologous group 1, the four CASLs (1AL, 1AS, 1BL, and 1BS) were crossed with BL. The F₁ plants, each heterozygous for one chromosome arm only, were crossed with the ditelosomic line of BL that lacks the critical arm substituted in the CASL to produce a monosomic-monotelosomic line, from which selfed disomic progeny plants were selected by counts of chromosome number in root tip cells. By this procedure, recombination which occurred at meiosis of the F₁ plants was fixed, and the resulting RSLs are true-breeding. RSLs of group 1 were produced in Israel and of groups 4 and 7 in California.

Field evaluation trials

Chromosome arm substitution lines. The CASLs were evaluated in 11 experiments over 4 years in Israel and California as follows:

Israel:	Beit-Govrin	1997, 1998
	Mivhor	1997, 1998, 1999
California:	Davis	1997, 1999
	Tulelake	1996, 1997, 1998, 1999

The experiments were planted in the autumn at Mivhor, Beit-Govin, and Davis and in the spring at Tulelake. The Mivhor site represents an irrigated field crops rotation with an annual rainfall of 370 mm and supplemental irrigation, whereas Beit-Govrin represents more arid conditions and extensive farming. Tulelake is located in northern California at an elevation of 1300 m with fertile soil, and annual rainfall of about 330 mm. Davis is located in the central valley of California with about 425 mm annual rainfall. Both California sites received supplemental irrigation and are considered to be favorable for wheat production with generally high grain yields.

At all sites, plots of about 1.6 x 5-m in size were planted by cone seeding machine and harvested by a combine-harvester. Experimental design was randomized blocks with 4 or 5 blocks. In Israel, the plots were organized in long blocks to facilitate nearest neighbor analysis. Regular farming practices were used in the experimental fields. Grain yield was determined in all experiments. Yield component measurements were made in one experiment (Davis, 1997). Grain protein percentage (GPP) was determined from a ground 1-g grain sample using Technicon InfraAnalyzer 450 in the Weizmann Institute in Israel. Samples were sent to Israel from California for protein determination. Results for each CASL were analyzed by ANOVA using SAS and presented as per experiment means and, for each CASL, as a percentage deviation from Bethlehem. An LSD ($P=0.05$) criterion was used to judge biological significance of differences.

Diallel cross. Since all of the hybrid seeds were produced by hand pollination, only a limited number could be produced and the field trials had to be limited in size. The field experiments were conducted at Tulelake with spring planting in 1997 and 2000. In 1997 a randomized block experiment with 4 replicates was planted with each plot being a single row of 5 plants spaced 30 cm between rows and within rows. Each plot was bordered at the ends with similarly spaced plants. Survival was variable,

resulting in an average of 3 plants per plot. One cross had no surviving plants (7BL/7AS). For the 2000 trial, a larger number of hybrid seeds had been produced and, as an extra precaution, the F_1 and parental seeds were planted in a greenhouse at Tulelake and transplanted to the field. The plots were hand-watered to assure survival. Each plot was a single row with 6 plants spaced 30 cm between plants and rows as in 1997. A randomized block design was used with 10 replicates. Plot ends were bordered with a similarly spaced plant. Survival and uniformity of growth was good. Average number of plants per plot was 5.5. In 1997 each plant was harvested and grain yield recorded; in 2000, grain yield was determined from the whole plot and converted to yield per plant. In 2000, 4 spikes per plot were collected and measured for length (mm) and number of spikelets, kernel number per spike was determined as the mean number obtained from the 4 spikes, kernels per spikelet was determined as number of kernel per spike/number of spikelets per spike, spike density was computed as mm per rachis internode as spikelet number/spike length, and weight per kernel (mg) was computed from the number of kernels from 4 spikes. Partial analyses of the data have been completed. Heterosis was computed as the first criterion of gene interaction as the percentage deviation of the F_1 from the midparent $[(P_1 + P_2)/2]$.

Recombinant substitution lines. The RSL characterization trial in California consisted of the parental lines Bethlehem and five CASLs 4AS, 4BL, 7AL, 7BS, and 7BL and the RSLs grown at Tulelake in the 2000 season as single 1-m rows spaced 30 cm apart in a randomized complete block design with 7 replicates. The amount of seed of each RSL was limited, so a light seeding rate was used. The low seeding rate and unevenness in the soil preparation resulted in variable stands so that grain yield per plot could not be obtained. Four spikes per plot of the first 4 replicates were collected from competitive plants to obtain data on the same six traits mentioned above for the diallel cross. The measurements are partially completed at present and some of the results from the group 7 RSLs will be presented here. All plants in each of 4 replications were harvested to provide for sufficient seed for a future field trial.

Mapping of chromosome arms

First, probe by enzyme combinations that showed RFLPs between Bethlehem and TTD140 were selected. Then the chromosome location of a selected probe (usually known from the producer of the probe) was validated in our material by analyzing

CASLs of a desired group with this probe. Mapping was carried out using both RSL population and F₂ population (progeny of F₁ CASL x BL) with the appropriate Mapmaker population definition in Israel. Mapping with only the RSLs was done at Davis. At Davis a smaller number of probes were used resulting in less precision in mapping. All RSL populations were relatively small for mapping purposes.

Section 3. Results

Objective 1. Evaluation of the contribution of CASLs to grain yield and its components and to grain protein concentration and yield

Grain yield. The evaluation trials were successful in both Israel and California. The basic genotype, Bethlehem, developed in Israel, proved to be well-adapted to growing conditions in California, as evidenced by comparisons to California check varieties. In all 11 experiments statistical significance of differences among the CASLs was found. Similarly, differences were maintained in the over-years analyses of both Israel and California experiments and for the combined results over years and locations. Genotype x year interactions were significant at all 4 locations, but the interaction effect was smaller than the genotype effect. The grain yield results are presented for Bethlehem and each CASL as percentage of the yield of Bethlehem (Table 3.1). Tests of significance of the differences (CASL_i – Bethlehem) were made on grain yields in kg/ha, but expressed in Table 3.1 as percentages of CASL/Bethlehem.

Of most interest was to determine if TTD chromosome arms contributed positively to grain yield. There were five CASLs showing significant positive effects in the Israel trials (4AS, 5AS twice, 5BS twice) and five in the California trials (4AS, 6BL, 7AL twice, 7AS twice). While not significant at P=0.05, in the Beit-Govrin site in both years, there was a trend of positive effects from arm 3BL. These results were not consistent and means over all experiments were not significant. More general were the negative effects of the TTD chromosome arms, including 20 of 106 comparisons in Israel trials and 40 of 132 comparisons in California and 11 CASLs had significantly lower grain yields than Bethlehem over the 11 experiments. Two CASLs were particularly noteworthy. 3AS introduces a gene to Bethlehem causing brittle or disarticulating rachis causing severe seed losses before harvest, thus accounting for the poor yields in the reported trials. 7BL was uniformly about 20%

lower-yielding than Bethlehem over all sites. This is remarkable when comparing 7BL with other members of the group 7 family of lines. 7BS had no effect on grain yield while 7AL and 7AS were variably significant positively and negatively in the 11 experiments. This suggests that the group 7 chromosomes of TTD introduce new and interesting variability to Bethlehem.

Grain protein concentration. Grain protein percentage (GPP) was determined for all replications of the Israel field trials, for the four replications of the 1997 Davis field trial, and for 1 replication of the Tulelake 1997 field trial. (Table 3.2). Tests of significance of the differences (CASL_i – Bethlehem) were made on GPP, but expressed in Table 3.2 as percentages of CASL/Bethlehem. CASL7BS consistently had significantly higher GPP in Israel and in the 1997 Davis trial. While no LSD could be calculated for the one replication at Tulelake 1997, GPP was also higher for CASL7BL.

Yield components and other spike traits. Grain yield is the end product of the multiplicative effects of several traits which are called yield components and can be expressed as number of spikes per unit area X number of seeds per spike X weight per seed. Seeds on a spike are formed in florets which are in turn produced in a spikelet, thus seed number per spike is the product of number of spikelets per spike X number of seeds per spikelet.

In the Davis 1997 trial, several spike traits were measured on 4 spikes obtained from each plot. No data could be obtained on number of spikes per unit area. Significant differences among the CASLs and between Bethlehem were found for six traits. The data are summarized graphically in Figure 2.1 as percentages of Bethlehem. The darkened bars indicate significant difference from Bethlehem and the shaded ones were not significantly different. For the chromosomes tested, significant effects were found for all chromosomes except those of group 6; group 3 showed limited contribution from TTD, only 3BL caused a reduction in spikelet number. With respect to the yield components, kernel number per spikelet was significantly increased by 4 chromosome arms and substantial, but not significant, increases were exhibited by 7 additional chromosome arms. The number of spikelets per spike was increased by 6 chromosome arms and decreased by 2 arms. The number of kernels per spike mirrors the results obtained for spikelet number and kernels per spikelet, but not entirely. Finally, weight per kernel was improved over Bethlehem by at least four chromosome arms and decreased by one. 7BL shows a

large decrease in kernel weight. This observation has been made in other experiments (data not shown). Recall that 7BL showed the greatest yield decrease of all of the CASLs. Low kernel weight must be contributing to this low yield because kernel number per spike is increased by this line. It is inferred therefore that kernel weight and spike number (not measured) are the principal contributors to the low yield performance of 7BL.

Yield components are generally negatively correlated, making yield improvement a complex task. TTD appears to contribute differently to the yield components thus making it a potentially useful genetic resource for yield improvement. Since there are positive effects contributed by TTD on chromosome arms throughout the A and B genomes, it will be useful to target specific chromosome arms for selection in breeding programs. As an example, based on the limited data provided here, the following chromosome arms could be selectively introduced into Bethlehem and other arms of TDD eliminated.

TTD chrom. arm	Kernel wt	Kernel no	Spikelet no
1AL			X
1AS	X		
1BL		X	X
2AS	X		X
4AS		X	
4BL		X	X
5AS		X	
7AL	X		
7AS	X		
7BL		X	

The results obtained from yield component analyses suggest that the apparent negative effects of TTD genes on grain yield in Bethlehem, a highly productive hexaploid wheat, are due to interacting genes for each trait and for combinations of traits. The use of *T. t. dicoccoides* wheat as a gene resource for hexaploid wheat can be greatly aided by information on the nature of the interactions among chromosome arms and on the distribution of the those genes within chromosome arms, hence the study of interactions and recombinant inbred lines discussed below is critical to developing an effective strategy for exploiting *T. t. dicoccoides*.

Table 3.1. Grain yield (kg/ha) obtained from 11 field experiments in Israel and California for cultivar Bethlehem (BL) and the CASLs (expressed as percent of Bethlehem).

Identlty	Beit Govrin			Mihvor				Israel mean
	1997	1990	2-year	1997	1998	1999	3-year	
			mean				mean	
1AL	3.5	-9.6*	-2.7	4.2	-8.8	-16.0	-6.6	-4.8
1AS	7.0	-3.5	2.1	-4.0	9.9	-11.4	-1.3	0.2
1BL	—	-7.8	—	1.7	-8.8	-9.8	-5.5	—
1BS	-6.7	-12.8*	-9.6*	7.6	-9.3	-17.7	-6.2	-7.7
2AS	-16.7*	-4.2	-10.8*	-4.1	2.6	-63.6*	-20.0*	-15.9*
2BL	6.9	7.2	7.0	2.9	2.2	-8.6	-0.9	2.7
2BS	-29.5*	-37.8*	-33.4*	0.7	-20.5	-38.1*	-18.7*	-25.3*
3AL	7.7	-9.1	-0.2	-3.1	-9.9	-28.7	-13.4*	-7.4
3AS	-59.6*	—	—	-55.5*	—	—	—	—
3BL	7.9	4.4	6.3	5.2	-1.9	-10.9	-2.2	1.6
3BS	-0.6	-1.6	-1.1	-12.2	-1.1	-10.6	-7.8	-4.7
4AL	—	—	—	—	—	—	—	—
4AS	-2.6	-7.0	-4.7	18.6*	6.5	-27.7	0.1	-2.0
4BL	-44.6*	-25.1*	-35.5*	-17.7*	-13.8	-37.1*	-22.2*	-28.2*
5AS	15.5*	-1.7	7.4	19.3*	0.6	1.3	7.2	7.3
5BS	14.8*	-3.7	6.1	12.4*	0.5	-18.4	-1.3	2.1
6AL	-12.6	-11.5*	-12.1*	-2.5	-11.3	-35.4	-15.7*	-14.1*
6AS	-0.4	-7.8	-3.9	3.9	-13.7	-21.4	-10.1	-7.3
6BL	-6.0	-11.0*	-8.4*	8.4	-26.6*	-26.0	-14.6*	-11.8*
7AL	-8.5	-9.9*	-9.2*	10.3	0.5	-21.7	-3.0	-5.8
7AS	11.4	6.4	9.1*	1.9	-16.7	-26.7	-13.5*	-3.3
7BL	-26.9*	-18.8*	-23.1*	-28.3*	4.0	-35.2	-18.9*	-20.8*
7BS	-6.9	6.6	-0.6	-2.8	-16.8	7.9	-4.5	-2.8
BL	3132	2768	2950	3652	3833	3309	3598	3274
Mean	2923	2569	2786	3599	3603	2624	3305	3043
CV, %	10.0	7.3	8.8	10.0	15.7	31.6	17.6	15.6
LSD (0.05) kg/ha	412.6	264.5	241.5	454.5	802.3	1173.4	431.2	286.3
LSD (0.05) % BL	14.1	10.3	8.7	12.6	22.3	44.7	13.0	9.4

* -0.05≤P≤0.05 based on LSD test on yield in kg/ha, rather than as % Bethlehem.

Table 3.1. Continued.

Identity	Davis			Tulelake					California mean	Grand mean of 11 trials
	1997	1999	2-year mean	1996	1997	1998	1999	4-year mean		
1AL	-9.3	-5.5	-7.0	-26.0*	-14.7*	-5.9	-5.2	-10.9*	-9.2*	-7.8*
1AS	-0.7	-12.0	-7.7	-10.4	-9.8*	-6.4	-7.2*	-8.1*	-7.9*	-5.3*
1BL	-26.1*	-3.7	-12.3	-34.0*	-11.3*	-4.4	-16.0*	-13.4*	-12.9*	—
1BS	-7.6	-10.0	-9.1	-3.8	-11.5*	-1.6	-2.0	-4.9	-6.7*	-7.0*
2AS	-3.7	-12.3	-9.0	12.9	-2.1	-8.2	-3.0	-2.3	-5.2	-8.6*
2BL	2.4	-16.8*	-9.4	4.7	-2.4	-4.5	-3.5	-2.4	-5.5	-2.9
2BS	-8.6	-15.4	-12.8	-5.0	-20.4*	-20.7*	-10.4*	-15.8*	-14.5*	-17.9*
3AL	-13.4*	-18.8*	-16.7*	-12.5	-18.4*	3.6	-7.3*	-7.9*	-11.8*	-10.4*
3AS	-63.5*	-9.5	-30.3*	-21.9*	-46.4*	-24.6*	-15.8*	-28.2*	-29.1*	—
3BL	0.5	-13.3	-8.0	13.6	-7.3	-5.8	-12.1*	-5.5	-0.0*	-4.0
3BS	-11.3	-14.0	-12.9	5.2	-9.4	7.6	-6.7*	-1.6	-6.6*	-6.0*
4AL	—	-6.3	—	—	—	-4.2	-23.9*	—	—	—
4AS	3.6	-10.9	-5.3	-13.8	-4.8	11.4*	-0.5	0.1	-2.3	-2.2
4BL	-9.8	-5.8	-7.3	-20.8*	-7.2	-1.1	-8.6*	-7.5*	-7.4*	-14.0*
5AS	11.4	-10.1	-1.8	5.4	-11.0*	-3.2	-0.4	-3.6	-2.8	0.4
5BS	-1.0	-4.5	-3.1	-2.5	-9.2	-2.8	-1.0	-4.1	-3.7	-1.9
6AL	-3.0	-10.9	-7.9	-1.7	-14.9*	-7.8	-8.9*	-9.4*	-8.7*	-10.4*
6AS	-1.7	-15.5*	-10.2	10.6	-6.6	0.7	-0.4	-0.5	-4.8	-5.6*
6BL	—	-9.5	—	—	—	13.3*	-4.9	—	—	—
7AL	12.2*	-9.0	-0.9	20.0*	-7.3	-0.4	-8.8*	-2.1	-1.5	-2.9
7AS	12.1	-11.0	-2.1	22.1*	-9.5	-4.5	-9.5*	-3.9	-3.2	-3.2
7BL	-54.2*	-15.8*	-30.6*	-23.2*	-25.2*	-11.3*	-20.0*	-19.3*	-24.3*	-23.2*
7BS	-4.8	-4.4	-4.6	17.2	-12.5*	3.4	-5.1	-1.9	-3.0	-3.0
BL	4729	7573	6151	4026	9232	9510	8611	7845	6998	5136
Mean	4349	6800	5566	3909	8133	9203	7961	7298	6432	4738
CV, %	9.3	17.5	18.8	9.0	7.6	10.8	6.1	10.4	13.5	15.3
LSD (0.05) kg/ha	571.9	1169.4	836.2	732.5	878.0	972.4	474.4	450.6	412.3	270.41
LSD (0.05) % BL	13.1	17.2	15.0	18.7	10.8	10.6	6.0	6.2	6.4	5.7

* -0.05≤P≤0.05 based on LSD test on yield in kg/ha, rather than as % Bethlehem.

Table 3.2. Protein percent obtained from 7 field experiments in Israel and California for cultivar Bethlehem (BL) and the CASLs (expressed as percent of Bethlehem).

Identity	Beit-Govrin			Mihvor				Israel mean	Davis 1997	Tulelake 1997	Cali- fornia mean	Grand mean of 7 trials
	1997	1998	2-year mean	1997	1998	1999	3-year mean					
1AL	0.7	0.0	0.4	-7.2*	1.5	6.1	0.0	0.0	1.5	1.4	1.5	0.5
1AS	2.9	0.8	1.9	3.2	0.0	4.6	2.5	2.2	0.8	-2.2	-0.7	0.0
1BL		2.4		-4.0	2.2	6.9	1.8		0.8	-7.2		
1BS	4.3	9.4*	6.8*	-0.8	2.9	4.6	2.3	4.5	3.1	-5.8	-1.5	1.1
2AS	3.6	5.5	4.5	-1.6	2.2	9.9	3.6*	4.0	3.1	-0.7	1.1	2.2
2BL	-6.5	6.3	0.0	-2.4	-2.2	8.4	1.3	0.4	1.5	-1.4	0.0	0.0
2BS	2.2	14.2*	7.9*	5.6	5.1	2.3	4.3*	6.1	6.1*	2.9	4.4*	4.9
3AL	-0.7	1.6	0.4	4.8	-2.2	5.3	2.5	1.5	6.9*	10.1	8.5*	4.6
3AS	2.2			1.6					-1.5	3.6	1.1	
3BL	-1.4	-6.3	-3.8	3.2	2.2	-3.8	0.5	-1.6	4.6*	0.0	2.2	0.0
3BS	-4.3	1.6	-1.5	-2.4	0.0	-8.4	-3.6*	-2.5	-2.3	2.2	0.0	-1.6
4AS	0.7	1.6	1.1	-4.0	2.9	6.1	1.8	1.5	-1.5	-10.8	-6.3*	-2.8
4BL	5.0	4.7	4.9	0.0	5.1	-3.1	0.8	2.8	3.1	2.2	2.6	2.3
5AS	-3.6	-0.8	-2.3	-3.2	-5.8	-1.5	-3.6*	-2.9	-1.5	-5.8	-3.7*	-3.7
5BS	0.7	5.5	3.0	-0.8	1.5	-3.1	-0.8	1.1	3.8*	5.0	4.4*	2.4
6AL	0.0	5.5	2.6	3.2	7.3*	8.4	6.4*	4.5	3.8*	3.6	3.7*	3.7
6AS	2.9	2.4	2.6	-0.8	8.8*	0.8	3.1*	2.8	3.8*	-5.0	-0.7	0.7
6BL	6.5	0.0	3.4	-2.4	-1.5	-2.3	-2.0	0.7				
7AL	1.4	3.1	2.3	-3.2	-2.9	7.6	0.5	1.4	3.1	0.0	1.5	1.1
7AS	-5.8	0.0	-3.0	-1.6	2.2	3.8	1.5	-0.8	2.3	2.2	2.2	0.4
7BL	10.8*	7.9*	9.4*	9.6*	10.9*	7.6	9.4*	9.4*	8.4*	18.7	13.7*	11.2*
7BS	1.4	3.1	2.3	1.6	0.0	-5.3	-1.3	0.5	1.5	-2.9	-0.7	-0.5
BL	13.9	12.7	13.3	12.5	13.7	13.1	13.1	13.2	13.1	13.9	13.5	13.4
Mean	14.0	13.1	13.6	12.5	14.0	13.4	13.3	13.4	13.4	14.0	13.7	13.6
CV, %	5.7	5.0	5.5	5.2	5.0	8.9	3.7	11.3	2.7		4.4	4.4
LSD (0.05)	1.14	0.93	0.74	0.81	0.98	1.69	0.36	0.92	0.50		0.45	0.83
% prot.												
LSD (0.05)	8.1	7.1	5.4	6.5	7.0	12.6	2.7	6.9	3.8		3.3	6.1
% BL												

* $-0.05 \leq P \leq 0.05$ based on LSD test on yield in GPP, rather than as % Bethlehem.

Figure 2.1. Spike morphology from one of 11 yield trials (Davis 1997). Each CASL is identified by the number of the chromosome arm that was substituted. Values given as percentage deviation from Bethlehem. Solid bars indicate significant difference at $P=0.05$.

Objective 2. Determination of the extent and nature of gene interactions (epistatic effects) with TTD and Bethlehem chromosome arms of groups 1 and 7

The diallel cross data from the Tulalake 2000 experiment was partially analyzed up to now. The experiment was of good quality with CVs ranging from 5.9 to 12.7% for spike data and 18.9% for grain yield.

Grain yield. Comparisons of the F_1 s to Bethlehem showed that three were significantly higher and none were significantly lower for grain yield. Midparent heterosis was taken as a first good indicator of interaction of genes between the specified chromosome arms of TTD and Bethlehem. Table 3.3 shows grain yield heterosis data as percentage of the midparent (MP) value. Sixteen out of 36 crosses showed significant MP heterosis and eight of those crosses had significant high-parent heterosis. The mean MP heterosis over 36 crosses was 16.1%. These results show clearly that TTD contributed genetic diversity to the Bethlehem genetic background. This diversity is not contributed evenly by each chromosome arm. One of eight CASLs X Bethlehem showed significant heterosis, chromosome 1 X 1 CASLs gave 3 of 6, chromosome 7 X 7 gave 5 of 6, and 1 X 7 gave 7 of 16 significant MP heterosis values. Another way to look at this is to compare the mean % heterosis values for various groups of crosses. This is shown in Table 3.4. For grain yield, those results show rather clearly that group 1 TTD chromosome arms are less heterotic than group 7 TTD arms. Also shown is that heterosis is greater with two heterozygous arms (1 X 1, 1 X 7, and 7 X 7) than with one (Bethlehem X 1 and 7).

Kernel number per spike. Detailed data on heterosis will not be presented for kernel number or other traits that were analyzed. For kernel number 20 of 36 crosses showed heterosis, with a mean over all crosses being 4.5%, considerably lower than for grain yield. Table 3.4 shows less diversity in heterosis values among the various groups of crosses.

Table 3.3. Grain yield heterosis as percentage of the midparent (MP) value. Bold-faced values are the parental mean yields in grams per plant.

	P ₁	BL	1AL	1AS	1BL	1BS	7AL	7AS	7BL	7BS
P ₂		1	2	3	4	5	6	8	7	9
BL	1	42.2	4.4	8.4	-1.4	-1.4	5.4	25.8*	11.0	-5.2
1AL	2		40.4	23.1*	-5.2	15.8*	2.0	18.7	28.7*	0.4
1AS	3			37.9	10.4	22.2*	36.4*	15.4	51.6*	22.2*
1BL	4				34.9	1.1	13.3	26.3*	19.7	-0.6
1BS	5					44.4	14.6*	7.5	30.2*	9.9
7AL	6						39.4	6.1	39.2*	28.4*
7AS	8							35.4	30.1*	17.4*
7BL	7								24.1	37.9*
7BS	9									37.7

* $-0.05 \leq P \leq 0.05$ based on LSD test on difference of midparent from F₁.

Table 3.4. Mean MP heterosis (%) for various groups of crosses for three traits.

Hybrid group	No. hybrids	Grain yield/plt.	Kernel weight	Kernel no./spike
Bethlehem x group 1	4	2.5	-2.3	10.3
Bethlehem x group 7	4	9.2	-0.7	7.8
group 1 x group 1	6	11.3	-4.0	11.3
group 1 x group 7	16	18.5	1.4	11.8
group 7 x group 7	6	26.6	4.2	14.0

Kernel weight. Heterosis for kernel weight was much lower than for the kernel number or yield. Five of 36 crosses had MP heterosis and one of them was negative. The average over all crosses was 0.1%. Of the four significant positive heterosis values, three of them involved CASL 7BL. Recall that this CASL had very low kernel weight. Since it is dominating the heterosis values in this diallel, it might be suspected that kernel weight is under rather simple genetic control in these materials. Further information on this point will be evident when the analysis of the RSL lines is completed.

Objective 3. Production of recombinant substitution lines (RSLs).

Chromosome group 1. Following the procedure described in the Materials and Methods, 39 disomic plants were obtained from the cross BL x CASL 1AS, 47 from 1AL, 43 from 1BS and 50 from 1BL. These plants were analyzed by all DNA (RFLP and SSR) probes that showed polymorphism between BL and TTD, and by grain protein (glutenin and gliadin) markers. The results of this analysis are shown in Fig 2.2a-d. Most of the progeny of each chromosome group 1 CASLs were identified as parental type (genotype 1=BL; 2= CASL). Of the rest, most showed a single recombination point as evident by a single TTD segment with one edge overlapping one of the edges of the TTD segment in the parental CASL. Few others showed two or more recombination points (up to 3 segments or a segment edge not overlapping the parental segment edge).

No contiguous RSL series could be derived from any of the progenies. Yet, selection of RSLs with partially overlapping TTD segment size or complementary segments allowed a preliminary dissection of the TTD segment in the CASL, thereby localizing of the effect of the TTD into a smaller segment.

Chromosome group 4. Following the procedure described in the Materials and Methods, 31 disomic plants were obtained from the cross BL x CASL 4AS and 31 from BL x CASL 4BL. These plants were analyzed by RFLP probes that showed polymorphism between BL and TTD (two probes for the 4AS lines and four for the 4BL lines. The results of this analysis are shown in Table 3.5. For 4AS lines, 71% had a parental genotype and 55% of the 4BL lines did. For 4BL with 4 markers analyzed, there was apparently 1 double-crossover event. For the 4AS arm, the two markers are 29cM apart, but no order can be derived from our data. The order for these two markers determined in other RFLP maps (Dubcovsky et al. 1996a,b) is A–B. For the 4BL arm, the order determined for the four markers (B–A–C–D) differs from that (A–B–C–D) found in two RFLP maps that used the same markers (Dubcovsky et al. 1996a, b), however the difference is between the very close A and B markers (3.2 cM). No contiguous RSL series could be derived from either of the lines, primarily due to the low number of markers used.

Fig. 2.2.a Constitution of chromosome 1A in the recombinant substitution lines derived from the cross between the common wheat cv. Bethlehem (BL) and the chromosome arm substitution line 1AS of the wild emmer accession TTD140 in the background of BL. Centromeres are indicated by dashed-line.

0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 cM

Genotyp

RSL

BL TTD Not identified

Fig. 2.2.b Constitution of chromosome 1A in the recombinant substitution lines derived from the cross between the common wheat cv. Bethlehem (BL) and the chromosome arm substitution line 1AL of the wild emmer accession ITD140 in the background of BL. Centromeres are indicated by dashed-line.

Fig. 2.2.c Constitution of chromosome 1B in the recombinant substitution lines derived from the cross between the common wheat cv. Bethlehem (BL) and the chromosome arm substitution line 1BS of the wild emmer accession TTD140 in the background of BL. Centromeres are indicated by dashed-line.

Fig. 2.2.d Constitution of chromosome 1B in the recombinant substitution lines derived from the cross between the common wheat cv. Behtlehem (BL) and the chromosome arm substitution line 1BL of the wild emmer accession TTD140 in the background of BL. Centromeres are indicated by dashed-line.

Table 3.5. Arrays of genotypes after marker analysis of potential recombinant lines for chromosome group 4. Suggested marker order is from proximal to distal, left to right. (BL=Bethlehem parent, TDD=*Triticum turgidum dicoccoides* parent)

4AS	No. of lines	Markers		rec freq (cM)
		A	B	
		Xpsr922 (A)	Xwg622 (B)	
	10	BL	BL	AB: 29.0
	12	TTD	TTD	
	8	BL	TTD	
	1	TTD	BL	

4BL	No. of lines	Markers				rec freq (cM)
		A	B	C	D	
		Xbcd1652	Xpsr914	Xbcd1302	XksuH11	
	5	BL	BL	BL	BL	AB: 3.2
	12	TTD	TTD	TTD	TTD	AC: 16.1
	3	BL	BL	TTD	TTD	AD: 41.9
	5	BL	BL	BL	TTD	BC: 19.4
	2	TTD	TTD	BL	BL	BD: 45.2
	3	TTD	TTD	TTD	BL	CD: 25.8
	1	TTD	BL	TTD	TTD	151.6

Chromosome group 7. Following the procedure described in the Materials and Methods, 6 disomic plants were obtained from the cross BL x CASL 7AS, 29 from 7AL, 30 from 7BS and 28 from 7BL. (It was difficult to match parents for crossing BL x CASL7AS.) These plants (except for the 7AS lines with too few disomic plants for analysis) were analyzed by RFLP probes that showed polymorphism between BL and TTD (three probes for 7AL lines, two for 7BS lines, and four for 7BL lines). The results of this analysis are shown in Table 3.6. For the 7AL lines, 39% had a parental genotype, 50% for the 7BS lines, and 29% for the 7BL lines. For 7AL, there were apparently 4 double-crossover events and for 7BL, there were 7. The marker order implied for the 7AL arm from these data (A–C–B) differs from that (A–B–C) in RFLP maps that used the same markers (Langridge et al. 1995, Dubcovsky et al. 1996b). For the 7BL arm, these data provide no clear indication of marker order, however, they do not contradict the marker order (A–B–C–D) determined in RFLP maps that used the same markers (Gale et al. 1995, Langridge et al. 1995, Dubcovsky et al. 1996). For the 7BS arm, the two markers are at least 50cM apart and no order can be derived from our data. The order for these two markers determined by Dubcovsky et al.

(1996b) is that A is proximal to B. No contiguous RSL series could be derived from any of the lines, primarily due to the low number of markers used.

Table 3.6. Arrays of genotypes after marker analysis of potential recombinant lines for chromosome group 7. Suggested marker order is from proximal to distal, left to right. (BL=Bethlehem parent, TTD=*Triticum turgidum dicoccoides* parent)

7AL	No. of lines	Markers			rec freq (cM)
		A Xcdo673	B Xwg420	C Xpsr680	
	6	BL	BL	BL	AB: 53.6
	5	TTD	TTD	TTD	AC: 46.4
	8	BL	TTD	TTD	BC: 21.4
	1	BL	BL	TTD	121.4
	3	TTD	BL	BL	
	1	TTD	TTD	BL	
	3	TTD	BL	TTD	
	1	BL	TTD	BL	

7BS	No. of lines	Markers		rec freq (cM)
		A Xabc455	B Xwg834	
	3	BL	BL	AB: 53.5
	13	TTD	TTD	
	1	BL	TTD	
	15	TTD	BL	

7BL	No. of lines	Markers				rec freq (cM)
		A Xcdo673	B Xpsr547	C Xwg420	D Xpsr680	
	4	BL	BL	BL	BL	AB: 46.4
	4	TTD	TTD	TTD	TTD	AC: 53.6
	1	BL	BL	BL	TTD	AD: 46.4
	2	BL	BL	TTD	TTD	BC: 28.6
	2	BL	TTD	TTD	TTD	BD: 42.9
	1	TTD	TTD	TTD	BL	CD: 21.4
	2	TTD	TTD	BL	BL	239.3
	5	TTD	BL	BL	BL	
	1	BL	BL	TTD	BL	
	2	BL	TTD	TTD	BL	
	2	BL	TTD	BL	BL	
	1	TTD	BL	BL	TTD	
	1	TTD	BL	TTD	TTD	

Objective 4. Characterization of the RSLs for quantitative traits and mapping and tagging chromosome segments producing significant effects by RFLP markers.

Chromosome group 1. A few RSLs altogether representing the whole substituted TTD segment of the studied arm were selected from each progeny of chromosome group 1 CASLs and propagated. Each group included two RSLs showing the segmental constitution of the parents BL and the CASL. The RSLs were evaluated in the field during two seasons (1998 and 1999) in Israel. In 1998 due to a small seed amount we were not confident with the grain yield results (see 2nd year report). In 1999 the same RSLs were evaluated in a larger scale (four replicates of 10 sq. m). The results (grain yield and grain protein percentage) are shown in Table 3.7. For each group (representing one of the group 1 chromosome arms) no significant difference in grain yield (and almost none in grain protein percentage) was found among BL, the relevant CASL, and any of the RSLs. The differences probably reflect a high experimental variation (for example, parental type RSLs were found different from their corresponding CASL or from BL, and some RSLs that are higher yielders differ from others that contain a larger TTD segment). Indeed, the season of 1999 was deprived from rainfall, the experiment was irrigated several times which introduced a high level of variation and weed contamination. It should be remembered that chromosome group 1 was selected because of encouraging grain yield data obtained in some CASLs grown in pot experiments. This yield advantage was not repeated later when the CASLs were evaluated in field plots. Therefore alleles for increased grain yield should not be expected in chromosome group 1 of TTD140.

Table 3.7. Grain yield and grain protein percentage in the CASLs of TTD140 group 1 chromosomal arms and in 18 RSLs derived from them in the background of cv. Bethlehem in Mivhor 1999.

Entry	Grain yield (kg/ha)	Protein percent
Bethlehem	4450	13.4a
CASL 1AS	3980	11.3b
1AS7-78	5020	12.3ab
1AS2-86	4130	12.4ab
S.E.	314	0.48
Bethlehem	4400	11.9
CASL 1AL	5090	12.4
1AL1-23	4670	12.5
1AL2-30	4730	12.7
1AL7-9	5030	12.6
1AL10-1	4580	12.5
1AL14-38	4420	12.0
S.E.	378	0.48
Bethlehem	3150	14.4
CASL 1BS	2610	15.2
1BS6-197	3400	14.8
1BS11-195	2870	14.9
1BS13-192	3350	14.0
1BS11-175	3140	14.6
S.E.	339	0.47
Bethlehem	1860	15.6
CASL 1BL	1930	16.4
1BL2-126	1650	16.4
1BL1-147	2310	16.0
1BL10-133	1530	16.0
1BL12-109	1650	14.9
1BL13-125	2270	16.0
1BL14-137	1610	15.4
1BL15-134	2280	14.7
S.E.	268	0.50

All values within each arm group are not significantly different ($P=0.05$) except protein among 1AS genotypes (common letter denotes nonsignificant difference).

Chromosome groups 4 and 7. No yield data were taken because of nonuniform stands. Height data have not yet been analyzed. Seed was increased, which will allow yield data to be obtained in a future nursery. Spike morphology traits (length, numbers of spikelets, total kernels, and weight of total kernels) were scored for the parents and potential RSLs in four replications of the experiment. These data for 7BS and 7BL arms have been analyzed (Table 3.8).

In contrast to the results of the grain yield trials (objective 1), there was no significant difference in mean kernel weight from this analysis of four spikes among Bethlehem, CASL 7BL, and CASL 7BS. However, mean kernel weight of Bethlehem of 7BL was higher than 7BL by 3.4 mg, even though not significant at $P=0.05$. Several 7BS and 7BL RSLs were either significantly lower or greater than that of Bethlehem. One 7BL RSL (7BLrec-9) had a lower mean kernel weight than Bethlehem and one (7BLrec-20) had a higher mean kernel weight. Four 7BS RSLs (7BSrec-10, -14, -21, and -30) had higher mean kernel weights than Bethlehem. The transgressive segregation for kernel weight in the 7BL X Bethlehem cross was distinct with one-fourth of the lines having heavier kernels than Bethlehem and one-fourth having lighter kernels than 7BL. One-half of the lines were intermediate around the values of the two parents. Since these lines are expected to be homozygous, these results suggest a simple genetic control, such as two interacting loci controlling kernel weight. This can create problems for mapping kernel weight QTLs and these issues will be addressed in further analysis of the data and perhaps further experimentation. As was the case with group 1, no apparent relationship could be found among the values for kernel weight and the recombination genotypes as indicated by the molecular markers used to confirm recombinants.

Table 3.8. Mean spike traits obtained from field experiment with Bethlehem, CASL7BL, CASL7BS, and 7BL and 7BS RSLs at Tulelake, California, 2000.

Line	Spike length	No. of spikelets	Spike density	Kernels/ spike	Kernels/ spikelet	Kernel weight
Bethlehem	89.9	16.2	5.6	48.6	3.0	29.2
CASL 7BL	88.7	17.4	5.1	49.3	2.8	25.8
7BLrec-1	81.6	15.0	5.4	50.1	3.3	29.6
7BLrec-2	93.9	16.4	5.7	54.7	3.3	35.6
7BLrec-3	85.6	16.1	5.3	53.0	3.3	30.3
7BLrec-4	74.7*	16.7	4.5*	39.5	2.4	24.2
7BLrec-5	87.4	16.4	5.3	52.5	3.2	27.5
7BLrec-6	88.5	16.4	5.4	48.4	3.0	31.1
7DLrec-7	95.3	14.9	6.4*	54.9	3.7*	33.8
7BLrec-8	93.5	16.2	5.8	52.1	3.2	34.7
7BLrec-9	87.6	17.1	5.1	45.4	2.7	20.6*
7BLrec-10	90.3	16.5	5.5	53.3	3.2	33.4
7BLrec-11	84.1	16.8	5.0*	52.9	3.1	22.9
7BLrec-12	92.1	15.4	6.0	51.1	3.3	33.5
7BLrec-13	90.3	16.8	5.4	55.3	3.3	28.3
7BLrec-14	78.5*	17.0	4.6*	40.5	2.4	27.9
7BLrec-15	91.8	16.1	5.7	48.8	3.0	31.1
7BLrec-16	98.8	17.8*	5.5	54.3	3.0	31.3
7BLrec-17	83.1	15.6	5.3	36.1*	2.3*	30.6
7BLrec-18	88.5	16.6	5.3	45.3	2.7	23.6
7BLrec-19	80.3*	17.4	4.6*	46.6	2.7	25.0
7BLrec-20	90.6	15.2	6.0	51.6	3.4	36.4*
7BLrec-21	84.8	16.1	5.3	50.7	3.1	30.6
7BLrec-22	91.1	16.8	5.4	47.9	2.9	26.7
7BLrec-23	86.6	17.8*	4.9*	56.4	3.2	26.0
7BLrec-24	90.7	18.7*	4.9*	51.6	2.8	24.8
7BLrec-25	87.9	18.8*	4.7*	53.9	2.9	28.4
7BLrec-26	93.3	16.7	5.6	48.0	2.9	33.5
7BLrec-27	94.1	16.9	5.6	52.9	3.1	28.9
7BLrec-28	86.6	16.3	5.3	50.8	3.1	27.2
Mean	92.1	16.7	5.5	51.4	3.1	30.8
cv	7.3	6.6	6.1	16.9	15.1	15.8
LSD	9.3	1.5	0.5	12.1	0.6	6.7

* -0.05≤P≤0.05

Table 3.8. Continued.

Line	Spike length	No. of spikelets	Spikelet density	Kernels/ spike	Kernels/ spikelet	Kernel weight
Bethlehem	89.9	16.2	5.6	48.6	3.0	29.2
CASL 7BS	90.5	16.0	5.7	47.8	3.0	29.1
7BSrec-1	95.8	17.1	5.6	50.0	2.9	31.7
7BSrec-2	95.9	17.1	5.6	56.2	3.3	32.7
7BSrec-3	95.0	18.1*	5.2	51.4	2.8	29.6
7BSrec-4	88.4	15.8	5.6	44.6	2.8	34.5
7BSrec-5	98.9	17.0	5.8	63.9*	3.8*	32.5
7BSrec-6	95.1	17.5	5.4	60.4	3.5	30.7
7BSrec-7	93.7	16.6	5.6	55.5	3.3	34.3
7BSrec-8	98.6	17.5	5.6	47.2	2.7	30.6
7BSrec-9	90.8	16.6	5.5	47.8	2.9	31.5
7BSrec-10	99.0	17.6	5.6	48.5	2.8	37.6*
7BSrec-11	91.2	17.9*	5.1	50.3	2.8	28.0
7BSrec-12	95.8	16.3	5.9	53.6	3.3	28.2
7BSrec-13	98.6	17.8*	5.5	55.9	3.1	33.0
7BSrec-14	107.1*	16.9	6.3*	53.3	3.1	37.9*
7BSrec-15	95.3	16.3	5.9	53.3	3.3	29.3
7BSrec-16	89.9	15.8	5.7	49.7	3.2	33.2
7BSrec-17	90.4	15.9	5.7	46.0	2.9	33.1
7BSrec-18	93.8	16.4	5.7	54.6	3.3	34.4
7BSrec-19	93.2	17.2	5.4	54.9	3.2	32.9
7BSrec-20	102.9*	16.9	6.1*	56.9	3.4	31.4
7BSrec-21	90.6	15.1	6.0	51.0	3.4	38.9*
7BSrec-22	94.1	17.0	5.5	44.3	2.6	29.4
7BSrec-23	98.6	16.8	5.9	54.9	3.3	34.8
7BSrec-24	93.0	17.1	5.4	49.7	2.9	31.1
7BSrec-25	95.4	16.6	5.8	50.3	3.0	32.5
7BSrec-26	101.8*	18.0*	5.7	61.8*	3.4	30.9
7BSrec-27	97.3	16.5	5.9	54.3	3.3	34.0
7BSrec-28	103.9*	17.6	5.9	62.1*	3.5	30.6
7BSrec-29	96.2	17.0	5.7	55.5	3.3	32.0
7BSrec-30	99.7*	17.5	5.7	56.3	3.2	36.8*
Mean	92.1	16.7	5.5	51.4	3.1	30.8
cv	7.3	6.6	6.1	16.9	15.1	15.8
LSD	9.3	1.5	0.5	12.1	0.6	6.7

* -0.05≤P≤0.05

Objective 5. Construction of recombination genetic maps of chromosome arms.

Chromosome 1A. Using the RSLs of 1AL, 13 markers (12 RFLP and one grain glutenin, *Glu-A1*), 3 on the short arm and 10 on the long arm were mapped on chromosome 1A (Fig. 2.3). Eight SSR markers (*Xgwm*) were also mapped on 1A using this population. Yet, only 15 different loci could be identified due to co-segregation of some of the markers. Using the RSLs of 1AS, 8 RFLP, 3 SSR, and one grain glutenin were mapped and revealed 9 different loci, 3 of which were on 1AS.

Some inconsistency in the marker distances prevailed between the two maps, especially regarding the distance between *Xgwm164* and *Xbcd454* and between *Xpsr391* and *Xcdo 312*. Also, *Glu-1A* that co-segregated with *Xbcd1150* in the 1AL population became separated in 1AS population.

Altogether, the map of chromosome 1A spanned about 147 cM and included 16 loci with a mean distance of 10 cM between markers.

Chromosome 1B. Using RSLs of 1BL, 26 markers (20 RFLP and one grain glutenin, *Glu-B1*), 4 on the short arm and 16 on the long arm were mapped on chromosome 1B (Fig. 2.3). Five SSR markers were also mapped on 1B using this population. This map resolved into 14 loci (two of which are on the short arm). Using RSLs of 1BS, 12 RFLP markers (5 on 1BL), 5 SSR markers (1 on 1 BL), and one grain gliadin, *Gli-B1* were mapped on chromosome 1B. This map resolved into 8 loci on 1BS and 5 on 1BL.

As for chromosome 1A, distances between markers on the undesired arm of each RSL (i.e., 1BL arm in 1BS RSL map and vice versa) tend to be bigger than the distances on the desired arm (i.e., 1BL arm in 1BL RSL map).

Altogether, the RSL map of chromosome 1B spanned about 113 cM and included 22 loci with a mean distance of 5.4 cM between markers.

F₂ mapping. Similar maps were constructed using the F₂ population although some differences in marker distances were evident (Fig. 2.4). While the total length of 1BL map remained the same (about 85 cM), F₂ 1BS map was longer than RSL 1BS map (41 vs. 29 cM, respectively).

Chromosome groups 4 and 7. Detailed mapping is in progress.

Fig. 2.4 RFLP-based linkage maps of chromosome 1B of bread wheat constructed from two populations of recombinant substitution lines (RSLs) and two F2 populations derived from crosses between the bread wheat cultivar BL and either of the two chromosome-arm substitution lines (CASIs) 1BL or 1BS. All loci on the RSL-population maps were ordered at a LOD score > 3.0.

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