J. G. Waines, UC Riverside.

Title: Determination of optimum root and shoot size in bread wheat for increased water and nutrient-use efficiency and grain yield.

Note: In this report the results of the long-tube (150 cm) experiment are given first as experiment No. 1 and the results from the short tube (80 cm) experiment are given second, as No. 2, which we are still analyzing. This reverses the order of last year's report.

> **Experiment 1.** *To determine the relationship between root and shoot biomass and grain yield of bread wheat grown in long (1.50 m) root tubes in a cooled glasshouse.*

A separate small pilot experiment conducted in 2011 season with 9 selected recombinant inbred lines (RILs) from the cross between bread wheat (*Triticum aestivum* L) 'Iran # 49' and 'Yecora Rojo', the 2 parents- # 49 and YR, and 2 California cultivars 'Blanca Fuerte' and 'Joaquin' was conducted in long sand tubes in the winter-spring growing season 2011 with 4 replications. Even though this was a short growing season, significant differences were observed between the two parents for total root biomass. There was also a positive association between root size and grain yield which we intended to investigate further in the 2012 season. Below follows the report of this 2012 season's experiment.

Introduction: Grain yield in spring bread wheat (*Triticum aestivum* L.) is influenced by shoot characteristics, morphological traits such as plant height and number of tillers, and phenological periods such as time to anthesis and maturity. Information on the relationship between grain yield and root characteristics, such as root biomass and shoot biomass is scarce in wheat. Our main objective was to determine optimum root and shoot biomass to maximize grain yield production.

Materials and methods: A set of genotypes including 12 (original 9 + 3 additional from spring 2011 growing season) F8 recombinant inbred lines (RILs) derived from a cross #49 x Yecora Rojo plus the two parents, along with two standard Californian wheat cultivars, namely 'Blanca Fuerte' and 'Joaquin' was used. Genotype Iran 49 is a tall landrace with a large root system and Yecora Rojo is a CIMMYT-derived two-gene dwarf cultivar with small root system grown in California. Grains from these lines were germinated in Petri dishes on January 13, 2012. Seven days later, seedlings with similar growth were transplanted in polyethylene tubing bags sleeved into polyvinyl chloride (PVC) tubes, 150 cm long and 10 cm in diameter. Two drainage holes made at the bottom of each bag were covered with a filter paper before being filled with 16 kg of dry silica sand #30 with 24% field capacity (w/w). A randomized complete block design with four replicates was used. Plants were grown under well watered conditions. Phenological periods such as days from germination to booting, heading, anthesis, and maturity were recorded. Plant height from the sand surface to the tip of highest spike (not including awns) along with number of tillers and spikes per plant was measured. Shoot biomass including grains was harvested from the sand surface, dried, grains were separated and counted, then weighed. The root system was washed without damage, longest root measured, then the root system separated into two parts;

shallow roots included all roots less than 30 cm and deep roots included all roots greater than 30 cm. Roots were dried and weighed. Root biomass was calculated as the sum of the two parts.

Data were subjected to ANOVA for each trait and simple correlation coefficients between pairs of traits were computed.

Results and Discussion: Significant differences were found among the genotypes for all the traits measured. Mean values for some important traits are shown in Table 1. In this study, correlation coefficients between time to maturity and plant height with grain yield were not significant. However, time to maturity and plant height showed moderate but significant correlation with root biomass and shoot biomass excluding grains (SBEG).

The relationship between root biomass and SBEG with grain yield is presented in Fig. 1. Variation in root biomass and in SBEG explained 93% and 87% of variation observed in grain yield, respectively. The two curves in Fig. 1 indicate that maximum grain yield was produced when root biomass and SBEG reached 9 and 22 g plant^{-1}, respectively. Grain yield leveled off beyond these values. The optimum ratio of SBEG to root biomass was 2.44.

Blanca Fuerte and L 31 had relatively high combinations of grain yield and harvest index, calculated as the ratio of grain yield to total plant biomass, including root biomass, shoot biomass and grains (Table 1). However, root biomass in L 31 (5.27 g plant^{-1}) was greater than that of Blanca Fuerte (3.06 g plant^{-1}). L 28 was similar to Joaquin and Yecora Rojo for root biomass, grain yield, and harvest index. L 27 had the highest shallow root weight and deep root weight with relatively high grain yield, but its harvest index was low.

Table1. Mean plant height (PH), days to maturity (DM), shallow-root weight (SRW), deep-root weight (DRW), root biomass (RB), shoot biomass excluding grains (SBEG), grain yield (GY), shoot biomass (SB), and harvest index (HI) for spring bread wheat genotypes grown under well watered conditions in a 1.5 m sand-tube experiment in glasshouse 2012.

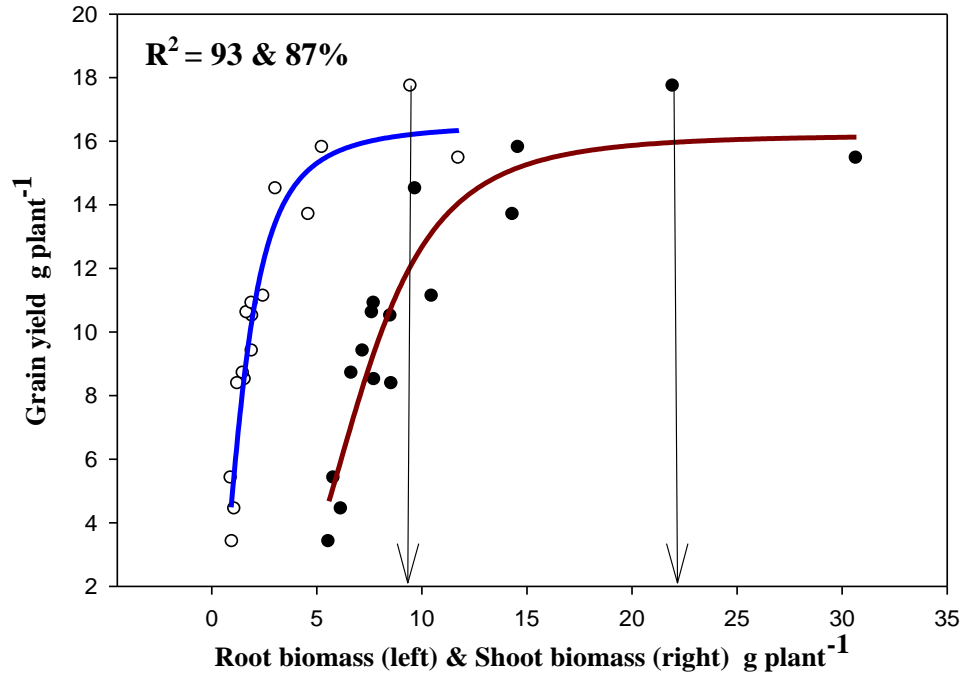| Genotype	PH cm	DM no.	SRW g plant^{-1}	DRW g plant^{-1}	RB g plant^{-1}	SBEG g plant^{-1}	GY g plant^{-1}	SB g plant^{-1}	HI %
Blanca Fuerte	62	118	1.97	1.09	3.06	9.70	14.5	24.20	53.2
Joaquin	62	114	1.17	0.76	1.93	7.20	9.4	16.57	50.6
#49	117	131	5.32	4.16	9.48	21.96	17.7	39.68	36.2
Yecora Rojo	57	117	1.20	0.72	1.92	7.73	10.9	18.62	53.0
L 8	71	114	0.96	0.54	1.5	6.67	8.7	15.35	51.5
L 9	102	114	1.32	1.15	2.47	10.94	11.1	21.61	46.2
L 19	96	117	0.76	0.48	1.24	8.57	8.4	16.94	46.0
L 21	94	118	0.74	0.37	1.10	6.17	4.4	10.59	36.9
L 22	78	122	1.06	0.53	1.59	7.74	8.5	16.28	47.7
L 23	78	122	0.53	0.45	0.98	5.58	3.4	8.92	33.8
L 27	94	141	7.47	4.29	11.76	30.68	15.5	46.14	26.6
L 28	63	121	1.19	0.75	1.94	8.52	10.5	19.06	50.3
L 31	60	129	3.28	1.99	5.27	14.60	15.8	30.42	43.8
L 34	99	119	3.02	1.60	4.62	14.34	13.7	28.02	41.9
L 49	59	133	0.46	0.46	0.92	5.81	5.4	11.17	44.4
L 51	55	118	0.93	0.75	1.68	7.65	10.6	18.23	53.1

Fig.1. Relationship between root biomass (left) and shoot biomass
(right, excluding grains) with grain yield.

As far as we are aware, this is the first time that the relationship of root biomass, shoot biomass and grain yield has been determined for California wheat, and possibly for US wheat. For the second year we found a positive correlation between of root biomass and grain yield in this set of RILS from Iran 49 x Yecora Rojo, even though the first year's results were from a shortened season.

We plan to repeat this experiment under well-watered conditions in 2013 (season 2 request for funding by California Wheat Commission) to confirm these results and also quantify the magnitude of year to year variation and genotype x year interaction. The next season's research project should also examine RILs with different root systems under droughted conditions to measure the effect of root system size on grain production under drought stress in a glasshouse experiment in long tubes (150 cm). We also plan to grow these genotypes, plus additional lines identified from Experiment 2 in 80 cm sand-tube experiment in the glass house, in a field experiment to compare results with those from the glasshouse.

Experiment 2. *Genetic analysis of root and shoot traits in bread wheat using recombinant inbred lines (RILs) in short tubes (80 cm) in a cooled greenhouse.*

Introduction: The root system is a vital part of a bread wheat plant absorbing water and nutrients for plant growth and development and eventually for production of grain yield. Information on inheritance of root traits such as shallow roots, deep roots, deepest root (longest root) and interrelationships of these root traits with shoot traits such as shoot biomass and grain yield are

3

scarce in wheat. The main reason for this is the difficulty of extracting a root system from the soil without damage, and to the heterogeneity of soil in a field that may make root and shoot data unreliable. Our main objective was to determine gene action and genetic variation for root traits along with important shoot traits in sand cultures that allow access to undamaged roots in a uniform environment.

Materials and Methods: A population of recombinant inbred lines (RILs) was produced by single-seed descent from crossing genotype #49 (a tall landrace from Iran with a large root system) and Yecora Rojo (YR, a modern Mexican-derived, two-gene dwarf cultivar with small root system) grown in California for over 40 years. Of the original 165 F8 RILs, 118 randomly chosen RILs plus the two parents were used in this study. The reason for using 120 lines was because only 60 tubes fit on a bench in a tube rack, and we occupied the two middle benches in the greenhouse section. We avoided placing tubes and plants too close to the sides of the greenhouse, especially the cooler intake and outtake, as temperature differences affect plant growth and development. Grains from these lines were germinated in Petri dishes on Dec. 15, 2011. Seven days later, seedlings with similar growth were transplanted into sand in polyethylene tube bags sleeved into polyvinyl chloride (PVC) tubes, 80 cm long and 10 cm in diameter. Two drainage holes made at the bottom of each bag were covered with a filter paper before being filled with 8.5 kg of dry silica sand #30 with 24% field capacity (w/w). A randomized complete block design with two replications was used. Plants were grown under well-watered conditions. Of the 118 RILs used, 104 RILS plus the two parental lines had plants that reached maturity in both replications. Phenological periods such as days from germination to booting, heading, anthesis, and maturity were recorded. Plant height from sand surface to the tip of highest spike (not including awns) along with number of tillers and spikes per plant was measured. Shoot biomass including grains was harvested from the sand surface, dried, grains were separated and counted, then weighed. The root system was washed without damage, longest root measured, then the root system was divided into two parts; shallow roots included all roots less than 30 cm and deep roots included all roots greater than 30 cm. Roots were dried and weighed. Root biomass was calculated as the sum of the two parts. Plant biomass was calculated as sum of root biomass and shoot biomass. Harvest index (HI) was calculated as the ratio of grain yield to plant biomass. The data were subjected to ANOVA for each trait.

Results and Discussion: Significant differences were found among the RILs including the parental lines for all the traits measured or calculated, except for longest root, which could be due to the restricted depth of 80 cm tubes. Distribution of some important traits is shown by boxplot (Figs. 2, 3). In Fig. 2, root biomass, shallow root weight, and deep root weight showed positive skewness. Positive skewness is associated with complementary gene interactions. These complementary actions were toward smaller root system. In Fig. 3, skewness was positive for plant biomass, was close to zero for grain yield as seen by the median line being at the middle of the box, and was negative for harvest index. Lack of skewness indicates normal distribution and negative skewness is associated with duplicate additive × additive epistasis (homozygote × homozygote interactions). The additive epistatic interactions observed for harvest index were toward lower values. The genes controlling the trait with skewed distribution tend to be of dominant gene action, irrespective of whether they have increasing or decreasing effect on the trait. The traits mentioned above showed significant transgressive segregation as seen by the presence of RILs with values outside of both parental means (Figs. 2, 3). The above results indicated that the cross #49 × Yecora Rojo generated a tremendous amount of genetic variation for many important agronomic traits.

Sample size has a big impact on the value of skewness. Therefore, additive × additive epistasis is detected by comparing the average of the RILs with the mid-parent value (Table 2).
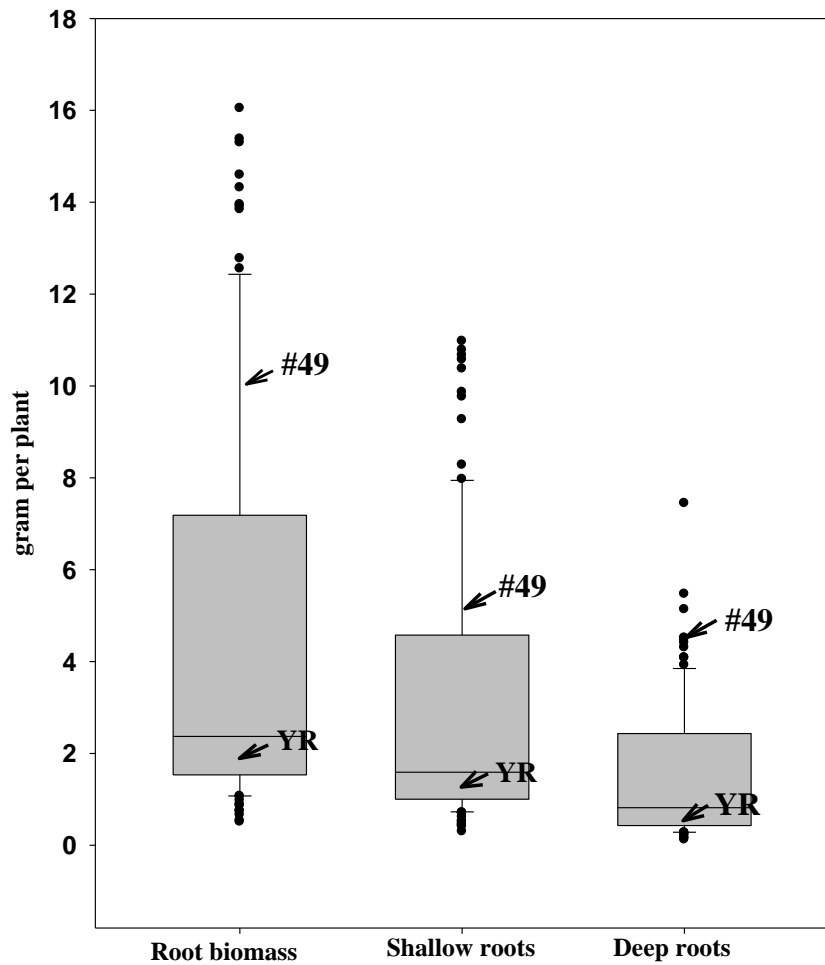

Fig. 2. Box plot for root biomass (g plant^{-1}), shallow root weight (g plant^{-1}), and deep root weight (g plant^{-1}) for 106 bread wheat RILs. The horizantal line in the box is the median value.
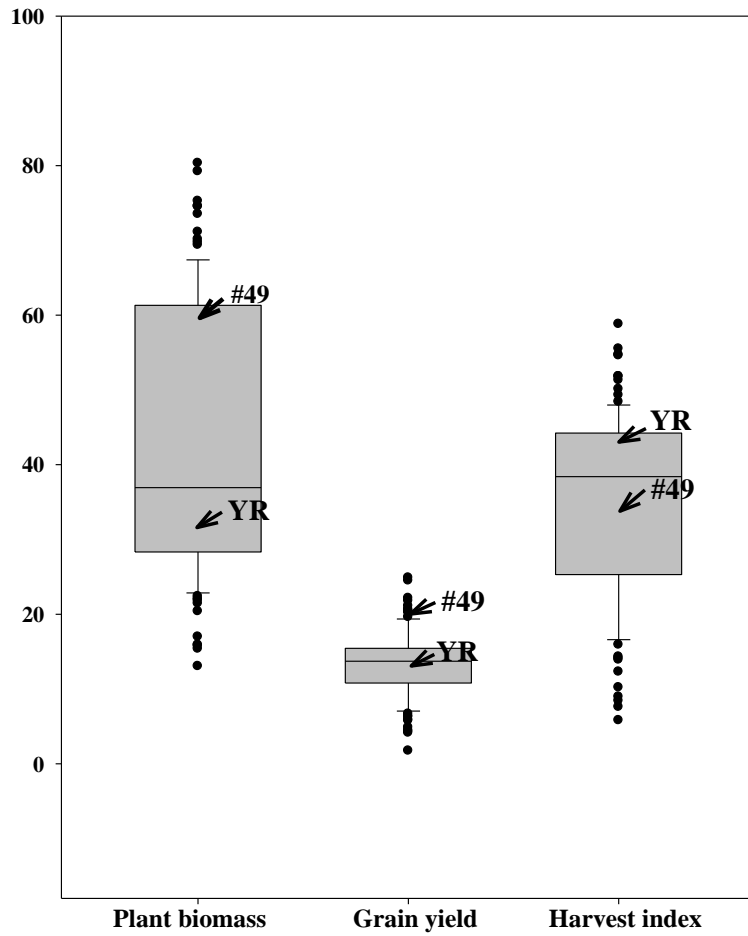
Fig. 3. Box plot for plant biomass (g plant^{-1}), grain yield (g plant^{-1}), and harvest index (%) for 106 bread wheat RILs. The horizantal line in the box is the median value.

Table 2. Means for 104 RILs derived from a #49 × Yecora Rojo cross of bread wheat grown and evaluated in a sand-tube experiment in a glasshouse in 2011-2012 season.

Trait	#49	Yecora Rojo	Mid-parent	RILs
Plant height, cm	137.0[†]a	60.0b	99.0	89.0[**]
Days to maturity, d	127.0a	113.0b	120.0	127.0
Root biomass, g plant^{-1}	9.8a	1.8b	5.8	4.5
Shallow roots, g plant^{-1}	5.3a	1.3b	3.3	3.0
Deep roots, g plant^{-1}	4.5a	0.5b	2.5	1.5[*]
Plant biomass, g plant^{-1}	50.2a	30.3b	46.1	42.2
Grain yield, g plant^{-1}	20.4a	13.8b	17.1	13.2[**]
Harvest index, %	34.4a	43.6b	39.0	35.1[*]

[†] Means for the same trait followed by different letters were different at the 0.05 level.
[*],[**] Means different from their respective mid-parent values at the 0.05 and 0.01 level, respectively.

The parental means were different from each other for all the traits measured (Table 2). The average of the 104 RILs was not different from the mid-parental value for days from germination to maturity, root biomass, shallow root weight, and plant biomass. However, the average of the 104 RILs was different from the mid-parental value for plant height, deep root weight, grain yield, and harvest index. Therefore, these results suggest the presence of additive × additive epistasis for these traits (Table 2).

Only one RIL (#134) had the desirable combination of all traits: GY = 17.2 g plant^{-1}, HI = 46.0%, root biomass = 4.81 g plant^{-1}, shallow roots = 2.92 g plant^{-1}, deep root = 1.89 g plant^{-1}, plant height = 94 cm, days to maturity = 127 d, and plant biomass = 38.0 g plant^{-1}. Additional RILs with a desirable combination of characters may be present in the 165-104 = 61 lines that have not been fully genotyped. Or they might be generated by crossing lines with desirable contrasting characters and selecting out F2 or F3 plants.

Conclusions from Research in 2011-2012 Season:
1. 104 out of a possible 165 RILs were phenotyped for root and shoot characters. In large tubes, there was a positive relationship between root biomass, shoot biomass and grain yield in this RIL population between Iran #49 and Yecora Rojo. The optimum root biomass was 9 g with a shoot to root biomass ratio of 2.44. The relationship may vary with parents and we suspect with the field soil. That California cultivar Blanca Fuerte had larger root biomass and grain yield than Joaquin or Yecora Rojo in this experiment tends to support this conclusion. Some California cultivars may have root systems that are too small to absorb optimum levels of water and nutrients, even under irrigation, which in turn will affect grain yield. This may mean that lines with large root biomass and grain yield should be chosen as parents in future breeding programs. However there are important shoot characters that need to be integrated into a breeding scheme, including shoot and spike characters, disease resistance and harvest index.

2. Information was obtained on gene action and inheritance of root and shoot traits in this RIL population, which may be of use to wheat breeding programs at UC Davis.