Positional cloning of a rye QTL responsible for water stress resistance in wheat based on radiation mapping and comparative genomics
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Project award year: 2016
Three year research project
Overall summary

**Background:** Approximately 900 Mt of wheat (*Triticum aestivum* and *T. turgidum*) are produced worldwide every year (FAO), but further increases are required to feed a growing human population. One understudied area that can contribute to these yield increases is the role of different root architectures on wheat adaptation to different soils. Although some progress has been made in the understanding of root development and architecture in Arabidopsis, this knowledge is limited in grass species.

The introgression of a small segment of wheat (*Triticum aestivum* L.) chromosome arm 1BS in the distal region of rye (*Secale cereale* L.) 1RS.1BL arm translocation in wheat (henceforth 1RS<sup>RW</sup>) was previously associated with reduced grain yield, carbon isotope discrimination and stomatal conductance, suggesting reduced access to soil moisture.

**Original objective:** Our long-term goal is to identify the gene(s) responsible for the differences in drought tolerance and yield under stress.

**Major conclusions, solutions, achievements:** Here we show that lines with the normal 1RS arm have longer roots than lines with the 1RS<sup>RW</sup> arm in both field and hydroponic experiments. In the 1RS<sup>RW</sup> lines, differences in seminal root length were associated with a developmentally regulated arrest of the root apical meristem (RAM). Approximately 10 days after germination, the seminal roots of the 1RS<sup>RW</sup> plants showed a gradual reduction in elongation rate, and stopped growing a week later. Seventeen days after germination, the roots of the 1RS<sup>RW</sup> plants showed altered gradients of reactive oxygen species and emergence of lateral roots close to the RAM, suggesting changes in the root meristem.

The improved phenotyping method using hydroponics allowed us to establish consistently, and in less than 3 weeks, the presence or absence of the gene variant causing loss of apical dominance and short roots. Using this methodology we have made significant progress in the identification and validation of the 1RS<sup>RW</sup> candidate gene(s). An RNAseq study demonstrated that our candidate gene is a master regulator affecting the transcript levels of multiple genes involved in meristem development, auxin, jasmonic acid and cytokinin metabolism, and distribution of reactive oxygen species along the root.

**Implications, both scientific and agricultural:**

The shorter roots of the 1RS<sup>RW</sup> lines relative to the 1RS lines were associated with reduced biomass (estimated by Normalized Differences Vegetation Index) and grain yield, with larger differences under reduced or excessive irrigation than under normal irrigation. These results suggest that this genetic variation could be useful to modulate root architecture and mitigate the negative impacts of excess or reduced water in wheat production.
Summary Sheet

Publication Summary

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Training Summary

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Description of the cooperation

The USA and Israel labs have established a productive collaboration during the last 20 years that continued during this project as demonstrated by the publication list below. The group from Israel has provided their expertise in wheat genomics and their resources for the IBS physical map. They have also provided the annotation of the genes present in the candidate regions. The USA group has performed the hydroponic and field experiments and the RNAseq and radiation mutants. Dr. Gilad Gabay, who finished his PhD at the Hebrew University of Jerusalem and ARO, Volcani center in Israel, will be the new lead postdoc at UCD laboratory, which will further facilitate communication among the US and Israeli laboratories. In summary, we have made significant progress in the first two years of the project advancing all the proposed objectives in spite of personnel changes. We have identified a promising group of candidate genes that have been validated by physiological experiments and that are now being validated using mutants and transgenic approaches for final publication.

Prof. Fahima visited Prof. Dubcovsky at Davis California in January 2018 and they met also at the International Wheat Genetics Symposium in Saskatoon, Canada (July 21st to 26th, 2019). Since October 2019, Dr. Fahime is in a Sabbatical visit at Davis, which facilitates frequent meetings. The PIs and postdocs coordinate activities and exchange results via regular email and skype communications. A new PhD student from Israel will stay for one year in Dr. Dubcovsky’s laboratory to be trained in the root phenotyping and genotyping technologies.
Achievements

Published Results

We published a joint paper describing the root phenotypes of the Hahn-1RS (a complete 1RS arm from rye) and Hahn-1RS\(^\text{RW}\) (complete 1RS plus a distal insertion of a small segment from wheat chromosome arm 1BS) in hydroponic and field experiments in the *Journal of Experimental Botany* 70: 4027–4037. This study was fully supported from by this BARD grant, and additional publications generated from this and previous support are listed at the end of this final report.

**1RS\(^\text{RW}\) lines have shorter roots than 1RS lines in the field**

To compare the length of the roots of the 1RS and 1RS\(^\text{RW}\) NILs in the field we planted an experiment in the field organized in an RCBD with six blocks and four genotypes per block. Plots were machine sown in 4.5 m\(^2\) plots, and we excavated ~2 m deep trenches cutting perpendicular across the middle of plots including complete blocks one, three and six (Figure 1A) to expose the root system. We took horizontal soil core samples from the center of each block at 20 cm intervals using a thin-walled copper pipe. Total root length densities were consistently higher in the 1RS than in 1RS\(^{\text{RW}}\) NILs through the soil profile, with the largest absolute differences detected at 40 cm (Fig. 1B). The overall ANOVA for total root length density showed significant differences between the 1RS and 1RS\(^{\text{RW}}\) genotypes ($P = 0.004$). The differences in root length explained our previous results showing reduced drought tolerance and reduced yield under stress in the 1RS\(^{\text{RW}}\) genotype relative to the 1RS isogenic line. These differences were associated with a greater access of the 1RS genotype to stored soil moisture (Howell et al. 2014).
Figure 1. Change in total root length in 1RS\textsuperscript{RW} and 1RS\textsuperscript{WR}. (A) Excavation of the first block including the four genotypes (1RS, 1RS\textsuperscript{RW}, 1RS\textsuperscript{WR}, 1RS\textsuperscript{WW}). The holes below the center of each plot (20 to 140 cm deep, and 180 cm deep in the other two blocks) indicate were the horizontal soil core samples were taken. (B) Total root length density. Error bars represent the SE of the means across blocks. Asterisks or numbers above error bars are P-values for the difference between genotypes at individual depths. *$P<0.05$, **$P<0.01$, ***$P<0.001$.

**Improved phenotyping method**

We made substantial progress in phenotyping and characterizing the differences between the 1RS and 1RS\textsuperscript{RW} genotypes. We implemented a hydroponic culture system in which we observed consistent differences in main root length and root apical dominance (Fig. 2A). Differences start approximately 10 days after germination when the primary root of the 1RS\textsuperscript{RW} genotype starts to slow down and eventually ceases to grow (Fig. 2B). The root apical dominance disappears in the 1RS\textsuperscript{RW} genotype and lateral branches are formed close to the root apical meristem (Fig. 2C). By contrast, the 1RS showed consistent root elongation with a relatively large distance between the root tip and the lateral root initiation zone. Using this improved phenotyping method in hydroponic culture we reduced the phenotyping time from once a year (field conditions) to approximately 3 weeks (hydroponic conditions).
1RS and 1RS\textsuperscript{RW} lines differ in and reactive oxygen species (ROS)

Since ROS gradients affect root elongation, we estimated their distribution in seminal roots 17 DAG by measuring the amount of formazan produced from the reduction of NBT and fluorescence in DCF-DA staining. Formazan intensity, associated with superoxide anions, was similar between genotypes near the root tips (distal region) but was significantly lower in the 1RS\textsuperscript{RW} than in the 1RS roots starting at 650 $\mu$m from the root tip ($P < 0.05$), and becoming even more pronounced after 860 $\mu$m ($P < 0.001$, Fig. 3A).

Fluorescence intensity from the DCF-DA staining, associated mainly with hydrogen peroxide, peroxynitrite and hydroxyl radicals, showed a very different pattern. We observed significantly higher intensities in 1RS\textsuperscript{RW} relative to the 1RS roots between 250 and 950 $\mu$m, and even higher differences between 350 and 640 $\mu$m measured from the root tip ($P < 0.001$, Fig. 3B).

The transition from cell proliferation to cell elongation and differentiation and the subsequent development of lateral roots depends on the distribution of ROS along the root axis, specifically on the opposing gradients of superoxide and hydrogen peroxide. Superoxide is predominant in
dividing cells in the meristematic zone, while hydrogen peroxide is predominant in elongated cells in the differentiation zone. The balance between these ROS modulates the transition between root proliferation and differentiation zones. Therefore, the different ROS distribution detected between 1RS and 1RS\textsuperscript{RW} roots reflect the major developmental changes between these genotypes.

**Fig. 3.** Distribution of ROS along the main roots of plants carrying the distal rye (1RS) or wheat chromosome segments (1RS\textsuperscript{RW}) 17 d after germination. (A) Formazan signal intensity along the roots as determined with NBT (mainly detects superoxide anions). (B) Fluorescence signal intensity along the root as determined by DCF-DA (mainly detects hydrogen peroxide). Zero indicates the most distal point of the root (root tip). Representative NBT- and DCFDA-stained roots from the corresponding experiment (number indicates growth rate in mm h\textsuperscript{-1} of the individual roots at the last sampling time). Circles connected with a line indicate the significant interval (an arrow indicates that the significant interval continues).

**Reference used in this report**
Changes in direction from that in the original proposal

The project proceeded as planned with the initial results already published. Most of the proposed experiments are completed or in progress. The genomic sequencing and RNAseq studies have been completed and are being analyzed for publication. In the new project, we will complete the validation of the candidate genes using CRIPR-Cas9 mutants and overexpression of the candidate genes. We have already generated CRISPR and overexpression transgenic plants for three of the candidate genes.
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<td>Krugman, T., Nevo, E., Beharav, A., Sela, H., &amp; Fahima, T.</td>
<td>The Institute of Evolution Wild Cereal Gene Bank at the University of Haifa</td>
<td>Israel Journal of Plant Sciences</td>
<td>65 : 129-146</td>
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<td>Klymiuk, V., Fatiukha, A., &amp; Fahima, T.</td>
<td>Wheat tandem kinases provide insights on disease-resistance gene flow and host–parasite co-evolution</td>
<td>The Plant Journal</td>
<td>98 : 667-679</td>
<td>2019</td>
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<td>Yaniv, E., L. Huang, V. Klymiuk, D. Raats, L. Feng, S.</td>
<td>The cloned Yr15 gene (WTK1) encodes two kinase-like protein domains, both required for</td>
<td>International Plant and Animal Genome</td>
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<td>Fahima T., Klymiuk V., Fatiukha A., Huang L., Krugman T.</td>
<td>Transferring of exotic resistance alleles from wild emmer into bread wheat using durum as a bridge</td>
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<td>Fahima T.</td>
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<td>Klymiuk V, Fatiukha A, Lin Huang, Wei ZZ, Kis-Papo T, Saranga Y, Krugman T. and Fahima T.</td>
<td>Diversity and evolution of disease resistance genes derived from wild emmer wheat.</td>
<td></td>
<td>; 2017</td>
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<tr>
<td>Published</td>
<td>T.R. Howell, A. Lukaszewski, and J. Dubcovsky.</td>
<td>Characterization of two isogenic chromosome arm translocations from rye into wheat that show differential drought resistance.</td>
<td></td>
<td>; 2016</td>
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APPENDIX

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Unpublished data

Epistatic interactions of 1RS<sup>RW</sup> with other genes

In our initial study, we observed that the differences between the 1RS /1RS<sup>RW</sup> lines on grain yield were stronger in the Hahn than in the Attila genetic background (Howell et al. 2014). In the hydroponic experiments, we observed that the effects of the 1RS<sup>RW</sup> chromosome on root length disappeared when the 1RS<sup>RW</sup> chromosome was introgressed in the variety Pavon, and this correlated with the absence of differences in grain yield in the field. To study the genes that control these differences in the presence of the 1RS<sup>RW</sup> effect, we made a cross between Hahn-1RS<sup>RW</sup> and Pavon-1RS<sup>RW</sup> and analyzed the differences in root length in the F<sub>2</sub> population. We genotyped this population and identified two strong QTL for root length on chromosome arms 4BL (LOD 4.7, 32.8% variation explained) and 5BL (LOD 3.1, 15.3% variation explained).

Then, we generated a second F<sub>2</sub> population from the cross Hahn-1RS x Pavon-1RS<sup>RW</sup> to study the interactions of the 1RS and 1RS<sup>RW</sup> polymorphism with the 4BL and 5BL QTL detected in the first population. The 4BL QTL showed an interaction with the 1RS/1RS<sup>RW</sup>, with larger differences in the 4BL Hahn allele. The 5BL QTL showed the same trend as in the previous population, with the Pavon allele associated with longer roots. We did not detect any significant interaction between 1RS/1RS<sup>RW</sup> and the 5BL QTL. These results indicate that the 4BL QTL allele from Hahn might be important for the detection of the effect of the 1RS/1RS<sup>RW</sup> polymorphism.

Identification of wheat genes in the distal 1BS introgressed segment

We sequenced and assembled 1RS and 1RS<sup>RW</sup> telocentric arms sorted by flow cytometry (1RS: 80X coverage, 1RS<sup>RW</sup>: 100X coverage). Assembled sequences covered 145.3 Mb for the 1RS arm and 153.8 Mb for the 1RS<sup>RW</sup> arm. We compared the two assemblies with the recently
released wheat genome RefSeq v1.0 and identified a 4.6 Mb segment of 1BS chromatin present in the 1RS\textsuperscript{RW} arm (Fig. 4).

All rye homologs the genes present in the 1BS segment were present in both the 1RS and 1RS\textsuperscript{RW} lines, which indicated that the short 1BS segment in the 1RS\textsuperscript{RW} arm was the result of an insertion into the 1RS arm, rather than a replacement of the orthologous rye segment. Since no rye gene was missing in the 1RS\textsuperscript{RW} arm, we concluded that the short-root phenotype of the 1RS\textsuperscript{RW} lines was not caused by the deletion of a favorable rye gene.

To test if a gene in the introgressed wheat segment was responsible for the short-root phenotype, we characterized the 1RS-1BS recombinant line T-21, which has a recombinant 1RS chromosome with a distal 1BS segment encompassing all the 1BS genes present in 1RS\textsuperscript{RW} and replacing all the orthologous genes (a normal homeologous recombination event). We backcrossed these recombinant lines into the Hahn background, and found no significant differences in root length between T-21 and the 1RS line ($P = 0.64$), which demonstrated that short roots were not caused by a particular wheat gene present in the 1BS region. Based on this
result, we hypothesize that changes in gene dosage caused by the genes present in the inserted 1BS segment in 1RS\textsuperscript{RW} were responsible for the short-root phenotype.

**Dissection of the 4.6-Mb 1BS introgression using deletion mapping**

To dissect the introgressed 1BS region, we generated radiation mutants. We identified one deletion affecting the proximal region of the 1BS insertion, designated as C1del (Fig. 5A). We sequenced the coding regions of C1del using exome capture and mapped the sequencing reads back to the reference wheat genome. Using this strategy, we determined that C1del has a 1.46 Mb deletion of the 1BS introgressed segment (Fig. 5A).

To test if the deleted region included the genes responsible for short roots, we backcrossed this deletion 4 times to Hahn-1RS\textsuperscript{RW} to reduce background mutations. We genotyped and phenotyped the segregating BC\textsubscript{4}F\textsubscript{2} progeny for root length and found that plants homozygous for the deletion had significantly longer roots than the plants heterozygous or homozygous for the 1RS\textsuperscript{RW} chromosome. In addition, we found no significant differences in root length between the C1del homozygous and Hahn-1RS control plants (Fig. 5B). This result was validated in a separate BC\textsubscript{2}F\textsubscript{2} population (C1del x Hahn-1RS). Taken together, these results demonstrated that the duplicated gene(s) that caused the short roots were located within the 1.46 Mb 1BS deletion.

Since the elimination of some of the duplicated genes restored the long-roots, we interpreted this result as additional evidence supporting the hypothesis that the changes in gene dosage caused by the insertion of the 1BS segment were responsible for the short-root phenotype of the Hahn-1RS\textsuperscript{RW} plants.
Gene expression profile in the distal 1BS introgressed segment

To prioritize the genes within the 1.46 Mb region, we analyzed their expression profiles using RNAseq. We collected the apical part (1.0-1.5 cm) of the main roots from 1RS and 1RS\textsuperscript{RW} plants (lateral roots were eliminated) at 6, 9 and 16 days after germination (DAG). The samples collected at 16 DAG showed the largest differences between genotypes and were the most divergent among the three days. A significantly larger proportion of the total analyzed genes (82,602) were differentially expressed (FDR < 0.01) between the two genotypes at 16 DAG (39.2%) than at 6 (0.05%) or 9 DAG (0.04%). We detected 32,381 differentially expressed genes between 1RS and 1RS\textsuperscript{RW} (FDR \(P < 0.01\)) in seminal roots at 16 DAG. This result indicates that the 1BS insertion in 1RS\textsuperscript{RW} triggered a major root developmental switch in the roots.

It is not possible to compare the relative expression of the 1BS genes between the 1RS\textsuperscript{RW} and 1RS genotypes because the 1BS genes are absent in 1RS. Therefore, we compared the expression levels at different time points and prioritized the genes that showed a large fold change between 6 DAG (when no differences were observed in main root length or ROS distribution) and 16 DAG (when those differences were evident).
The 1.46 Mb candidate region includes 38 high-confidence annotated genes. We found that 14 of those genes were expressed at very low levels or were not expressed at all in roots. Among the 24 genes expressed in roots, we prioritize 11 candidate genes for further functional characterization. We have initiated the generation of CRISPR and overexpression transgenic plants for these gene for functional validation.

Additional results obtained as part of the BARD collaboration

Positional cloning of an additional gene residing on chromosome 1BS: Cloning of the wheat 

Yr15 resistance gene sheds light on the plant tandem kinase-pseudokinase family (Klymiuk et al. 2018)

In parallel to the efforts devoted to the cloning of the wheat/rye QTL responsible for water stress resistance, we have continued our collaboration, stemming from our previous BARD grant, on the cloning of resistance gene Yr15 that is effective against most races of stripe rust. This gene was introgressed into bread and durum wheats from the wheat progenitor Triticum dicoccoides. The 1BS resources developed in the Yr15 and current project complemented each other and accelerated both projects. The Yr15 results were recently published in Nature Communications (Klymiuk et al., 2018). In this paper we report the cloning of Yr15, a broad-spectrum resistance gene derived from wild emmer wheat, which encodes a putative kinase-pseudokinase protein designated as WHEAT TANDEM KINASE 1 (WTK1).

Abstract: Yellow rust, caused by Puccinia striiformis f. sp. tritici (Pst), is a devastating fungal disease threatening much of global wheat production. Race-specific resistance (R)-genes are used to control rust diseases, but the rapid emergence of virulent Pst races has prompted the
search for a more durable resistance. Here, we report the cloning of Yr15, a broad-spectrum R-
gene derived from wild emmer wheat, which encodes a putative kinase-pseudokinase protein,
designated as wheat tandem kinase 1, comprising a unique R-gene structure in wheat. The
existence of a similar gene architecture in 92 putative proteins across the plant kingdom,
including the barley RPG1 and a candidate for Ug8, suggests that they are members of a distinct
family of plant proteins, termed here tandem kinase-pseudokinases (TKPs). The presence of
kinase-pseudokinase structure in both plant TKPs and the animal Janus kinases sheds light on the
molecular evolution of immune responses across these two kingdoms.

Publications and invited talks at International meetings

We published a joint paper fully supported from this grant describing the root phenotypes in
hydroponic and field experiments in the Journal of Experimental Botany (1).


We also published a joint paper in Nature Communications, describing the positional cloning of
Yr15, which used the physical mapping and sequencing of the 1BS chromosome supported by
this grant. This project was supported by our previous and current BARD grants:

2. Klymiuk V, Yaniv E, Huang L, Raats D, Fatiukha A, Chen S, Feng L, Frenkel Z,
Bariana H, Sela H, Saleem K, Sørensen CK, Hovmoller MS, Distelfeld A, Chalhoub B,
resistance gene sheds light on the plant tandem kinase-pseudokinase family. Nature
Communications 9:3735.

The approach used for the cloning of Yr15 was described in a review paper, published by the
Israeli PhD student and PI, as a book chapter:


Presentation in scientific conferences:


11. **Fahima T.,** Klymiuk V., Yaniv E., Huang L., Raats D., Fatiukha A., Chen S., **Krugman T.,** Jääskeläinen M., Chang W., Schudoma C., Bariana H., Khadgi-Sørensen C.,...

