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Introduction

Wheat is the most strategic crop that provides about 20% of the daily dietary protein intake in the world. The productivity and production of durum wheat (*Triticum turgidum* subsp. *durum*) is affected by stem rust (*Puccinia graminis* f. sp. *tritici*: Pgt), leaf rust (*Puccinia triticina*: Pt) and yellow rust (*Puccinia striiformis* f. sp. *tritici*: Pst) in different countries (Silva et al. 2023). Virulent races of (Pgt), such as TTTF, and races of (Pst), such as PstS7 and PstS14, have been recently identified in Morocco (El Hanafi, S. et al. 2018). Across North Africa and the Middle Eastern regions where durum wheat is a major staple crop, rust diseases pose a serious threat to wheat production, often leading to severe yield losses under favorable disease development conditions. The most prevalent diseases in farmers' fields were leaf rust (LR) and stripe rust (SR), highlighting the need to develop elite germplasm with multiple disease resistance. Efforts have been made to develop multiple resistance to three rust diseases in ICARDA durum wheat breeding pipelines. The objective of this work is to identify new sources of resistance to three rust diseases of durum wheat.

Materials and methods

Seedling stage screening: Forty-eight elite durum wheat genotypes were evaluated for their resistance to leaf rust (LR), stem rust (SR) and yellow rust (YR) under controlled conditions. For LR and SR, urediniospores were mixed with talc powder and dusted onto 12-days old plants. Inoculated seedlings were incubated for 24 hours in a growth chamber under dark conditions at 18–20 °C. After incubation, seedlings were maintained in the growth chamber at 20 °C with a 16-hour light/8-hour dark photoperiod (Figure 1).

For yellow rust (YR), inoculated seedlings were incubated for 24 hours under dark conditions at 10 °C, followed by incubation at 17 °C with a 16-hour light/8-hour dark photoperiod for 17 days.

Field screening: The 48 genotypes were planted at Sidi Allal Tazi research station of INRA, Morocco, favorable for LR development under natural infection in 2023/2024 cropping season.

Disease evaluations: For LR and SR, seedlings were evaluated 12 days after inoculation for infection types (IT) using a 0–4 rating scale (Stakman et al. 1962). Where 0 to 2+ were considered low IT (resistance), and 3 to 4 were considered as high IT (susceptible). Under field condition, genotypes were evaluated for percent diseases severity following the modified Cobb's scale (Peterson et al. 1948) and infection types of R, MR, MS, and S at heading stage (Roelfs et al. 1992).

Genome-Wide Association Study (GWAS) of Rust Resistance was carried out by combining genotypic data and disease severity scores from the seedling and the adult plant stages. Marker-trait association(s) (MTA) were determined using a mixed linear model (MLM) in TASSEL association(s) (MTA) were determined using a mixed linear model (MLM) in TASSEL version 5.2.53

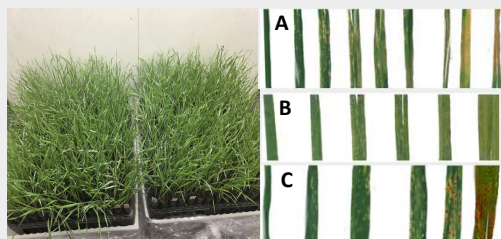


Figure 1. Leaf rust inoculation at seedling stage.

Figure 2. Variations in wheat genotype responses to yellow rust (A), leaf rust (B), and stem rust (C) at the seedling stage.

Results

- Genotypes showed varying levels of responses for the three rust diseases (Figure 2).
- At seedling stage, 39 genotypes were resistant to yellow rust, 31 genotypes were resistant to leaf rust, and 26 genotypes were resistant to stem rust.
- At the adult plant stage, 35 genotypes were resistant to leaf rust, and 16 genotypes were resistant to stem rust across two environments.
- 13 genotypes were resistant to all three rust diseases at the seedling stage (Figure 3).
- 7 genotypes were resistant to LR, SR and YR at both the seedling and adult plant stages.
- Genome-wide association analysis identified several markers with strong associations to rust disease resistance. Among these, only one marker showed a significant association with resistance to leaf rust (LR), and one with stem rust (SR). A single-marker regression analysis for resistance to the three types of rusts confirmed the presence of major known markers.

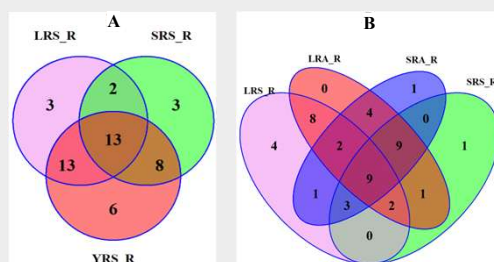


Figure 3: A) Venn diagram of infection responses (R) of 48 durum wheat genotypes to the three rust diseases yellow rust-resistant (YRS_R), stem rust-resistant (SRS_R) and leaf rust-resistant (LRS_R) at the seedling stage. B) Venn diagram of infection responses of 48 durum wheat genotypes to leaf rust and stripe rust at the seedlings stage (LRS_R and SRS_R) and at the adult plant stage (LRA_R and SRA_R).



Figure 4. Variations in adult plant stage resistance at the Sidi Allal Tazi Field Station for stem rust (A), and leaf rust (B)



Figure 5. Leaf rust resistant (R) and Susceptible (S) reaction.



Figure 6. Stem rust resistant (R) and Susceptible (S) reaction.

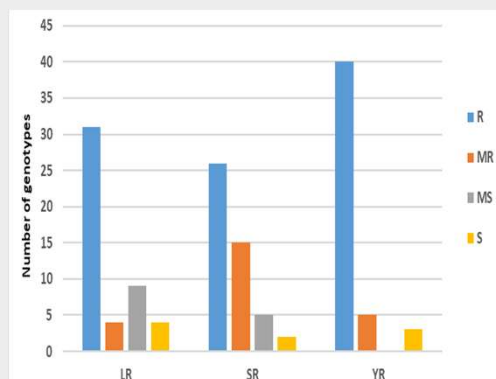


Figure 7. Number of durum wheat genotypes with different reaction responses to leaf rust (LR), stem rust (SR), and yellow rust (YR) under controlled condition. Here R = resistant, MR = moderately resistant, MS = moderately susceptible, and S = susceptible.



Figure 8. Durum wheat infected with yellow rust at adult plant stage.

Figure 9. Yellow rust under control condition.

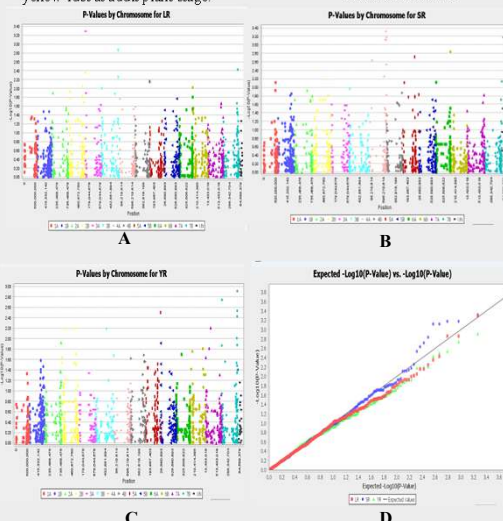


Figure 10. Genome-wide association mapping of durum wheat rust resistance at the seedling stage: A) leaf rust (LR), B) Stem rust (SR), and C) yellow rust (YR). The Manhattan plots display significant marker-trait associations on various chromosomes of durum wheat $-\log_{10}$ of p-values. (D) Quantile-Quantile (Q-Q) plots of marker-trait associations at the seedling stage using MLM model in TASSEL.

Conclusion

- The elite durum genotypes showed varying levels of reactions to the three diseases.
- Some genotypes were resistant to all tested rust diseases at seedling.
- Seven genotypes showed resistance to all three rust diseases under both controlled conditions and at the adult plant stage
- In general, there is high potential to develop multiple rust disease resistant durum wheat elite genotypes