

Case Study

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Multi-Environment Trial Analysis of Elite Rye Cultivars under Rainfed Conditions

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Received: 20 Apr., 2025

Accepted: 30 May, 2025

Published: 19 Jun., 2025

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Preferred citation for this article:

Zhang G.P., and Wang W., 2025, Multi-environment trial analysis of elite rye cultivars under rainfed conditions, Triticeae Genomics and Genetics, 16(3): 130-137 (doi: [10.5376/tgg.2025.16.0014](https://doi.org/10.5376/tgg.2025.16.0014))

Abstract Rye (*Secale cereale* L.), as an important crop with both food and feed value, has strong adaptability in arid and semi-arid regions and is a key variety resource for ensuring food security and the development of animal husbandry. However, under dryland conditions, the yield and quality of rye are highly constrained by environmental factors, showing a significant genotype-environment interaction (G×E) effect. To deeply assess the performance of superior rye varieties in different ecological regions, this study, based on the multi-environment test (MET) method, systematically analyzed the yield, quality, stress resistance and resource utilization efficiency of multiple elite rye varieties under typical arid conditions. By comparing the yield differences, stability of quality traits and water and nitrogen utilization efficiency under different ecological environments, Combining GGE-Biplot and stability analysis methods, varieties with high yield, high quality and wide adaptability were screened out. The research results show that multi-environment trials can not only reveal the restrictive factors of the environment on rye production, but also provide a theoretical basis for regionalized variety recommendation and the establishment of breeding goals in dryland farming areas. This study aims to promote the application and popularization of superior varieties by scientifically evaluating the comprehensive performance of elite rye varieties under dryland conditions, and to provide references for future rye breeding and sustainable agricultural development in arid areas.

Keywords Rye; Dryland farming conditions; Multi-environmental tests; Output stability; Variety adaptability

1 Introduction

Rye (*Secale cereale* L.) plays a very important role in global food security and animal husbandry, especially in areas with poor soil and little rainfall. It has strong adaptability and tenacious vitality, and is suitable for both human consumption and animal feed. In Central and Eastern Europe, rye is often planted in sandy soil, which has poor water retention capacity, but rye can still grow well (Schittenhelm et al., 2014). It is precisely because it can grow under less favorable conditions that rye has helped the economy of rural areas and made the supply of food and feed more stable.

Although there are many benefits to growing rye under rain-fed conditions, there are also some problems. Drought caused by climate change is one of the biggest problems. When drought comes early or is particularly severe, the output will drop significantly, sometimes even by 14% to 50% (Hubner et al., 2013; Kottmann et al., 2016). Unstable rainfall, coupled with poor soil fertility, makes it even more difficult for rye to maintain high yields continuously. Therefore, the focus of breeding work is to select varieties that are more drought-tolerant and have stronger adaptability. Against this backdrop, multi-environment testing (MET) becomes particularly important. It can help us understand the performance of different varieties in different environments and see which varieties can maintain stable yields under multiple conditions. This is a very useful tool for breeders (Haffke et al., 2015).

This study conducted a comprehensive analysis of superior rye varieties evaluated through multiple environmental trials under dryland conditions, including assessing the yield performance and stability of the selected varieties, analyzing the interaction between genotypes and the environment, and providing suggestions for variety breeding to enhance regional adaptability and resilience. This study aims to provide references for breeding strategies and support the sustainable production of rye in the face of increasingly severe climate challenges.

2 Yield Performance of Rye Cultivars across Different Environments

2.1 Yield variation and its dependence on environmental factors

What exactly affects the yield of rye? Ultimately, the differences can be quite significant in different places and years. There are quite a few factors influencing it, including climate, soil and management methods, but the most crucial one is actually the environment. Many studies have long pointed out that things like weather that change every year, as well as natural conditions in different locations, often have a greater influence than genes themselves (Laidig et al., 2017). It is even said that 70% of the changes in production can be attributed to environmental factors, especially when the temperature is extreme or there is too much sand in the soil (Zymaroieva and Nykytiuk, 2023). Of course, management is not optional. For instance, how to apply nitrogen fertilizer and how to manage the land can also create a gap in yield. Moreover, in many cases, hybrid varieties perform better, especially when encountering adverse conditions or marginal plots (Ghafoor et al., 2024).

2.2 Stability analysis and identification of high-yielding and stable cultivars

Hybrid varieties are good because they have a high average yield, usually 17.9% to 24.9% higher than population varieties. There is no dispute about this in terms of data. But whether it is stable or not is another matter. Especially in scenarios with diseases and high management intensity, their performance may not be so stable (Sulek et al., 2024). Not all varieties are like this. Some exceptions are still worth mentioning. For instance, "Grafinya" and "Lika" have been identified as representatives that balance high yield and stability. They not only have strong adaptability but also perform well in multiple locations and years (Parfenova and Psareva, 2024). The stability indicators commonly used in breeding, such as Shukla variance and the stability index of multiple traits, are precisely designed to select such reliable varieties, making their application more reassuring.

2.3 Main characteristics of genotype \times environment ($G \times E$) interactions

As for the interaction between varieties and the environment, to put it simply, "relying on the weather for a living" also depends on the variety. The ranking of rye crops may not be the same in different environments - the top spot can change with a different location or year. This kind of "cross-interaction" is not uncommon (Parfenova and Psareva, 2024; Sulek et al., 2024). This is precisely why it is necessary to conduct numerous environmental tests. Otherwise, relying solely on data from one area, it is very difficult to determine whether a variety is truly "universal". Some varieties rush forward as soon as conditions improve, while others can hold their ground under pressure. This difference stems from the $G \times E$ effect. Its significance also lies in reminding us that variety recommendations should not be a one-size-fits-all approach; they should be classified by region if necessary. To analyze these complex interaction relationships, some advanced statistical models are needed, so as to more reliably determine which varieties are suitable for cultivation in which ecological environments (Laidig et al., 2017; Zymaroieva and Nykytiuk, 2023).

3 Environmental Adaptability of Quality Traits

3.1 Regional variation in protein content and nutritional quality

The amount of protein it contains is not known only after it is grown, but is closely related to where it is grown, in which year it is grown, and whether there is water or not. This matter cannot be determined by genotype alone. The influence of the environment is greater, especially the two variables of harvest year and regional location. When they change, the protein level varies significantly (Siekman et al., 2021; St wowost pniewska et al., 2024). To give an extreme example, the protein content of rye grown in the arid areas of Mexico can be as high as 20.3% - 22.8% (Yanez et al., 2023), which to some extent indicates its "tough" ability in harsh environments. By the way, although hybrid varieties produce more, they are not so outstanding in terms of protein and dietary fiber, and are usually lower than population varieties (Figure 1) (Brzozowski et al., 2023). For those who want to pursue high nutritional value, this might require more thought.

3.2 Performance patterns of grain physical traits (thousand-kernel weight, test weight)

In addition to nutritional value, another aspect of grain quality that is often examined is physical indicators, such as 1000-grain weight (TKW) and bulk density. These characteristics may seem simple on the surface, but in fact, they are quite influenced by the environment and the variety itself. Multi-environmental experiments have found

that not only do different varieties exhibit different behaviors, but G×E interactions also "get involved", making the results more variable (Miedaner et al., 2012). Interestingly, in those arid or semi-arid plots, rye does not completely fail. It can still maintain a commercially acceptable grain shape and weight (Sabaghnia and Janmohammdi, 2024), indicating that this crop still has some foundation in an environment with unstable water content. Moreover, the heritability of these physical traits is not low, and it is not difficult to select them for breeding. As long as the direction is right, it is entirely possible to pick out stable genotypes.



Figure 1 Images of cereal rye production. Clockwise from top left: emerging from winter dormancy, anthesis, mature grain prior to harvest and grain maturation. Photos by Elżbieta Szuleta (Adopted from Brzozowski et al., 2023)

3.3 Stability of feeding and processing quality

The requirements for processing and feeding are not that simple. For instance, dietary fiber, viscosity, and baking effect - none of them can fail. However, the performance differences among different varieties in these aspects are indeed quite significant. Interestingly, group varieties stand out more in terms of fiber and viscosity indicators, which are quite advantageous whether for human consumption or as feed (Brzozowski et al., 2023; St Wodaniewska et al., 2024). Of course, there are also balanced varieties, such as "Zilant", which has a decent yield and stable baking quality. It can maintain a high standard in many regions for several years (Ponomareva et al., 2021). Although most quality traits are susceptible to the G×E interaction, on the other hand, through multi-environment testing and with the addition of some advanced genetic analysis methods, it is still possible to select varieties with wide adaptability and stable quality.

4 Stress Resistance and Resource Use Efficiency

4.1 Water use efficiency under rainfed conditions

It has long been recognized that rye can survive quite well in dry land, especially in some marginal areas where there is little rainfall, yet it can still produce some yield (Moskal et al., 2021). But saying it is "drought-resistant" doesn't mean there will be no problem at all when there is a drought. If drought really comes, rye will still be affected, both in terms of yield and physiological response. Moreover, the ability of different varieties to withstand drought varies, which is actually closely related to how water is managed and how water shortages are dealt with. Some varieties are a bit smarter. Their root systems are deeply rooted and their osmotic regulation is well done. Even if the water is insufficient, they can still maintain the yield (Makhramova and Urokov, 2024). In addition, some research has found that when certain chromosomal fragments of rye are introduced into wheat, the water use efficiency also improves. This "borrowing genes" approach to some extent indicates that there is a genetic basis behind drought resistance.

4.2 Disease resistance and genotype × environment interaction effects

Rye is not only drought-resistant, but it is also quite capable in resisting pests and diseases. The main reason is that its genetic foundation is rich enough, containing many disease-resistant genes. However, whether one can

resist diseases or not also depends on whether the environment is suitable or not. Once G×E interaction occurs, it may cause varieties that originally performed well to "fail" somewhere else (Rakoczy-Trojanowska et al., 2021). For instance, some rye varieties have been able to maintain a stable disease defense line for several years and multiple pilot projects, but others are "picky about the environment", and their performance immediately changes when the climate or the type of pathogen is changed (Safonova and Aniskov, 2022). Of course, there are now new tools in breeding. For instance, genomics has been able to identify some key genes involved in stress responses, which presents a great opportunity for breeding new disease-resistant varieties (Lin et al., 2024).

4.3 Nitrogen use efficiency and its contribution to yield and quality

While other grains are still relying on fertilization to boost their yields, rye has long been doing more work with less fertilizer. Especially in the application of nitrogen fertilizers, its efficiency is so high that it is enviable (Milczarski et al., 2011). Part of the reason is that its physiological structure is "energy-saving", and the other part is that it has the ability to save fat in its genes. In many low-investment systems, it can grow steadily and well, which is very attractive to those engaged in sustainable agriculture. If rain-fed conditions are encountered, improving nitrogen utilization efficiency is quite crucial. On the one hand, the yield is less likely to drop; on the other hand, the quality can also be maintained. After all, when there is too much nitrogen, the processing characteristics of grains may be affected instead (Moskal et al., 2021). Fortunately, with the availability of tools such as molecular markers and genomic resources, breeders are increasingly capable of precisely identifying varieties with higher nutrient utilization efficiency, ensuring both yield and quality are not compromised.

5 Case Studies: Multi-Environment Trial Results in Typical Regions

5.1 Rye cultivar adaptability in Eastern European rainfed areas

The climate changes year by year, and the output fluctuates up and down accordingly. In Eastern Europe, especially in the winter rye growing areas of Russia, this phenomenon is actually not new. Safonova and Aniskov (2023) analyzed a large amount of multi-environmental test data and found that climate change almost "determines" the fluctuations in production, with an impact proportion as high as 79%. However, not all varieties "rise and fall together" in such an environment. In the Kirov region, varieties like "Rafinha" and "Lika" have performed steadily, with yields reaching 5.09 and 5.07 tons per hectare respectively. Their overall adaptability is also good, and their stability is not bad (Parfenova and Psareva, 2024). Of course, there is also a bit of "differentiation". Varieties like "Baptiste" and "Perepel" are also quite good overall, with strong adaptability and stable yields. But teams like "Flora" and "Talica", although they charge ahead fiercely under favorable conditions, may fall behind in a different environment. These differences are actually a reminder to breeders that in rain-fed systems like those in Eastern Europe where the weather is the key, high yields alone are not enough; stable yields are more important.

5.2 Trials and performance of elite cultivars in rainfed regions of Northern China

When it comes to growing rye on dry land, northern China is no exception. The planting problems here are well known to all: the climate is unstable, water is tight, and the soil is not ideal either. But precisely under such conditions, rye has become a promising "breakthrough point". Rye is capable of carrying heavy loads and also saves water and fertilizer. These two features are exactly to the taste of the arid regions in the north (Ghafoor et al., 2024). And it's not just the domestic claim; experiments in Europe have also confirmed this point. The ideas and strategies that perform well in those environments, such as focusing on both yield and stability simultaneously, can also be fully applied to variety selection in northern China (Shawon et al., 2024). In rain-fed conditions, this combination of "resilience and efficiency" for rye is clearly useful for both growing and sustaining.

5.3 Experiences and outcomes of international multi-location trials

The German side is doing it more systematically. They conducted multi-point experiments using data from over 180 environments to adjust the model and calibrate parameters. Finally, they used these results to guide breeding and management (Figure 2) (Shawon et al., 2024). This is not merely a simple trial planting of a few plots in the field, but involves different soil types and climatic backgrounds. The aim is to select those varieties that have a wide range of adaptability and are not picky about the environment. One more point worth mentioning is that

these experiments are not only for research but also have eventually developed a toolkit that can assist breeders and farmers in making decisions. The experience of Germany shows that if the data from multiple environmental tests is used well, it can not only help you select good varieties but also make management more precise. This approach is now being promoted globally. In the face of climate change, everyone is looking for more stable and stress-resistant rye varieties (Ghafoor et al., 2024).

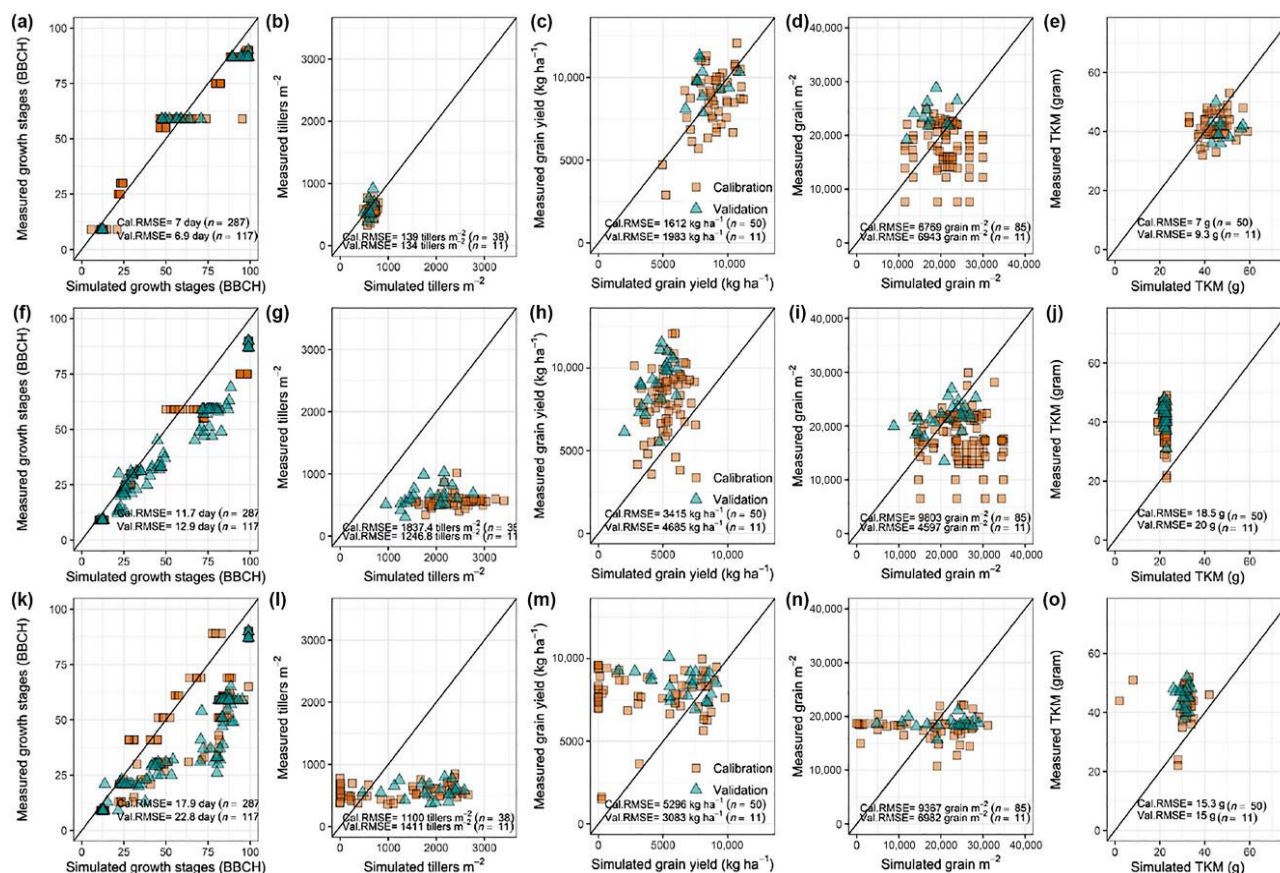


Figure 2 CSM-CERES-Wheat, where CSM is cropping system model and CERES is crop and environment resource synthesis, comparison of calibration scenarios (a-e), Röhl et al.'s (2020) approach (f-j), and default settings (k-o) for growth stages, tiller density (tiller/m²), grain yield (kg/ha), grain density (grain/m²), and 1 000-kernel mass (TKM). BBCH, Biologische Bundesanstalt, Bundessortenamt and Chemische industrie; RMSE, root mean square error (Adopted from Shawon et al., 2024)

6 Comprehensive Evaluation and Cultivar Selection Strategies

6.1 Integrated evaluation index system for yield, quality, and stability

How to select a reliable rye variety under rain-fed conditions? Just looking at the output is not enough; quality must also be taken into account, and stability cannot be ignored either. Therefore, a multi-dimensional evaluation standard is needed. Tools such as Shukla variance and multitrait stability index have been widely used to measure how stable a variety is in different environments (Safonova and Aniskov, 2023). Of course, these values are just an entry point. To have a comprehensive understanding of the performance of a set of materials, the GT double-plot analysis method is more practical. It can simultaneously compare agronomic traits and quality indicators on one plot (Yari et al., 2018). However, not all indicators can play a decisive role. Some structural parameters such as the number of grains per spike, the number of spikes per unit area, and the harvest index, along with output items like grain quality, are the key factors that truly determine the ultimate fate of a variety (Su Leek et al., 2024).

6.2 Selection of elite cultivars and regional recommendations

Choosing varieties is not about who can run faster, but about who can "run steadily" on all kinds of plots. "High yield + stability + adaptability" is basically an iron triangle. Any shortcoming in it is prone to failure. These indicators are not set on a whim; they are supported by a large number of environmental test data and

comprehensive evaluation results. Like in the experiments in Russia, several varieties such as "Rushnik 2", "Bereginya" and "Novaya Era" performed quite well. They were not picky about the environment and the yield was stable. In Poland, the conditions were a bit more complex. Hybrid varieties such as KWS Vinetto and SU Performer demonstrated obvious advantages, with yields nearly 18% higher than those of population varieties (Safonova and Aniskov, 2023). Of course, recommendations cannot be copied directly. Ultimately, how to select still depends on the "field conditions" such as local soil, water and fertilizer, and management methods. Only when all these factors are matched can the variety truly perform at its best (Ghafoor et al., 2024; Sulek et al., 2024).

6.3 Application of multi-environment trial results in breeding and dissemination

The purpose of completing the experiment is not to write a report, but more importantly, to apply it to breeding and promotion. Multi-environment trials (MET) have now become an indispensable part of rye breeding. If you don't do it, the varieties selected later might not adapt to the local environment. Nowadays, the breeding approach is also different from before. Relying solely on field observations is not enough; high-throughput phenotypic and even genomic data must also be incorporated. With the application of hyperspectral imaging techniques, the prediction of complex traits has become more accurate, and the breeding process can also be significantly accelerated (Galan et al., 2020). Methods such as genomic selection and marker-assisted selection are increasingly being used in combination with traditional methods to help breeders maintain a comprehensive balance of yield, quality and stress resistance (Hawliczek et al., 2023). Ultimately, the varieties truly pushed to farmers should not only be easy to grow and have high yields, but also be able to withstand external variables.

7 Conclusion and Outlook

Over the past few years, environmental tests have indeed made it clearer for everyone whether rye can be stably produced under rain-fed conditions. The screening results of which varieties are more stable and better adapted to local conditions have also been gradually released. Indicators such as yield, quality and stability are now basically evaluated together, and genomic tools have also begun to be widely involved in the breeding process. It seems that there has been considerable progress. But things are not that simple. There are still many problems: the reporting standards are not uniform, the analysis methods are scattered here and there, and in addition, the practical operations such as how to recruit people and synchronize data in cross-regional trials are also not easy to handle. Some studies have pointed out that methods supported by highly certain evidence are actually in the minority. Many so-called comparative studies still lack reproducibility and design quality, which has reduced the promotional value of some research results.

Where should I go next? Standardization is clearly inevitable. Statistical analysis must be rigorous and repeatability must also be improved. Especially when multi-environment data becomes complex, if issues such as cluster analysis and repeated measurements are not handled clearly, the credibility of the results becomes a question mark. One more point - the report must be transparent. What methods were used, where the data came from, and how the analysis was conducted cannot be kept under wraps. On the other hand, integrating new technologies such as genomic selection, high-throughput phenotyping, and modeling into the breeding process will also accelerate genetic improvement and enhance adaptability. But technology alone is not enough. To truly address the demands of regional breeding, it is still necessary to rely on a collaborative network, cross-location collaboration, and open data sharing. Only such large-scale cooperation can possibly cope with the challenges of complex environments.

The connection between experiments and digital technology is actually already taking place. Remote sensing technology can monitor the growth conditions and environmental changes in the fields in real time. Big data methods can also help us understand those difficult G×E interactions, which ones are more compatible with each other, and speak with data. These new tools have indeed significantly enhanced the efficiency of experiments, data accuracy, and decision-making judgment, and are very helpful for more precise breeding and more targeted variety recommendations. Ultimately, it's not a matter of one replacing the other. Instead, it's about how to better

integrate traditional field experiments with digital agriculture, especially in rain-fed systems. For sustainable rye breeding, the real breakthrough lies in the synergy of "old methods + new technologies".

Acknowledgments

We appreciate Dr Xu from the Hainan Institution of Biotechnology for her assistance in references collection and discussion for this work completion.

Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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