DOI 10.5281/twist.10049652#570

TWIST, 2026, Vol. 21, Issue 1, pp. 228-233

TWIST

Journal homepage: www.twistjournal.net

Marker-Assisted Selection for Enhancing Iron and Zinc Content in Tef (*Eragrostis tef*): A Comprehensive Review

Zeleke Keimeso*
[1] – Ethiopian Institute of Agricultural Research (EIAR),
Ambo Agricultural Research Center, Ambo P.O. Box 37, West Shoa, Ethiopia

[2] – Pan African University Life and Earth Sciences Institute (Including Health and Agriculture),
Ibadan, Nigeria
[**Corresponding author*]

Victor O. Adetimirin
Department of Crop and Horticultural Sciences,
University of Ibadan, Ibadan, Nigeria

Abstract

Tef (*Eragrostis tef*) is a vital staple crop in Ethiopia, consumed daily by over 50 million people. Despite its agronomic importance and nutritional potential, tef grain typically contains suboptimal levels of iron (Fe) and zinc (Zn), contributing to widespread micronutrient deficiencies. Enhancing the mineral content of tef through biofortification is therefore a crucial breeding objective. This review highlights the role of marker-assisted selection (MAS) in identifying and improving traits related to Fe and Zn content in tef. It discusses the use of various molecular markers, including SSRs, AFLPs, and SNPs, in assessing genetic diversity and trait associations. Insights from studies on pearl millet, lentil, and other cereals are also considered to inform tef improvement strategies. The discovery and validation of markers linked to grain Fe and Zn concentrations offer promising avenues for accelerating the development of nutrient-rich, high-yielding tef varieties. Overall, this review underscores the potential of integrating molecular tools into conventional breeding programs to address micronutrient malnutrition in tef-growing regions.

Keywords

Tef, marker-assisted selection, biofortification, iron, zinc, genetic diversity

INTRODUCTION

Tef is a cereal crop that is known for its nutritional value, but its low iron (Fe) and zinc (Zn) content has been a concern for many years. Deficiencies in these micronutrients can lead to a range of health problems, including impaired immune function, anemia, and stunted growth. Tef is an important crop in Ethiopia, where it is a daily food staple for about 50 million people, accounting for 14% of all calories consume (Abraham, 2015). However, the Zn and Fe content of tef grain is often below recommended levels, which can contribute to nutrient deficiencies and food insecurity in the country (Ereful et al., 2022a). The use of molecular markers and candidate genes in marker-assisted selection for Zn and Fe content in tef could provide a powerful tool for improving the nutritional value of this important crop (Raza et al., 2020). Marker-assisted selection (MAS) is a technique that has been gaining attention in recent years for its ability to identify and select plants with specific traits based on molecular markers. In the case of tef, molecular markers near target genes have been utilized for MAS or marker-assisted breeding (Pujar et al., 2020) to improve productivity (Numan et al., 2021). This approach can help breeders to select plants with high Fe and Zn content more efficiently and accurately than traditional breeding methods(Khan et al., 2023).

In recent years, MAS has been successfully applied in various crops, including rice and wheat, to improve their Fe and Zn content (Dixit et al., 2019; Singh et al., 2022). By identifying molecular markers linked to these traits, breeders can select plants with the desired genetic characteristics more efficiently (Bhat et al., 2016). Molecular markers near target genes are utilized for MAS or MAB, enabling the effective use of genetic diversity in tef breeding (Numan et al., 2021). This approach has the potential to accelerate the development of tef varieties with higher Fe and Zn content, thus contributing to improved nutritional outcomes.

The review will explore the use of MAS for Zn and Fe content in tef. The current state of research in this area, including the identification of molecular markers linked to Fe and Zn content in tef, and the potential applications of MAS in tef breeding programs will be discussed. The challenges and limitations of using MAS for Zn and Fe content in tef, and the future prospects of this technology in tef breeding will be examined. By examining the current research and developments in marker-assisted selection for Fe and Zn content in tef, this review aims to provide valuable insights into the potential of this technique for enhancing the nutritional value of tef grains and improving human health.

QUALITY TRAITS OF TEF

Tef is a highly versatile crop with broad trait diversity in the germplasm, offering ample opportunities for genetic improvement through either direct selection or intra-specific hybridization (Assefa et al., 2000). The quality traits of tef encompass grain yield, injera quality, nutritional quality, and straw quality(Jifar et al., 2022; Kebede, 2021; Tietel et al., 2020). Injera, a traditional Ethiopian flatbread made from tef flour, is evaluated based on factors such as texture, taste, and aroma (Kebede, 2021). Nutritional and health-related quality traits of tef grain include mineral content, fatty acid composition, and hydrophilic and lipophilic content (Tietel et al., 2020). Tef straw is used as fodder, and the quality traits of tef straw include yield, digestibility, and crude protein content (Jifar et al., 2022). The broad trait diversity in tef offers ample opportunities for genetic improvement through breeding programs, and enhancing these quality traits can contribute to the overall value and desirability of tef as a crop.

BIOFORTIFICATION

The deficiency of key vitamins and minerals, such as vitamin A, iodine, iron, and zinc, is a major underlying cause of numerous human health problems globaly, particularly in developing country (Singh et al., 2016). Conventional and molecular breeding-based biofortification approach, which involves exploiting genetic diversity and marker-assisted selection to develop mineral-dense crop varieties, mainly for iron and zinc are crucial. Thus, biofortification is the process of increasing the nutritional value of crops by increasing the bioavailable concentrations of essential elements in edible portions of crop plants through agronomic practices, conventional breeding, or genetic engineering (Singh et al., 2016).

Tef is a staple crop in Ethiopia, and biofortification of tef can help address micronutrient deficiencies, such as iron and zinc deficiencies, which are prevalent in the country (Gunaratna et al., 2019). It increase the concentration of essential micronutrients, such as iron and zinc, in the grain, which can help improve the nutritional status of people who rely on tef as a staple food (De Valença et al., 2017). The crop that is biofortified must sufficiently contribute to the total diet so that substitution of a non-biofortified variety with a biofortified variety will markedly increase intake of the target nutrient and meaningfully reduce an existing nutrient deficiency (Gunaratna et al., 2019). Biofortification techniques includes conventional breeding, agronomic practices, transgenic approaches and marker-assisted selection(Garg et al., 2018; Listman et al., 2019)

Bouis and Welch (2010) reported that crops such wheat, rice, maize, cassava, pearl millet, beans, and sweet potato in Asia and Africa have been bifortified for key nutrients-vitamin A, iron, and zinc through conventional breeding.

GENETIC DIVERSITY OF ZN AND FE CONTENT IN TEF

Tef is a highly diverse crop with significant genetic and phenotypic variation in grain iron (Fe) and zinc (Zn) contents (Assefa et al., 2015; Assefa et al., 2013). This variation allows for breeding gains in many nutritional traits of importance, and molecular markers such as simple sequence repeat (SSR) markers can help assess genetic diversity in grain Fe and Zn content (Raza et al., 2020). Molecular breeding approaches employing molecular markers are being extensively utilized for marker-assisted selection (MAS) to select and develop crop cultivars denser in iron and zinc (Kumar et al., 2015).

Ereful et al. (2022a) reported that a study focused on Ethiopian tef varieties found significant variation in nutritional and genetic traits, including Zn content which is potential for breeding enhanced nutritional traits in tef, such as Zn content. Assefa et al. (2015) stated the presence of genetic diversity in tef accessions that facilitate the development of improved varieties with high productivity and yield stability. This suggests that genetic diversity plays a crucial role in the breeding of tef varieties with desirable traits, including Zn and Fe content. Moreover, whole-genome sequencing of 24 Ethiopian tef varieties revealed significant genetic and phenotypic variation in nutritional traits, including Zn and Fe content (Ereful et al., 2022b). This variation provides opportunities for breeding programs to develop tef varieties with improved Zn and Fe content.

Overall, numerous studies have shown the genetic variability of Zn and Fe concentration in tef. With the help of marker-assisted selection and breeding techniques, it may be possible to use this diversity to create tef varieties with improved nutritional features, thereby addressing the problem of nutrient deficits in areas where tef is a main crop.

CONVENTIONAL BREEDING OF TEF

Conventional tef breeding efforts started in the late 1950s, since then about 42 tef varieties have been released (Assefa et al., 2011). Early breeding efforts concentrated on genetic improvement through the selection of pure lines from pre-existing germplasm as well as germplasm enhancement through collection, characterization, evaluation, and conservation (Ferede et al., 2020) (Figure 1). Since 1974, when the tef revealed the characteristics of flower opening (Berehe, 1975), hybridization was used as a means of tef improvement. However, conventional breeding efforts have focused on developing varieties with improved yield, resistance to biotic and abiotic stresses, and fitness for various cropping systems (Assefa et al., 2011). Thus, initiated between 1995 and 1998, molecular methods for tef included marker development, genetic linkage maps, and genetic and molecular diversity analysis (Assefa et al., 2011). There has been advancement in the study of tef genetic architecture and genomics over the previous two decades (Belay et al., 2008) (Figure 1). The conventional tef varietal refinement efforts should be supported by modern molecular tools and scientific techniques to speed up the tef breeding program (Assefa et al., 2015).

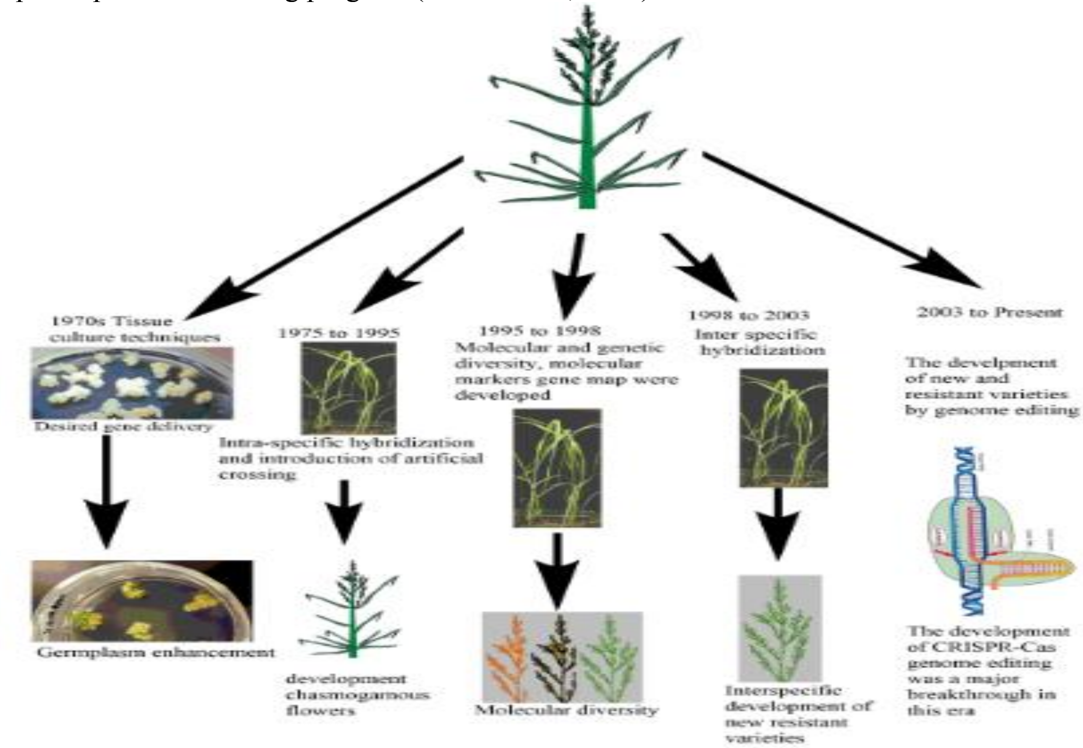

Fig. 1 Improvement of tef varieties over the last 50 years (Numan et al., 2021)

MOLECULAR MARKER ASSISTED SELECTION

Molecular breeding approaches employing molecular markers are being extensively utilized for marker-assisted selection (MAS) to select and develop crop cultivars denser in iron and zinc (Kumar et al., 2015). Molecular marker-assisted selection (MAS) is a technique that utilizes molecular markers, such as DNA markers, to assist in the selection of desirable traits in plant breeding programs. This approach has gained significant attention in recent years due to its ability to enhance the efficiency and precision of conventional breeding methods (Hasan et al., 2021). In the context of biofortification of tef, molecular marker-assisted selection can play a crucial role in accelerating the development of biofortified varieties with improved nutritional traits. Traditional breeding methods for improving nutritional traits in crops can be time-consuming and labor-intensive. By using molecular markers, breeders can identify and select plants with desired traits more efficiently and accurately (Numan et al., 2021). Molecular markers allow breeders to target specific genes or genomic regions associated with the desired nutritional traits. This precision breeding approach enables the selection of plants with the highest potential for improved nutritional value, increasing the chances of success in developing biofortified tef varieties (Numan et al., 2021).

The application of molecular markers in tef improvement was initiated during 1995–1998 (Assefa et al., 2011). Molecular markers near target genes are utilized for marker-assisted selection (MAS) or marker-assisted breeding (Ibitoye & Akin-Idowu, 2010; Pujar et al.). They enable the effective use of alleles during the selection of phenotypes. The most commonly used markers are microsatel-lites (simple sequence repeats; SSRs), amplified fragment length polymorphism (AFLPs)and single nucleotide polymorphisms (SNPs) (Ibitoye & Akin-Idowu, 2010). In tef, the SSRs and expressed sequence tag, restriction fragment length polymorphisms (RFLPs) and random amplified polymorphic DNA (RAPD) havebeen developed (Yu et al., 2006). (Numan et al., 2021) reported that through SSR analysis, some important traits in tef, including grain yield, days to maturity, panicle length and plant height were identified and improved. Similarly, these scholars reported using AFLP markers, the variability in tef accessions was identified. Assefa et al. (2011) described that different molecular markers were applied to tef genetic and genomic studies (Table 1).

For Zn and Fe content, Hindu et al. (2018) reported 11 SNPs for both Zn and Fe content. Similarly, a study conducted on lentil identified two SNP markers tightly linked to seed Fe and one linked to seed Zn concentration (Khazaei et al., 2017). Pujar et al. (2020) also reported that SNP markers associated with grain Fe, Zn, and protein for pearl millet. They suggested that the discovery of these markers and their validation could help in identifying the genomic regions and candidate genes influencing Fe and Zn content in tef.

Table 1 Summary of validated locus specific molecular markers in tef

Marker types	Clone/sequences	No. markers	Originating species
RFLP	cDNA	151	Tef
RFLP	cDNA	133	Barley, oat, rice
EST-SSR	EST	106	Tef
EST-SSR	EST	770	Rice, wheat, tall fescue, rye
SNP/INDEL	EST	18	Tef
gSSR	Genomic SSR	561	Tef
gSSR	Genomic SSR	47	Tall fescue

EST-SSR, expressed sequence tag derived simple sequence repeat; SNP/INDEL, single nucleotide polymorphism/insertion and deletion; RFLP, restriction fragment length polymorphism; gSSR, genomic simple sequence repeat.

CHALLENGES AND FUTURE DIRECTIONS

Marker-assisted selection (MAS) is a powerful tool for plant breeding, but it also has its challenges and limitations. Some of these challenges include the need for validation, the complexity of trait inheritance, and the cost and time required for development and validation of markers (Bhat et al., 2016; Boopathi & Boopathi, 2013; Cobb et al., 2019). Identifying markers linked to a trait of interest is a crucial step in MAS, but it can be difficult to find markers that are tightly linked to the trait, especially for traits that are controlled by multiple genes or influenced by environmental factors (Boopathi & Boopathi, 2013; Cobb et al., 2019). Additionally, developing and validating markers for MAS can be a time-consuming and expensive process, which can limit its applicability in certain crops (Bhat et al., 2016; Cobb et al., 2019)14. Despite these challenges, MAS remains an important tool for plant breeding, and it can be used in conjunction with other breeding methods to achieve the best results.

CONCLUSION

The use of molecular markers in tef improvement through marker-assisted selection (MAS) or marker-assisted breeding has shown promising results. The study reports the identification of important traits in tef, including grain yield, days to maturity, panicle length, and plant height through SSR analysis. Additionally, the study identifies variability in tef accessions using AFLP markers. The discovery and validation of SNP markers associated with grain Fe, Zn, and protein for pearl millet and lentil could help in identifying the genomic regions and candidate genes influencing Fe and Zn content in tef. Overall, the study provides valuable insights into the use of molecular markers in tef improvement and could aid in the development of high-yielding and nutrient-rich tef varieties.

FUNDING INFORMATION

This research review did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

DECLARATION OF CONFLICT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

1. Abraham, R. (2015). Achieving food security in Ethiopia by promoting productivity of future world food tef: A review. *Adv Plants Agric Res, 2*(2), 00045.
2. Assefa, K., Cannarozzi, G., Girma, D., Kamies, R., Chanyalew, S., Plaza-Wüthrich, S., Blösch, R., Rindisbacher, A., Rafudeen, S., & Tadele, Z. (2015). Genetic diversity in tef [Eragrostis tef (Zucc.) Trotter]. *Frontiers in plant science, 6*, 177.
3. Assefa, K., Chanyalew, S., & Tadele, Z. (2013). Achievements and prospects of tef improvement. International Workshop Proceedings, University of Bern, Institute of Plant Sciences, Switzerland,
4. Assefa, K., Ketema, S., Tefera, H., Kefyalew, T., & Chundera, F. (2000). Trait diversity, heritability and genetic advance in selected germplasm lines of tef [Emgmstis tef (Zucc.) Trotter]. *Hereditas, 133*(1), 29-37.
5. Assefa, K., Yu, J. K., Zeid, M., Belay, G., Tefera, H., & Sorrells, M. (2011). Breeding tef [Eragrostis tef (Zucc.) trotter]: conventional and molecular approaches. *Plant breeding, 130*(1), 1-9.

6. Belay, G., Tefera, H., Getachew, A., Assefa, K., & Metaferia, G. (2008). Highly client-oriented breeding with farmer participation in the Ethiopian cereal tef [Eragrostis tef (Zucc.) Trotter]. *African Journal of Agricultural Research, 3*(1), 022-028.
7. Berehe, T. (1975). Breakthrough in tef breeding technique. FAO Inf. *Bull. Cerealimprovement Prod. Near East Proj, 3*, 11-23.
8. Bhat, J. A., Ali, S., Salgotra, R. K., Mir, Z. A., Dutta, S., Jadon, V., Tyagi, A., Mushtaq, M., Jain, N., & Singh, P. K. (2016). Genomic selection in the era of next generation sequencing for complex traits in plant breeding. *Frontiers in genetics, 7*, 221.
9. Boopathi, N. M., & Boopathi, N. M. (2013). *Marker-assisted selection*. Springer.
10. Bouis, H. E., & Welch, R. M. (2010). Biofortification—a sustainable agricultural strategy for reducing micronutrient malnutrition in the global south. *Crop science, 50*, S-20-S-32.
11. Cobb, J. N., Biswas, P. S., & Platten, J. D. (2019). Back to the future: revisiting MAS as a tool for modern plant breeding. *Theoretical and Applied Genetics, 132*, 647-667.
12. De Valença, A., Bake, A., Brouwer, I., & Giller, K. (2017). Agronomic biofortification of crops to fight hidden hunger in sub-Saharan Africa. *Global food security, 12*, 8-14.
13. Dixit, S., Singh, U. M., Abbai, R., Ram, T., Singh, V. K., Paul, A., Virk, P., & Kumar, A. (2019). Identification of genomic region (s) responsible for high iron and zinc content in rice. *Scientific reports, 9*(1), 8136.
14. Ereful, N. C., Jones, H., Fradgley, N., Boyd, L., Cherie, H. A., & Milner, M. J. (2022a). Nutritional and genetic variation in a core set of Ethiopian Tef (Eragrostis tef) varieties. *BMC plant biology, 22*(1), 1-14.
15. Ereful, N. C., Jones, H., Fradgley, N., Boyd, L., Cherie, H. A., & Milner, M. J. (2022b). Whole-Genome Sequencing and Analysis of Nutritional Traits in a Core Set of Ethiopian Teff (Eragrostis tef) Varieties.
16. Ferede, B., Mekbib, F., Assefa, K., Chanyalew, S., Abraha, E., & Tadele, Z. (2020). Evaluation of drought tolerance in Tef [Eragrostis tef (Zucc.) trotter] genotypes using drought tolerance indices. *Journal of crop science and biotechnology, 23*, 107-115.
17. Garg, M., Sharma, N., Sharma, S., Kapoor, P., Kumar, A., Chunduri, V., & Arora, P. (2018). Biofortified crops generated by breeding, agronomy, and transgenic approaches are improving lives of millions of people around the world. *Frontiers in Nutrition*, 12.
18. Gunaratna, N. S., Moges, D., & De Groote, H. (2019). Biofortified maize can improve quality protein intakes among young children in southern Ethiopia. *Nutrients, 11*(1), 192.
19. Hasan, N., Choudhary, S., Naaz, N., Sharma, N., & Laskar, R. A. (2021). Recent advancements in molecular marker-assisted selection and applications in plant breeding programmes. *Journal of Genetic Engineering and Biotechnology, 19*(1), 1-26.
20. Hindu, V., Palacios-Rojas, N., Babu, R., Suwarno, W. B., Rashid, Z., Usha, R., Saykhedkar, G. R., & Nair, S. K. (2018). Identification and validation of genomic regions influencing kernel zinc and iron in maize. *Theoretical and Applied Genetics, 131*, 1443-1457.
21. Ibitoye, D., & Akin-Idowu, P. (2010). Marker-assisted-selection (MAS): A fast track to increase genetic gain in horticultural crop breeding. *African Journal of Biotechnology, 9*(52), 8889-8895.
22. Jifar, H., Assefa, K., Tadele, Z., Tesfaye, K., Dagne, K., Negawo, A. T., Blümmel, M., & Jones, C. S. (2022). Exploring the variation in grain and straw yield and straw quality traits of improved varieties of Tef [Eragrostis tef (Zucc.) Trotter]. *Advances in Crop Science and Technology, 10*(2), 494.
23. Kebede, W. (2021). Quality traits; measurement and possible breeding method to improve the quality traits of tef [Eragrostis tef (Zucc.) Trotter]. *International Journal of Novel Research in Life Sciences, 8*, 29-40.
24. Khan, M. I. R., Nazir, F., Maheshwari, C., Chopra, P., Chhillar, H., & Sreenivasulu, N. (2023). Mineral nutrients in plants under changing environments: A road to future food and nutrition security. *The Plant Genome*, e20362.
25. Khazaei, H., Podder, R., Caron, C. T., Kundu, S. S., Diapari, M., Vandenberg, A., & Bett, K. E. (2017). Marker–trait association analysis of iron and zinc concentration in lentil (Lens culinaris Medik.) seeds. *The Plant Genome, 10*(2), plantgenome2017.2002.0007.
26. Kumar, S., Thirunavukkarasu, N., Singh, G., Sharma, R., & Kulkarni, K. S. (2015). Biofortification for selecting and developing crop cultivars denser in iron and zinc. *Nutrient use efficiency: From basics to advances*, 237-253.
27. Listman, G. M., Guzmán, C., Palacios-Rojas, N., Pfeiffer, W. H., San Vicente, F., & Govindan, V. (2019). Improving nutrition through biofortification: Preharvest and postharvest technologies. *Cereal Foods World, 64*(3), 1-7.
28. Numan, M., Khan, A. L., Asaf, S., Salehin, M., Beyene, G., Tadele, Z., & Ligaba-Osena, A. (2021). From traditional breeding to genome editing for boosting productivity of the ancient grain tef [Eragrostis tef (Zucc.) Trotter]. *Plants, 10*(4), 628.
29. Pujar, M., Gangaprasad, S., Govindaraj, M., Gangurde, S. S., Kanatti, A., & Kudapa, H. (2020). Genome-wide association study uncovers genomic regions associated with grain iron, zinc and protein content in pearl millet. *Scientific reports, 10*(1), 19473.
30. Raza, Q., Riaz, A., Saher, H., Bibi, A., Raza, M. A., Ali, S. S., & Sabar, M. (2020). Grain Fe and Zn contents linked SSR markers based genetic diversity in rice. *PloS one, 15*(9), e0239739.

31. Singh, R., Saripalli, G., Gautam, T., Kumar, A., Jan, I., Batra, R., Kumar, J., Kumar, R., Balyan, H. S., & Sharma, S. (2022). Meta-QTLs, ortho-MetaQTLs and candidate genes for grain Fe and Zn contents in wheat (Triticum aestivum L.). *Physiology and Molecular Biology of Plants, 28*(3), 637-650.
32. Singh, U., Praharaj, C., Chaturvedi, S., & Bohra, A. (2016). Biofortification: Introduction, approaches, limitations, and challenges. *Biofortification of food crops*, 3-18.
33. Tietel, Z., Simhon, E., Gashu, K., Ananth, D. A., Schwartz, B., Saranga, Y., & Yermiyahu, U. (2020). Nitrogen availability and genotype affect major nutritional quality parameters of tef grain grown under irrigation. *Scientific reports, 10*(1), 14339.
34. Yu, J.-K., Sun, Q., Rota, M. L., Edwards, H., Tefera, H., & Sorrells, M. E. (2006). Expressed sequence tag analysis in tef (Eragrostis tef (Zucc) Trotter). *Genome, 49*(4), 365-372.