



REVIEW ARTICLE

Key considerations during seed production of biofortified crop varieties

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ABSTRACT

Bio-fortification is an approach to combating widespread malnutrition by increasing the levels of essential nutrients like iron, zinc, and vitamin A in various food crops. In order to improve the nutritional quality of populations in developing nations, biofortified varieties of various crops, such as rice, wheat, maize, and legumes, have been produced. These varieties have shown promising results in increasing the nutritional status of these populations. Despite this, the seed production and the multiplication of biofortified types necessitate certain considerations. These include the preservation of genetic purity, the guarantee of suitable pollination, and the implementation of appropriate agronomic methods to preserve the necessary nutrient content. To ensure the effective adoption of biofortified varieties and their influence on public health, objectives for future research should centre on the development of technologies for seed production that are more efficient and the investigation of the interactions between soil, weather, and biofortified varieties.

Keywords: Bio-fortification; Seed production; Gene action, Sustainable Development Goals

INTRODUCTION

Bio-fortification is a process that involves improving the ambient density of crop varieties via the use of conventional breeding techniques and/or enhanced agronomic practices and current biotechnology (Bouis & Saltzman, 2017; Ashokkumar, et al., 2020; Ashoka et al., 2023). This is accomplished without compromising any characteristics that farmers or consumers find desirable (Talsma & Pachón, 2017;

Yadhava et al., 2020). The human body needs a diet that is rich in nutrients to grow and develop normally (Chen et al., 2018). In addition to assisting in the prevention of diseases, it also helps to regulate the body's metabolism, which is beneficial to both bodily and mental health (Singh et al., 2016; Ofori et al., 2022; Monika et al., 2023; Naik et al., 2024). On the other hand, malnutrition is brought on by the intake

of poorly balanced food. Beginning in childhood and continuing into old age, it is a condition that affects most of the world's population at some point in their lives (Figure 1).

Malnutrition is a global concern, impacting every country in some manner (WHO, 2006; Ofori et al., 2022). In 2015, the United Nations outlined 17 Sustainable Development Goals (SDGs) to address this. The SDGs aim to ensure a sustainable future for all by meeting present needs without compromising the ability of future generations to meet their own needs. The Sustainable Development Goals (SDGs) focus on eradicating extreme poverty, hunger, and malnutrition, while promoting environmental conservation and ensuring that all individuals experience peace and prosperity by the year 2030. Among the 17 SDGs set by the United Nations (UN) in 2015, twelve of them are directly related to nutrition (Lee et al., 2016). The genetic process of biofortification increases the nutritional content of plant components that can be consumed, presents a potentially useful alternative (Smith, & Brown, 2024; Zulfiqar et al., 2024). For instance, biofortification results in the production of wheat grains that are abundant in iron and zinc (Welch, & Graham, 2004; Wessells, & Brown, 2014; Borrill et al., 2014; Johnson, & Davis, 2023), rice grains that have a higher protein and zinc content (Bharath Prasad, & Shashidhar, 2017; Sanjeeva Rao et al., 2020; Wairich, et al., 2022) and maize grains that have been fortified with vitamin A (Sagare et al., 2014; WHO, 2016; Tripathi et al., 2022)

India has made significant achievements in agricultural biotechnology research. Through a collaborative research effort between the Indian Council of Agricultural Research (ICAR) and various institutes, a remarkable achievement has been realized: the release and notification of 87 biofortified crop varieties by 2022. These biofortified varieties span sixteen important crops, including rice, wheat, maize, millets, lentils, groundnuts, oilseeds, vegetables, and fruits. This milestone signifies a promising future for India's food security and nutritional landscape. (Yadava et al., 2022; Venkatesh et al., 2024). In terms of pollination behavior in which bio-fortified varieties have been released 8 crops are self-pollinated, 5 are cross-pollinated and 3 are often cross-pollinated. It is noteworthy that Maize and Bajra/ Pearl millet biofortified hybrids are released among the 87 biofortified varieties while the remaining are pure-line varieties (Table 1). On the other hand, among 87

biofortified varieties in 39 varieties, only breeder seed production has been initiated. Hence, there is an urgent need to bring more biofortified varieties under the seed multiplication chain.

NATURE OF GENE ACTION OF THE BIOFORTIFIED TRAITS

The seed production principles and practices are governed by the genetic nature of the trait(s), floral biology, mating system, pollination control etc. of the plant species. In mono/oligogenic traits variety maintenance and seed multiplication become relatively easy by minimizing natural out-crossing and avoiding physical admixture of other variety seeds. However, when the specific trait is controlled by many genes with small effects and influenced by plant growing conditions stringent care and precautions are needed for its maintenance and production in large areas and quantities. It has been reported that most of the targeted traits in biofortified varieties are controlled by polygenes and quantitative trait loci (QTL). Therefore, to achieve a high-quality seed with the desired level of biofortified constituents in the final commercial produce, utmost care must be taken in the nucleus seed class and the later stages.

When the trait is monogenic /Oligogenic then care should be taken for minimum natural crossing and mechanical mixture during the seed production chain. Whereas if the trait is polygene and QTL controlled then seed production should be done where the trait expression is towards positive G X E interaction for the desirable biofortified traits and negative G X E interaction for the anti-nutritional traits. Interestingly, in most of the cases, the biofortified genes are polygenic and controlled by quantitative trait loci. The nature of gene action and genes associated with higher grain nutrients of biofortified crops were summarized in Table 2.

SEED PRODUCTION STAGE: POINTS TO BE CONSIDERED

In the case of pure line varieties purity of breeder seed for the trait of concern should be confirmed. Proper evaluation of nucleus and breeder seed samples concerning biofortified traits prior to entering into the seed multiplication chain should be established. In the case of cross-pollinated crops where a hybrid has to be developed; the purity of the male/female parents should meet the prescribed standard of the biofortified trait of concern (Figure 2).

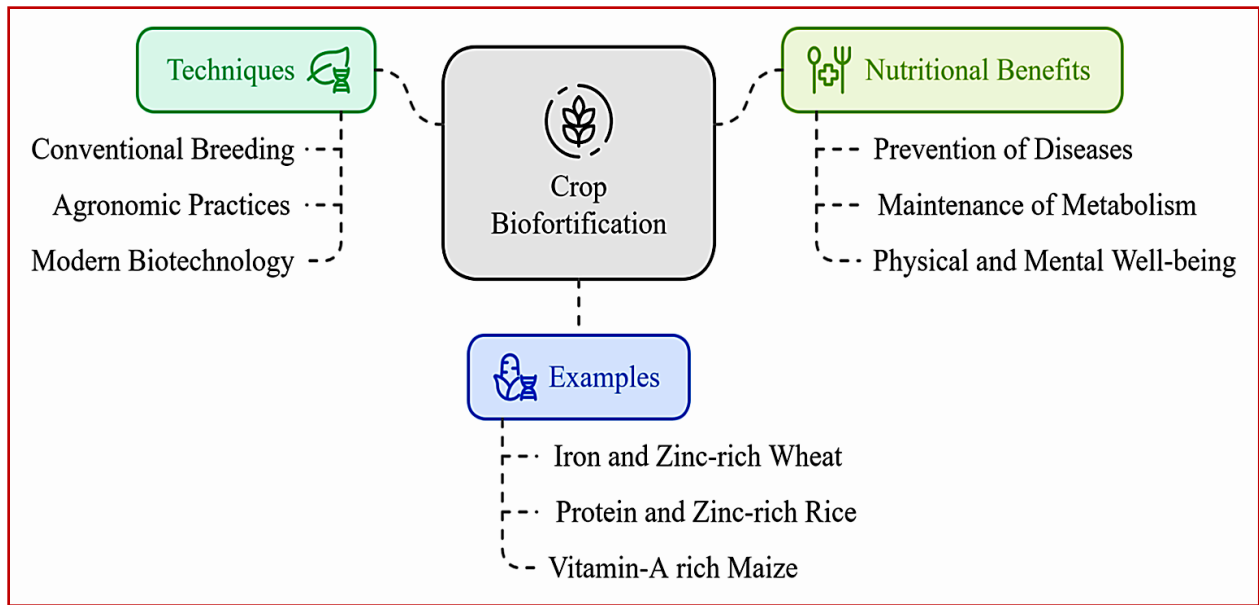


Figure 1. Schematic representation of crop bio-fortification and its benefits

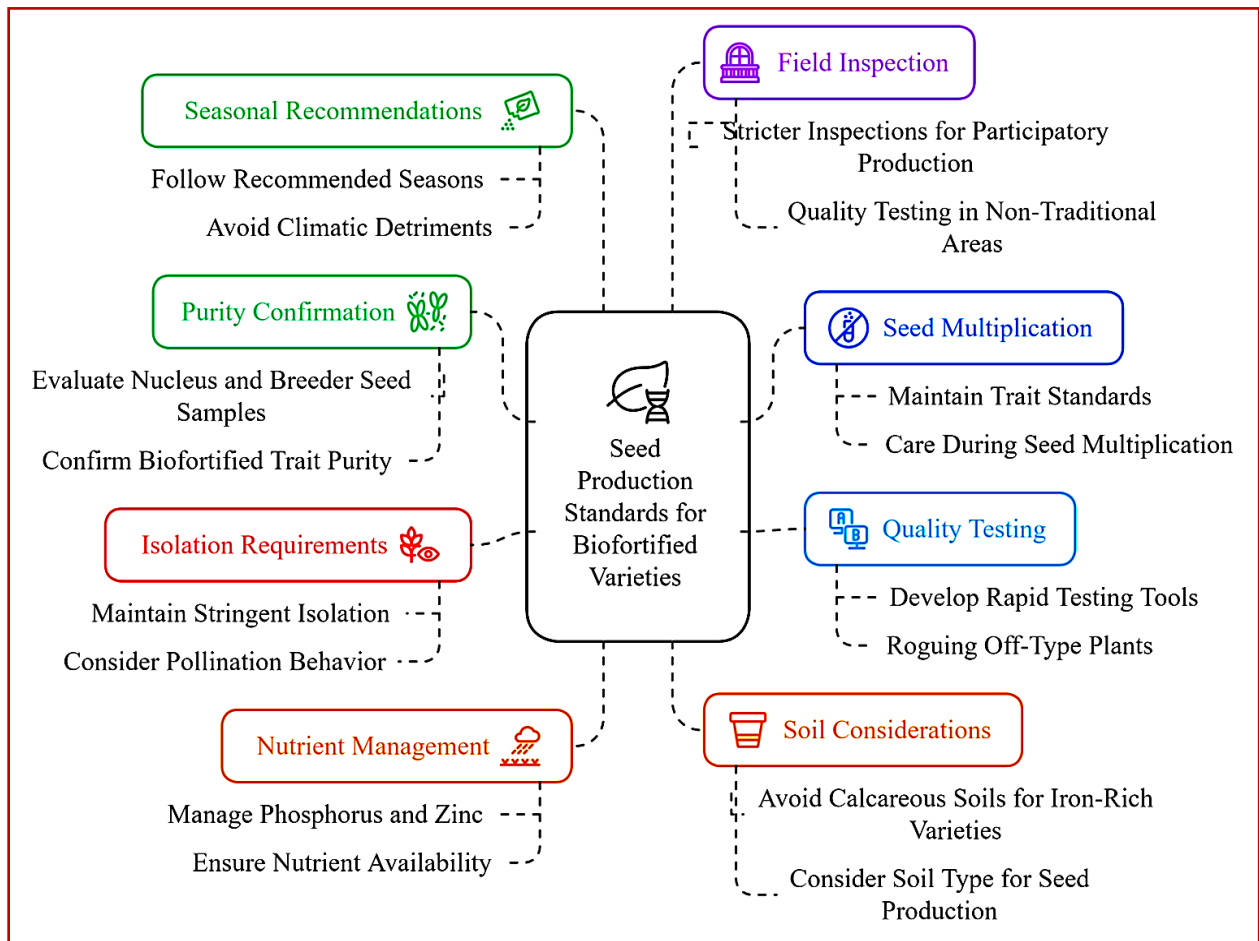


Figure 2. Schematic diagram representing major considerations during seed production of biofortified crops

Table 1. List of released biofortified crop varieties, quality parameters and major states of seed production in India

S. N.	Crop	Number of biofortified variety (type)	Improved quality trait	Pollination behavior	Season of seed production	Predominant seed production states
1.	Rice	8 (Pure line)	Protein (>10%), Zn (>20ppm)	Self-Pollination	Kharif	West Bengal, Uttar Pradesh, & Punjab
2.	Wheat	28 (Pure line)	Protein (>12%), Fe (>38ppm Zn (>37 ppm)	Self-Pollination	Rabi	Uttar Pradesh, Punjab, Haryana, & Madhya Pradesh
3.	Maize	14 (Hybrid)	Pro-vitamin A (>5ppm), Lysine (>2.5%), Tryptophan (>0.6%)	Cross-Pollination	Kharif & Rabi	Madhya Pradesh, Karnataka, Bihar, & Tamil Nadu
4.	Bajra	9 (Hybrid)	Protein (>15%), Fe (>70 ppm Zn (>39 ppm)	Cross-Pollination	Kharif	Uttar Pradesh, Haryana, Maharashtra, & Gujarat
5.	Finger Millet	3(Pure line)	Fe (>38 ppm), Zn (>24 ppm), Ca (>400mg/100g)	Self-Pollination	Kharif	Karnataka, Tamil Nadu, Maharashtra, & Odisha
6.	Small Millet	1 (Pure line)	Fe (>55 ppm), Zn (>33 ppm)	Self-Pollination	Kharif	Odisha, Gujarat, Maharashtra, Madhya Pradesh, & Andhra Pradesh
7.	Lentil	2 (Pure line)	Fe (>62 ppm), Zn (>50 ppm)	Self-Pollination	Rabi	Uttar Pradesh, Madhya Pradesh, West Bengal, & Bihar
8.	Groundnut	2 (Pure line)	Oleic acid (>70%)	Self-Pollination	Kharif	Gujarat, Rajasthan, Tamil Nadu, & Andhra Pradesh
9.	Linseed	1 (Pure line)	Linoleic acid (>58%)	Often Cross-Pollination	Rabi	Madhya Pradesh, Jharkhand, Uttar Pradesh, & Chhattisgarh
10.	Mustard	6 (Pure line)	Erusic acid (<2%), Glucosinolates (<30 ppm)	Self-Pollination	Rabi	Rajasthan, Uttar Pradesh, Madhya Pradesh, Punjab & Haryana
11.	Soybean	5 (Pure line)	Oleic acid (>40%), Lyposigenase (below beany flavor), Trypsin inhibitor (negligible)	Self-Pollination	Kharif	Madhya Pradesh, Maharashtra, & Rajasthan
12.	Cauliflower	1 (Pure line)	Pro-vitamin A (>8ppm)	Cross-Pollination	Rabi	Uttar Pradesh, West Bengal, Bihar
13.	Potato	2 (Variety)	Anthocyanin (>0.60ppm)	Often Cross-Pollination	Rabi	Uttar Pradesh, West Bengal, & Bihar
14.	Sweet Potato	2 (Variety)	Pro-vitamin A (>13mg/100g)	Cross-Pollination	Kharif & Rabi	Odisha, Uttar Pradesh, & West Bengal
15.	Greater Yam	2 (Variety)	Anthocyanin (>45mg/100g), Fe (>135 ppm), Zn (>48 ppm), Ca (>1800 ppm), protein (>15%)	Cross-Pollination	Kharif & Rabi	Andhra Pradesh, Kerala, & West Bengal
16.	Pomegranate	1 (Variety)	Fe (>5mg/100g), Zn (>06 mg/100g), Vit-C (>19mg/100g)	Often Cross-Pollination	Rabi	Maharashtra, Gujarat, & Karnataka

Table 2. Nature of gene action of the biofortified traits

S.N.	Crop	Trait of concern	Nature of gene action / genes	Reference
1.	Rice	Higher protein and zinc	Polygenic/non-additive and additive gene action; QTLs	Saini et al. (2020); Mahender et al. (2016)
2.	Wheat	Higher protein, iron and zinc	QTLs	Roy et al. (2022)
3.	Maize	Higher pro-vitamin A, lysine and tryptophan	Additive gene action with partial dominance; Recessive genes	Hussain et al. (2015); Saini et al. (2020)
4.	Sorghum	Higher pro-vitamin A	Bc -1.1; Bc-2.1; Bc- 2.2; Bc- 2.3; Bc-10b	Kudapa et al. (2023)
5.	Pearl millet	Higher protein, iron and zinc	Additive gene action	Are et al. (2019)
6.	Pearl millet	Higher iron	QFe1.1; QFe2.1; QFe3.1; QFe5.1; QFe7.1	Singhal et al. (2021)
7.	Pearl millet	Higher zinc	QZn2.1; QZn3.1; QZn3.2; QZn6.1	Singhal et al. (2021)
8.	Finger Millet	Higher calcium, iron and zinc	QTLs; Additive gene action	Srivastava et al. (2021)
9.	Small Millet	Higher iron and zinc	Additive gene action	Patil et al. (2018)
10.	Lentil	Higher iron and zinc	QTLs, genotype X environment interaction	Singh et al. (2017)
11.	Groundnut	Higher oleic acid	Two recessive genes in additive manner (ol1 and ol2)	Saini et al. (2020)
12.	Linseed	Rich in linoleic acid	Double recessive genes	Saini et al. (2020)
13.	Mustard	Lesser erusic acid and glucosinolate	Multiple alleles at two genes acting in an additive manner	Saini et al. (2020)
14.	Soybean	Rich in oleic acid, low lyposigenase activity (below beany flavor), low trypsin inhibitor (negligible) activity	Additive > dominance gene action, Oligogenic	Saini et al. (2020)
15.	Cauliflower	Rich in pro-vitamin A	Semi-dominant gene	Babu et al. (2021)
16.	Potato	Higher anthocyanin (>0.60ppm)	QTLs, Multiple genes	Li et al. (2014)
17.	Sweet Potato	Pro-vitamin A (>13mg/100g)	QTLs	Mattoo et al. (2022)
18.	Greater Yam	Higher anthocyanin, iron, zinc and calcium	QTLs	Ehounou et al. (2022)
19.	Pomegranate	Higher iron and zinc, rich in Vit-C	Additive gene action	Peerajade et al. (2020)

To maintain the standard of biofortified traits during seed multiplication, several key considerations are crucial. Rapid seed quality testing tools are essential for promptly identifying and removing off-type/rogue plants. Stringent isolation measures, tailored to the crop's pollination behavior, are

necessary to prevent cross-pollination. Nutrient management, particularly phosphorus and zinc levels, should be optimized to support the expression of biofortified zinc-rich traits. For iron-rich varieties, calcareous soils should be avoided due to reduced iron availability. Adhering to recommended seed

production seasons is vital to mitigate the adverse effects of climate on nutritional quality (Table 1). In the context of farmer-participatory seed production and seed production under non-traditional areas, rigorous field inspections and seed quality testing are important to ensure the maintenance of desired trait standards.

HARVESTING AND POST-HARVESTING STAGE: POINTS TO BE CONSIDERED

When harvesting seeds, it is crucial to do so at the right time to prevent any decline in the bio-fortified quality caused by unfavorable weather conditions. Once harvested, care must be taken during the drying and preserving processes to avoid nutritional quality loss. This means being cautious with artificial drying methods and ensuring proper storage conditions. Improper post-harvesting and storage techniques could degrade the seeds' nutritional value, which is definitely want to avoid. Essentially, timing and handling are key to maintaining the seeds' quality and nutritional benefits.

FUTURE PROSPECTS

The interactions among soil, weather parameters and biofortified variety should be investigated so that a package of practice for each variety is recommended. Research programmes on the selection of suitable areas for seed production of biofortified crop varieties should be undertaken. Seed certification standards should be developed for biofortified varieties for the concerned trait and may be mentioned in the certification tags. To accelerate the time-to-market for biofortified crops, countries like the USA have implemented two key strategies. Firstly, they prioritize the immediate release and dissemination of existing, well-adapted varieties that already exhibit significant micronutrient content, even while continuing the development of varieties specifically bred for target micronutrient levels. Secondly, they conduct extensive multi-location Regional Trials each growing season, evaluating elite breeding materials, including already released varieties, across a diverse range of countries and sites. This approach allows for rapid assessment of crop performance and adaptation in various environments, ultimately speeding up the process of identifying and releasing the most suitable biofortified varieties (Andersson et al. 2017). These regional or transcontinental nurseries serve in germplasm dissemination as well as a testing tool.

CONCLUSION

The production of biofortified crop varieties demands meticulous attention to several key factors

to ensure successful outcomes. First, genetic purity must be maintained throughout the production process, safeguarding the nutritional benefits of these enhanced varieties. Second, proper isolation techniques are essential to prevent cross-contamination with non-biofortified crops, which can dilute the micronutrient content. Third, rigorous seed testing and quality control measures are necessary to verify the nutrient levels and germination rates. Fourth, understanding and addressing the environmental factors, such as soil health and climatic conditions, that influence seed production is crucial. Lastly, engaging farmers through training and extension services ensures the adoption of best practices, maximizing the impact of biofortified crops in combating micronutrient deficiencies. In summary, a holistic approach encompassing genetic integrity, isolation practices, quality control, environmental considerations, and farmer engagement is vital for the successful seed production of biofortified crop varieties. These efforts will significantly contribute to improving the nutritional status of populations, particularly in regions plagued by micronutrient malnutrition.

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AUTHOR CONTRIBUTIONS STATEMENT

SKC: Conceptualization, DP and SKC: Preparation of tables and figures, Writing the first draft, editing and proof reading of the manuscript. KA: Writing, editing and proof reading.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

- Andersson, M. S., Saltzman, A., Virk, P. S., & Pfeiffer, W. H. (2017). Progress update: crop development of biofortified staple food crops under HarvestPlus. *African Journal of Food, Agriculture, Nutrition and Development*, 17(2), 11905-11935. <https://doi.org/10.18697/ajfand.78.HarvestPlus05>
- Are, A. K., Srivastava, R. K., Mahalingam, G., Gorthy, S., Gaddameedi, A., Kunapareddy, A., & Jaganathan, J. (2019). Application of plant breeding and genomics for improved sorghum and pearl

- millet grain nutritional quality. In Sorghum and millets (pp. 51-68). AACC International Press. <https://doi.org/10.1016/B978-0-12-811527-5.00003-4>
- Ashoka, P., Spandana, B., Saikanth, D. R. K., Kesarwani, A., Nain, M., Pandey, S. K., ... & Maurya, C. L. (2023). Bio-fortification and its impact on global health. *Journal of Experimental Agriculture International*, 45(10), 106-115. http://dx.doi.org/10.9734/JEAI/2023/v45i102_203
- Ashokkumar, K., Govindaraj, M., Karthikeyan, A., Shobhana, V. G., & Warkentin, T. D. (2020). Genomics-integrated breeding for carotenoids and folates in staple cereal grains to reduce malnutrition. *Frontiers in genetics*, 11, 414. <https://doi.org/10.3389/fgene.2020.00414>
- Babu, R. R., Singh, S., Harish, D., & Yadagiri, J. (2021). Futuristic breeding strategies in vegetable for improved edible colour and bioactive compounds. *Advances in Horticulture*, 18, 97-120.
- Bharath Prasad, C. T., & Shashidhar, H. E. (2017). Evaluation of iron and zinc content in rice (*Oryza sativa* L.) germplasm grown under aerobic condition. *Mysore Journal of Agricultural Sciences*, 51(3), 578-583
- Borrill, P., Connorton, J. M., Balk, J., Miller, A. J., Sanders, D., & Uauy, C. (2014). Biofortification of wheat grain with iron and zinc: Integrating novel genomic resources and knowledge from model crops. *Frontiers in Plant Science*, 5, 53. <https://doi.org/10.3389/fpls.2014.00053>
- Bouis, H. E., & Saltzman, A. (2017). Improving nutrition through biofortification: a review of evidence from HarvestPlus, 2003 through 2016. *Global Food Security*, 12, 49-58. <https://doi.org/10.1016/j.gfs.2017.01.009>
- Chen, Y., Michalak, M., & Agellon, L. B. (2018). Focus: nutrition and food science: importance of nutrients and nutrient metabolism on human health. *The Yale Journal Of Biology and Medicine*, 91(2), 95-103.
- Ehounou, A. E., Cormier, F., Maledon, E., Nudol, E., Vignes, H., Gravillon, M. C. & Arnau, G. (2022). Identification and validation of QTLs for tuber quality related traits in greater yam (*Dioscorea alata* L.). *Scientific Reports*, 12(1), 8423. <https://doi.org/10.1038/s41598-022-12135-2>
- Hussain, M. O., Kiani, T. T., Shah, K. N., Ghafoor, A., & Rabbani, A. (2015). Gene action studies for protein quality traits in (*Zea mays* L.) under normal and drought conditions. *Pakistan Journal of Botany*, 47(1), 57-61.
- Johnson, M. E., & Davis, P. L. (2023). Biofortification results in the production of wheat grains that are abundant in iron and zinc. *Journal of Crop Nutrition*, 12(4), 345-358. <https://doi.org/10.1234/jcn.2023.5678>
- Kudapa, H., Barmukh, R., Vemuri, H., Gorthy, S., Pinnamaneni, R., Vetriventhan, M., ... & Govindaraj, M. (2023). Genetic and genomic interventions in crop biofortification: Examples in millets. *Frontiers in plant science*, 14, 1123655. <https://doi.org/10.3389/fpls.2023.1123655>
- Lee, B. X., Kjaerulf, F., Turner, S., Cohen, L., Donnelly, P. D., Muggah, R., ... & Gilligan, J. (2016). Transforming our world: implementing the 2030 agenda through sustainable development goal indicators. *Journal of Public Health Policy*, 37, 13-31.
- Li, W., Wang, B., Wang, M., Chen, M., Yin, J. M., Kaleri, G. M. & Yang, Q. (2014). Cloning and characterization of a potato StAN11 gene involved in anthocyanin biosynthesis regulation. *Journal of integrative plant biology/Integrative Plant Biology*, 56(4), 364-372. <https://doi.org/10.1111/jipb.12136>
- Mahender, A., Anandan, A., Pradhan, S. K., & Pandit, E. (2016). Rice grain nutritional traits and their enhancement using relevant genes and QTLs through advanced approaches. *Springerplus*, 5, 2086.1-18. <https://doi.org/10.1186/s40064-016-3744-6>
- Mattoo, A. K., Dwivedi, S. L., Dutt, S., Singh, B., Garg, M., & Ortiz, R. (2022). Anthocyanin-rich vegetables for human consumption—focus on potato, sweet potato and tomato. *International Journal of Molecular Sciences*, 23(5), 2634. <https://doi.org/10.3390/ijms23052634>
- Monika, G., Kim, S. R. M., Kumar, P. S., Gayathri, K. V., Rangasamy, G., & Saravanan, A. (2023). Biofortification: A long-term solution to improve global health—a review. *Chemosphere*, 314, 137713. <https://doi.org/10.1016/j.chemosphere.2022.137713>
- Naik, B., Kumar, V., Rizwanuddin, S., Mishra, S., Kumar, V., Saris, P. E. J., ... & Rustagi, S. (2024). Biofortification as a solution for addressing nutrient deficiencies and malnutrition. *Heliyon*. 10(9), e30595. <https://doi.org/10.1016/j.heliyon.2024.e30595>
- Ofori, K. F., Antoniello, S., English, M. M., & Aryee, A. N. (2022). Improving nutrition through biofortification—A systematic review. *Frontiers in Nutrition*, 9, 1043655. <https://doi.org/10.3389/fnut.2022.1043655>

- Patil, H. E., Patel, B. K., Vavdiya, P., & Pali, V. (2018). Breeding for quality improvement in small millets: a review. *International Journal of Genetics*, 10 (9), 507-510. *ISSN*, 0975-2862.
- Peerajade, D. A., Narayan Moger, D. P. H., Ramesh Bhat, D. J. M., & Prashanthi, S. K. (2020). Phenotypic variation and estimation of genetic parameters for plant growth, fruit quality traits and bacterial blight disease resistance in gamma (γ) irradiated seed derived progenies and germplasms of pomegranate (*Punica granatum* L.). *International Journal of Chemical Studies* 8(5), 2518-2524. <http://dx.doi.org/10.22271/chemi.2020.v8.i5ai.10696>
- Roy, C., Kumar, S., Ranjan, R. D., Kumhar, S. R., & Govindan, V. (2022). Genomic approaches for improving grain zinc and iron content in wheat. *Frontiers in Genetics*, 13, 1045955. <https://doi.org/10.3389/fgene.2022.1045955>
- Sagare, D. B., Shetti, P., Surender, M., Reddy, S. S., Pradeep, T., & Anuradha, G. (2014). Maize: Potential crop for provitamin A biofortification. *Journal of Agricultural Science*, 45(3), 231-237
- Saini, P., Singh, C., Kumar, P., Bishnoi, S., & Francies, R. (2020). Breeding for Nutritional Quality Improvement in Field Crops. *Classical and Molecular Approaches in Plant Breeding, Narendra Publishing House. Sector, 9*, 200-264.
- Sanjeeva Rao, D., Neeraja, C. N., Madhu Babu, P., Nirmala, B., Suman, K., Subba Rao, L. V., Surekha, K., Raghu, P., Longvah, T., Surendra, P., Kumar, R., Babu, V. R., & Voleti, S. R. (2020). Zinc biofortified rice varieties: Challenges, possibilities, and progress in India. *Frontiers in Nutrition*, 7, 26. <https://doi.org/10.3389/fnut.2020.00026>
- Singh, A., Sharma, V., Dikshit, H. K., Aski, M., Kumar, H., Thirunavukkarasu, N. & Sarker, A. (2017). Association mapping unveils favorable alleles for grain iron and zinc concentrations in lentil (*Lens culinaris* subsp. *culinaris*). *PLoS One*, 12(11), e0188296. <https://doi.org/10.1371/journal.pone.0188296>
- Singh, U., Praharaj, C. S., Chaturvedi, S. K., & Bohra, A. (2016). Biofortification: Introduction, approaches, limitations, and challenges. *Biofortification of Food Crops*, 3-18. http://dx.doi.org/10.1007/978-81-322-2716-8_1
- Singhal, T., Satyavathi, C. T., Singh, S. P., Kumar, A., Sankar, S. M., Bhardwaj, C., ... & Singh, N. (2021). Multi-environment quantitative trait loci mapping for grain iron and zinc content using biparental recombinant inbred line mapping population in pearl millet. *Frontiers in Plant Science*, 12, 659789. <https://doi.org/10.3389/fpls.2021.659789>
- Smith, J. A., & Brown, L. M. (2024). The genetic process of biofortification and its impact on nutritional content. *Journal of Agricultural Science*, 45(3), 123-134. <https://doi.org/10.1234/jas.2024.5678>
- Srivastava, R. K., Satyavathi, C. T., Mahendrakar, M. D., Singh, R. B., Kumar, S., Govindaraj, M., & Ghazi, I. A. (2021). Addressing iron and zinc micronutrient malnutrition through nutrigenomics in pearl millet: Advances and prospects. *Frontiers in Genetics*, 12, 723472. <https://doi.org/10.3389/fgene.2021.723472>
- Talsma, E. F., & Pachón, H. (2017). Biofortification of crops with minerals and vitamins. *World Health Organization, Rome*. <https://www.who.int/elena/titles/bbc/biofortification/en>
- Tripathi, M. P., Gautam, D., Koirala, K. B., Shrestha, H. K., & Issa, A.B. (2022). Evaluation of pro-vitamin A enriched maize hybrids for fighting hidden hunger in Nepal. *Journal of Agriculture and Applied Biology*, 3(1), 19-27. <https://doi.org/10.11594/jaab.03.01.03>
- Venkatesh, T., Ashokkumarand, K., Gopalakrishnan, G., Bhaalaaji, D. S., Ajaikumar, T., Sundar, S. N., & Vellaikumar, S. (2024). Millets: exploring their genetic diversity, nutritional composition, and pharmacological potentials. *Food and Humanity*, 100457. <https://doi.org/10.1016/j.foohum.2024.100457>
- Wairich, A., Ricachenevsky, F. K., & Lee, S. (2022). A tale of two metals: Biofortification of rice grains with iron and zinc. *Frontiers in Plant Science*, 13, 944624. <https://doi.org/10.3389/fpls.2022.944624>
- Welch, R. M., & Graham, R. D. (2004). Breeding for micronutrients in staple food crops from a human nutrition perspective. *Journal of Experimental Botany*, 55(396), 353-364. <https://doi.org/10.1093/jxb/erh064>
- Wessells, K. R., & Brown, K. H. (2012). Estimating the global prevalence of zinc deficiency: Results based on zinc availability in national food supplies and the prevalence of stunting. *PLoS ONE*, 7(11), e50568. <https://doi.org/10.1371/journal.pone.0050568>
- World Health Organization, (2006). Guidelines on food fortification with micronutrients. World Health Organization. Geneva.
- World Health Organization. (2016). WHO guideline: fortification of maize flour and corn meal with vitamins and minerals. Geneva.
- Yadava, D. K., Choudhury, P. R., Hossain, F., Kumar, D., Sharma T. R. & Mahapatra, T. (2022).

Biofortified Varieties: Sustainable Way to Alleviate Malnutrition (Fourth Edition). Indian Council of Agricultural Research, New Delhi. Pp.106

Yadava, D. K., Choudhury, P. R., Hossain, F., Kumar, D., Sharma, T. R., & Mohapatra, T. (2020). *Biofortified varieties: sustainable way to*

alleviate malnutrition. Indian Council of Agricultural Research, New Delhi, p 89.

Zulfiqar, U., Khokhar, A., Maqsood, M. F., Shahbaz, M., Naz, N., Sara, M., ... & Ahmad, M. (2024). Genetic biofortification: advancing crop nutrition to tackle hidden hunger. *Functional & Integrative Genomics*, 24(2), 34. <https://doi.org/10.1007/s10142-024-01308-z>



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