

International Journal of Science and Research Archive

eISSN: 2582-8185 Cross Ref DOI: 10.30574/ijsra Journal homepage: https://ijsra.net/



(RESEARCH ARTICLE)

퇹 Check for updates

# Cytokinin: An invisible warrior to spot blotch disease: A new proposal to way forward

Anindita Roy \*

South Dakota State University, Brookings, SD, USA.

International Journal of Science and Research Archive, 2024, 11(02), 1414–1425

Publication history: Received on 26 February 2024; revised on 08 April 2024; accepted on 10 April 2024

Article DOI: https://doi.org/10.30574/ijsra.2024.11.2.0610

## Abstract

**Background of Abstract:** Wheat is threatened by spot blotch disease causing considerable yield loss, with existing genotypes lacking resistance and the necessary nutrients. Cytokinins (CKs) are key phytohormones that not only regulate plant development but also play an important role during spot blotch disease and in the nutrient metabolic pathway of crop plants.

**Body of Abstract:** Spot blotch disease, caused by *Bipolaris sorokiniana syn. Helminthosporium sativum syn. Cochliobolus sativus* is one of the dangerous diseases of wheat (*Triticum aestivum* L.) in Asia. Previous study identified the significant impact of zinc (Zn), sulfur(S), iron (Fe), nitrogen(N), potassium(P), and chlorine (Cl) fertilization on dipping the spot blotch acuteness in two wheat varieties Bhrikuti and Sonalika in Nepal. Cytokinins (CKs) are crucial phytohormones that not only control plant growth but also show a significant function during the nutrient metabolic pathway and in the stressed condition of the cereal crop plants. Lack of vital nutrients like Zn, S and Fe initiates severe injury of the body which is pleading the urgent requirement to improve the increase of these micronutrients in the cereal crop plant. Crop bio-fortification is one of the promising methods through which these nutrients could be boosted to a desirable volume. Cytokinin has a crucial role in controlling environmental stress and defense methods of plants. It is also discovered that it has an effect over Zn growth in cereal crop plants. Many studies identified that an augmentation of Zn, S and Fe in the seeds of the cereal crop plants ensuing the improvement of the root elongation which is also helpful for foraging nutrients to the innate ends of the soil and supports in the water absorbance in the drought area. Traveling micronutrients from the lithosphere through the root supports in the uptake of the micronutrients and transferring them to the cereal crop plants.

**Short Conclusion:** This review is exploring a crop bio- fortification method containing CRISPR-Cas9 through breeding methods could be a significant job for spot blotch stress management and developing the desirable traits to boost the nutritional rate of wheat which would be beneficial for mankind.

Keywords: Wheat; Spot blotch; Nutrients; Cytokinin (CK); Biofortification.

# 1. Introduction

Wheat (Triticum aestivum L.) is one of the most vital cereal crops in the world (1,2). Wheat is considered the most important cereal crop as it is broadly grown and eaten as food around the world among the field crops. It is also considered as the main food for almost 35 per cent of the world population. Wheat is probable to cultivate quicker than the other chief cereal crop plants (3). It is grown in a ample array of ecological conditions around the world and it is rich in nutrition apparatus and delivers around 20% of protein in human diet (4). Majority of the success was instigated by the amalgamation of high rates of venture in crop research, market expansion, and appropriate plan support that acquired place in the first Green Revolution, nonetheless still there is necessity to progress the crop yield to acquire the mandate of the hastily growing population (3). However, wheat is grown at a large measure and there is extreme

<sup>\*</sup> Corresponding author: Anindita Roy

Copyright © 2024 Author(s) retain the copyright of this article. This article is published under the terms of the Creative Commons Attribution Liscense 4.0.

demand around the world. Therefore, due to food security, fundamental alterations are required to enhance crop production due to converge the necessity of the deprived people (5). Instead of being contemplated as a main food. wheat is also a main font of nutrients for almost 40% of the world (6). Still, malnutrition is a thoughtful topic these days. Therefore, the advancement of the implying wheat genotypes via crop bio-fortification which improves the breeding method is necessary for nutritional security (7). Around 90%–95% of wheat grown in the world is bread wheat having 2n = 6x = 42 poised of three sub genomes like A, B, and D diploid genomes which is a rich pool of genes deciding production and its promoting desirable traits (Nadolska et al., 2017). However, wheat is susceptible by spot blotch disease which is caused by Bipolaris sorokiniana syn. Helminthosporium sativum syn. Cochliobolus sativus which is regard as one of the most overwhelming diseases in Southeast Asia and Eastern India (9,10). Internationally, it is considered as the most severe disease of wheat crops, mostly in warm areas of South America and South Asia (11). For this reason, attempts have been attained to identify resistance genotypes, but obtainability of immune plants is still absent (11,12). This spot blotch disease was identified during 19th century; however, it was achieved more attention after Green Revolution (13). Cultivars of wheat and barley are considered under Spartan attack from pathogens mainly at the time of flowering which severely hampers the grain filling and finally escorts to sinking the production of barley (14) and wheat crops (15). The usage of fungicide can decrease the spot blotch disease harshness (16), nevertheless frequent usage of fungicides not only upsurges the cost of agriculture production but also contaminates the environment. However, it is related to making fungicidal resistance pathogen as well (17). Therefore, development of resistance cultivars by molecular breeding method is a fruitful and cost-friendly approach for reducing the spot blotch problem. The accessibility of genetic data on spot blotch resistance genetics is limited as exposed by present literatures. Besides this spot blotch disease, wheat crop also suffers from deficiency of the essential nutrients for the human body. When the body does not acquire sufficient nutrients, it causes many health problems including fatigue, dizziness digestion, weight loss, and malnutrition which can trigger mental or physical disability. Previous studies identified that micronutrients are crucial for body functions, and their lack of them creates severe physical illnesses especially immune system disorder. Likewise, Verma et al. (18) identified that shortage of micronutrients triggers learning disability. decreases physical growth, and reproduction, and raises the percentage of infection. Even though the country has reached total crop yield calculated at 296.65 million tons in 2019–20, but it was higher by 26.87 million tons than the average yield of crop of the earlier 5 years. Likewise, wheat yield is calculated at 107.59 million tons in 2019-20 (Anonymous). Nevertheless, during the production of high yielding genotypes, adequate consideration has not been given to important nutrients; consequently, these developed high yielding genotypes have low amount of vital nutrients. Therefore, discovering the essential genotypes which deliberate a good source for spot blot disease resistance, and are rich in vital nutrients, and can harness the cytokinin hormone related genes would be a significant and useful method for developing crop production and other necessary traits by the application of molecular breeding methods such as multidisciplinary methods. For developing the high yielding wheat cultivars along with the essential nutrients, harnessing cytokinin is one of the evolving methods for the scientists under the changing global climate. The changing global climate raises greenhouse gases which are responsible for the decrease of crop yield, and quality which cause the nutritional lack in human body (19). Previous studies identified that drought stress decreases the accessibility of vital micronutrients, especially Fe and Zn (20). For supplying the demand of the fast-growing population, developing the production of crop plants is the main goal of plant scientists in the 21st century as well as increasing the vital nutrients level in the crop plants, through either bio-fortification (creating crops that have higher amount of the essential nutrients in their edible organs) or creating the genotypes along with vital nutrients via breeding methods is the present goal. In continuation, 28 bio-fortified wheat genotypes along with essential traits have been acquired (21). Production of wheat along with desirable traits can be developed by two key methods, the first is improving the high productive genotypes along with vital nutrients by joining all linked gene(s)/QTLs into a single genotype, and the second is decreasing the yield loss of potential genotypes caused by either biotic or abiotic stressors.

## 1.1. Symptoms and Diagnosis of Spot Blotch Disease

Symptoms of spot blotch naturally become visible on the sheath, leaf, glumes, and nodes as light brown lesions, typically elliptical in shape, measuring around 5–10 mm long and 3–5 mm wide. These lesions are dotted throughout the leaves and slowly raise in size and merge to create bigger necrotic patches which is indicated in Figure 1. The affected leaves quickly become chlorophyll deficient and ultimately die. During the most acute conditions, the spikes are also severely impacted and dark brown to black staining seems around the germinating point of the seed. The disease's level is modulated by various abiotic factors such as moisture, soil fertility, and temperature.

These symptoms stated above may normally be used for diagnosis of the disease, although this may require confirmation by microscopic identification of the pathogen. Therefore, alternative approaches for diagnosis by using either DNA-based probes or antibody have also been used (22). Like an example, an isolated sequence-categorized amplified zone (SCAR) marker was established for PCR-based recognition of *B. sorokiniana* in soil and plant tissues (23). This marker admitted the detection of the pathogen even before the visual symptoms occur in wheat leaves. This helps

in immediate detection and taking essential action for disease management. Amplified polymorphic DNA markers had been used for pathogen classification belonging to numerous groups of spot blotch established based on color and shape of the colonies (24). Recent study has been identified that fungal isolates from wheat plants were known to be *B. sorokiniana*, by using DNA markers based on (i)  $\beta$ -tubulin gene (ITS), (ii) ribosomal DNA gene and (iii) EF1- $\alpha$  gene (25).



Figure 1 Spot blotch disease symptoms on wheat leaves.

# 2. Harnessing the Role of Nutrient on the Alleviation of Spot Blotch Disease Severity in Wheat based on the Literature Review

Spot blotch disease in wheat triggered by *Bipolaris sorokiniana syn. Helminthosporium sativum syn. Cochliobolus sativus* - a non-specific foliar pathogen, is one of the most worrying wheat (*Triticum aestivum* L.) diseases recently in the temperate areas of South Asia (26). Due to this Spot blotch disease, Yield loss is very severe in the region of South Asia (27,28). Previous studies have identified that, spot blotch disease seriousness may be serious by soil nutrient failings (26,29). Previous studies suggested that application of mineral nutrients may decrease spot blotch disease severity in wheat (30,31). Application of nitrogen exhibited reduction in spot blotch severity of wheat under the field (31,32) and greenhouse conditions (33). Despite an early identification of the function of potassium in decreasing field crop diseases, its impact on spot blotch disease in wheat is inadequately identified. Inadequate earlier studies recommended that potassium treatment may be critical in decreasing spot blotch disease severity (26,31). Long-term experiments applying nutrient management tools in rice–wheat cropping systems have exhibited that K level in the soil is deteriorating (32,34). Therefore, in the rice–wheat system yield losses can be observed to increase when spot blotch pandemics appear in K deficient soil. Several studies identified the significance of reducing spot blotch disease for producing higher yields in humid and warmer regions (28). Restricted previous studies identified that K application can decrease spot blotch disease seriousness (29,35). Previous studies recommended that sufficient Cl nutrition acted as an important function in the leaf rust (*Puccinia triticina*) resistance of wheat (36,37).

## 2.1. Expression of Cytokinin Genes for the Development of Yield's Related Traits in Wheat

Previous study investigated the impact of wheat gene family members (GFMs) expressing the CKX enzyme, which degrades cytokinin oxide/dehydrogenase (CKX) irreversibly. Cytokinins, and as a result, it tightly controls the amount of cytokinin in various plant parts (38,39). Their results showed that, under controlled and typical field conditions, there was a natural variation in the expressive levels of the genes they tested. This variation was very high for yield and its contributing traits, suggesting that advantageous wheat genotypes could be selected for breeding purposes to increase crop yield. Additionally, cytokinins have a variety of functions in plant development and influence several crucial processes for agriculture (39). The well-documented practice of silencing specific CKX in cereal crops. Increased cytokinin levels affect yield and its component traits in rice, barley, and wheat (40,41,42). Depending on the species, there are different numbers of CKX GFMs; however, 11–14 gene family members have been proposed for bread wheat (44). On the other hand previous study investigated the CKX gene in wheat for the purpose of know the expression specificity of these genes for each other (43) The development of the plant's crops and based on this expression of genes, they have classified such genes into four groups: 1) The following groups are composed of leaf specific e.g., TaCKX9, TaCKX5, TaCKX4. 2) Inflorescence specific and evolving spikes, like TaCKX2.mined the HvCKX expression pattern in barley and found that these genes are essential. 3) Specific to seedling roots, such as TaCKX10, TaCKX7, and 4) Expressed in all organs examined at varying degrees, such as TaCKX11, TaCKX3, and TaCKX8. The effect of TaCKX1 silencing in wheat was also investigated by Jablonski et al. (43), who found that yield-related trait characteristics and various co-expression modes with other TaCKX GFMs affected the effect. TaCKX GFMs and phytohormones that were either up- or down-regulated controlled every yield-related attribute that was investigated. Earlier study state that cytokinins control the transcription of genes in targeted organs and that a variety of transcription factors (TFs) are linked to different stages of crop growth. NAC (for NAM, ATAF, and CUC) TFs are one of the biggest families of plant TFs involved in cytokinin-dependent regulation (44). It has been established that national advisory councils have a role to play in regulation. important agronomic traits. The field experiment was designed to investigate the situation, The effect of spraying cytokinins with various concentrations on them Growth and yield of wheat varieties, which were found to be 10. The growth, yield and quality of the cultivars have shown a marked difference. Higher concentrations of cytokinins have been detected a significant difference, for the majority of traits, such as flag leaf,number of grains, biological yield, and protein percentage respectively. Therefore, identified genotypes may be found: Under breeding programmes, used as a parent or donor Improvement in yield and attribute characteristics is also part of this.

# 2.2. Harnessing the Role of Cytokine as a Genetic Resource for Enhancing Wheat Nutrition

The developed wheat genotypes have adequate yield and improvement in the other desirable traits, but they have the necessary nutrients deficiency for the human body. Considering this occurrence in very recent past, 28 bio-fortified wheat genotypes (Table: 1) have been discovered by different agricultural research organization which are having sufficient essential nutrients and development in the other desirable traits (45). Along with this, many other agricultural research has been conveyed regarding the transference of micronutrients in the crop plants to increase their essential content (46,47,48). Therefore, to upgrade wheat genotypes for ensuring essential nutrients and other expected traits with having the resistance for different biotic and abiotic stresses, these upgraded desirable genotypes may be selected as donor parents in the breeding process. Besides this, previous studies identified that if chemical fertilizers are offered, then these genotypes can produce plentiful nutrients into grains. Therefore, the desirable genotypes could be studied by advanced breeding at a molecular level to realize the genetics of these desirable traits with the target of improving higher genotypes. Likewise, involvement of the physiological traits with the resistance to spot blotch disease in wheat such as leaf tip necrosis, leaf angle and stay green trait have been identified and suggested to discover breeding of the desirable challenging genotypes as these phenotypical characteristics are effectively associated with having the resistance to spot blotch disease (49,50,51). Stay-green is another important characteristic of wheat that can not only boost the production of wheat but is also able to influence having the resistance to spot blotch and other environmental stresses. This exceptional characteristic can be used as a structural marker for choosing the spot blotch disease resistance wheat from the big wheat populations and discover the breeding of favorable genotypes by the hybridization process. Cytokining are identified and considered as the most effective controller between the senescence and stavgreen characteristics of plants. (52) informed that expression of cytokinins can enhance grain numbers, and seed size of crops, and it also has a effective response against the different environmental stressors (53). Because of having wellknown properties on increasing seed number and seed size, and also having impact against different environmental abiotic and biotic stressors and producing vital nutrients, the cytokinins might be the phytohormone which supports the second "Green Revolution" have been reported by (54). Previous studies have identified the targeted breeding for essential micronutrients was invented by the promising international program of CGIAR (55) which is a biofortified breeding program including high-quantity of micronutrient phenotyping to advance the desirable genotypes by the biofortification process for ensuring specially the three vital micronutrients, namely iron (Fe), zinc (Zn), and provitamin A (PVA) which are very essential for the human health and they have gained momentum in 21st century. The plan for harvesting Plus, in conjunction with its Global Consortium partners, envisages substantially increasing the quantity of Fe, Zn and PVA in staple crops as well as releasing genetically modified wheat varieties that have great potential throughout the world. In the future, these bio-fortified genotypes may be donors or parents in crop enhancement breeding efforts. Excellent work has been done in creating better wheat genotypes with adequate zinc and other necessary nutrients by the CGIAR's core center, CIMMYT (56).

**Table 1** List of 28 bio-fortified wheat varieties released in country better for nutrients quality parameters.

Variety	Descriptions
HI 8823	HI 8823 wheat range is evolved through ICAR-Indian Agricultural Research Institute, Regional Station, Indore and launched in 2021. Rich in protein (12.1%) and zinc (40.1 ppm) in evaluation to famous varieties. It offers 38. five q/ha grain yield, adulthood in 122 days and appropriate for well-timed sown irrigated situations in rabi for the states like Madhya Pradesh, Chhattisgarh, Gujarat, Rajasthan, etc.
HUW 838	HUW 838 wheat range is evolved through Banaras Hindu University, Varanasi below ICAR-All India Coordinated Research Project on Wheat & Barley and launched in 2021. It incorporates excessive zinc (41. eight ppm), yielded 51. three q/ha grain yield, matured in 148 days and appropriate for early sown irrigated situations in rabi for the states including Punjab, Haryana, Delhi, Rajasthan, Western Uttar Pradesh, etc.

MP (JW) 1,358	This wheat range is evolved through Jawahar Lal Nehru Krishi Viswavidhyalaya, Zonal Agricultural Research Station, Powarkheda below ICAR-All India Coordinated Research Project on Wheat & Barley and launched in 2021. It is wealthy in protein (12.1%) and iron (40.6 ppm) in evaluation to famous wheat varieties. Its grain yield is 56.1 q/ha, adulthood time is one hundred and five days and appropriate for early sown irrigated situations in for Maharashtra, Karnataka and plains of Tamil Nadu.
DDW 40	It eight is durum wheat, evolved through ICAR-Indian Institute of Wheat & Barley Research, Karnal and launched in 2020. It is wealthy in protein (12.1%) in evaluation to 8%–10% protein in famous varieties, having grain yield 47. four q/ha, matured in 111 days, appropriate for well-timed sown irrigated situations in rabi season and endorsed for cultivation in Maharashtra, Karnataka, and plains of Tamil Nadu.
DBW 332	DBW 332: This variety is evolved through ICAR-Indian Institute of Wheat & Barley Research, Karnal which launched in 2021. This range is wealthy in protein (12.2%) and zinc (40.6 ppm) in evaluation to famous range. Its yield capability is 78. three q/ha, adulthood time is 156 days and appropriate for early sown irrigated situations in rabi season for one-of-a-kind states of the country.
DBW 327	This range is evolved through ICAR-Indian Institute of Wheat & Barley Research, Karnal and launched in 2021. It consists of excessive zinc (40.6 ppm), yielding 79. four q/ha, matured in one hundred fifty-five days and appropriate for its cultivation early sown irrigated situations in Punjab, Haryana, Delhi, Rajasthan, Western Uttar Pradesh, etc
HI 1636	It is evolved through ICAR-Indian Agricultural Research Institute, Regional Station, Indore and launched in 2021. Variety consists of excessive zinc (40. four ppm), has 56.6 q/ha grain yield, matured in 119 days and appropriate for well-timed sown irrigated situations in rabi for Madhya Pradesh, Chhattisgarh, Gujarat, Rajasthan, etc.
DBW 173	It is evolved via way of means of ICAR-Indian Institute of Wheat & Barley Research, Karnal launched in 2018. It is wealthy in protein (12.5%) and iron (40.7 ppm) in assessment to 8%–10% protein and 28. zero–32. zero ppm iron in famous varieties, having grain yield 47.2 q/ha, matured in 122 days, appropriate for past due sown irrigated situations in rabi season and endorsed for cultivation in Punjab, Haryana, Delhi, Rajasthan and different states.
UAS 375	This wheat range is advanced via way of means of University of Agricultural Sciences, Dharwad beneathneath ICAR-All India Coordinated Research Project on Wheat & Barley, launched in 2018, wealthy in protein (13.8%) in contrast to 8%–10% in famous varieties. Produces 21. four q/ha grain yield, matured in 103 days, appropriate for well-timed sown rainfed situations in rabi season and encouraged for cultivation in Maharashtra and Karnataka
DDW 47	DDW 47 is advanced via way of means of ICAR-Indian Institute of Wheat & Barley Research, Karnal, launched in 2020P. Variety wealthy in protein (12.7%) and iron (40.1 ppm) in contrast to 8%–10% protein and 28. zero–32. zero ppm iron in famous varieties, having grain yield 37. three q/ha, adulthood time 121 days, appropriate for well-timed sown limited irrigated situations in rabi season and encouraged for cultivation in Madhya Pradesh, Gujarat, Rajasthan, and Chhattisgarh.
Karan Vandana (DBW 187):	Karan Vandana wheat range is evolved with the aid of using ICAR-Indian Institute of Wheat & Barley Research, Karnal and launched in 2018 and 2020. It is wealthy in iron (43.1 ppm) in contrast to 28. zero–32. zero ppm in famous varieties, having grain yield 48. eight q/ha in Northeastern Plains Zone (NEPZ), 61. three q/ha in Northwestern Plains Zone (NWPZ) and 75. five q/ha in excessive fertility situations. Variety in matured in one hundred twenty days (NEPZ), 146 days (NWPZ) and 158 days (Highfertility) situations.
WB 02	WB 02 wheat range is advanced through ICAR-Indian Institute of Wheat & Barley Research, Karnal that's launched in 2017. It is wealthy in iron (40. zero ppm) and zinc (42. zero ppm) in contrast to 28. zero–32. zero ppm iron and 30. zero–32. zero ppm zinc in famous varieties. The grain yield of this range is 51.6 q/ha, matured in142 days, appropriate for irrigated well timed sown situations in rabi and endorsed for cultivation in Punjab, Haryana, Delhi, Rajasthan, Western Uttar Pradesh and oter states.
HPBW 1	HPBW 1 wheat range is evolved with the aid of using Punjab Agricultural University, Ludhiana beneathneath ICAR-All India Coordinated Research Project on Wheat & Barley that is launched in

HI 1633:	This was created in 2020 at the Indore Regional Station of the Indian Agricultural Research Institute (ICAR). It is higher in protein (12.4%), iron (41.6 ppm), and zinc (41.1 ppm) than common
DBW 303:	ICAR-Indian Institute of Wheat & Barley Research, Karnal is the developer of DBW 303, which is scheduled for delivery in 2020. Protein content is high (12.1%) compared to typical types' 8%–10% protein. This variety yields 81.2 q/ha of grain and matures in 156 days. It is suitable for early sowing under irrigation and grows well in high fertility conditions during Rabi. Punjab, Haryana, Delhi, Rajasthan, and other states are advised to cultivate it.
PB 757:	It is evolved with the aid of using Punjab Agricultural University, Ludhiana beneathneath ICAR-All India Coordinated Research Project on Wheat & Barley and launched in 2018. Contains excessive zinc (42. three ppm) in contrast to 30. zero–32. zero ppm zinc in famous varieties. The yield of this range is 36.7 q/ha, adulthood time is 104 days, appropriate for extremely overdue sown irrigated situations in rabi season and endorsed for cultivation in Punjab, Haryana, Delhi, Rajasthan, and different states.
PBW 752	PBW 752 wheat range is evolved with the aid of using Punjab Agricultural University, Ludhiana beneathneath ICAR-All India Coordinated Research Project on Wheat & Barley and launched in 2018. It is wealthy in protein (12. four%) in contrast to 8%–10% in famous varieties, having grain yield 49.7 q/ha, matured in one hundred twenty days, appropriate for overdue sown irrigated situations in rabi season and endorsed to domesticate for Punjab, Haryana, Delhi, Rajasthan, and different states.
MACS 4028	MACS 4028 is a durum wheat evolved with the aid of using Agharkar Research Institute, Pune beneathneath ICAR-All India Coordinated Research Project on Wheat & Barley and launched in 2018. It is wealthy in protein (14.7%), iron (46.1 ppm) and zinc (40. three ppm) in contrast to 8%–10% protein, 28. zero–32. zero ppm iron and 30. zero–32. zero ppm zinc in famous varieties. It offers grain yield of 19. three q/ha, maturated in 102 days, appropriate for rainfed, low fertility, well timed sown situations in rabi and endorsed for cultivation in Maharashtra and Karnataka
HI 8777	HI 8777 is durum wheat evolved with the aid of using ICAR-Indian Agricultural Research Institute, Regional Station, Indore and launched in 2018. It is wealthy in iron (48.7 ppm) and zinc (43.6 ppm) in contrast to 28. zero–32. zero ppm iron and 30. zero–32. zero ppm zinc in famous varieties. The grain yield of this range is 18. five q/ha, maturated in 108 days, appropriate for well-timed sown rain-fed situations in rabi season and endorsed for its cultivation in Maharashtra, Karnataka, and plains of Tamil Nadu.
HD 3171	HD 3171 wheat range is evolved with the aid of using ICAR-Indian Agricultural Research Institute, New Delhi and launched in 2017. This is wealthy in zinc (forty-seven.1 ppm) in contrast to 30. zero- 32. zero ppm in famous varieties. The grain yield of this range is 28. zero q/ha, adulthood time is 122 days, appropriate for well-timed sown rainfed situations in rabi and endorsed for cultivation in Eastern Uttar Pradesh, Bihar, Jharkhand, Odisha, West Bengal, Assam, and plains of Northeastern States
Pusa Ujala (HI 1605)	Pusa Ujala wheat range is evolved with the aid of using ICAR-Indian Agricultural Research Institute, Regional Station, Indore that is launched in 2017. It is wealthy in protein (13. zero%) and iron (43. zero ppm) in contrast to 8%–10% protein and 28. zero–32. zero ppm iron in famous varieties, having grain yield 30. zero q/ha, adulthood time one zero five days, appropriate for well-timed sown constrained irrigated situations in rabi season and endorsed for cultivation in Maharashtra and Karnataka
Pusa Tejas (HI 8759):	Pusa Tejas wheat range is likewise referred to as HI 8759 durum wheat, evolved with the aid of using ICAR-Indian Agricultural Research Institute, Regional Station, Indore and launched in 2017. It is wealthy in protein (12. zero%), iron (41.1 ppm) and zinc (42. eight ppm) in contrast to 8%–10% protein, 28. zero–32. zero ppm iron and 30. zero–32. zero ppm zinc in famous varieties. Its yield is 57. zero q/ha, matured is 117 days, appropriate for irrigated well timed sown situations in rabi and endorsed for its cultivation in Madhya Pradesh, Chhattisgarh, Gujarat, Rajasthan, and Uttar Pradesh.
	2017. It is wealthy in iron (40. zero ppm) and zinc (40.6 ppm) in contrast to 28. zero–32. zero ppm iron and 30. zero–32. zero ppm zinc in famous varieties. This range yielded 51.7 q/ha, matured in 141 days, appropriate for irrigated well timed sown situations and endorsed for cultivation in Punjab, Haryana, Delhi, Rajasthan, and different states of the country.

	variations, which have lower protein levels (8%–10%), iron levels (28.0–32.0 ppm), and zinc levels (30.0–32.0 ppm). This variety is ideal for late-sown, irrigation conditions and is recommended for cultivation in Maharashtra, Karnataka, and the plains of Tamil Nadu. It matures in 100 days and yields 41.7 q/ha of grain.
HD 3,298:	Developed in 2020 by the Indian Agricultural Research Institute (ICAR) in New Delhi. It is rich in iron (43.1 ppm) and protein (12.1%) compared to popular varieties that have 8%–10% protein and 28.0–32.0 ppm iron. It matures in 103 days, is suitable for very late sowing under irrigation, and is advised for cultivation in Punjab, Haryana, Delhi, Rajasthan, and other states.
MACS 4,058:	Agharkar Research Institute, Pune is the developer of this durum wheat, which will be made available in 2020 as part of the ICAR-All India Coordinated Research Project on Wheat & Barley. It is rich in protein (14.7%), iron (39.5 ppm), and zinc (37.8 ppm) compared to popular varieties that have 8%–10% protein, 28.0–32.0 ppm iron, and 30.0–32.0 ppm zinc. It matures in 102 days and is suitable for timely sowing under restricted irrigation during the Rabi season. Maharashtra and Karnataka are recommended to cultivate it.
HD 3,249:	This variety was created in 2020 and distributed by the Indian Agricultural Research Institute (ICAR), located in New Delhi. Compared to typical kinds that have 28.0–32.0 ppm of iron, this variety has 42.5 ppm of iron. It is advised for cultivation in Eastern Uttar Pradesh, Bihar, Jharkhand, and Odisha. It ripened in 122 days.
HI 8,805:	The 2020 release of HI 8,805 (durum wheat) is the result of research conducted by ICAR-Indian Agricultural Research Institute, Regional Station, Indore. It is higher in iron (40.4 ppm) and protein (12.8%) than typical cultivars, which have 28.0–32.0 ppm iron and 8%–10% protein. It is suitable for timely sowing in rainfed circumstances and has a grain yield of 30.4 q/ha. It matures in 105 days and is advised for cultivation in the plains of Tamil Nadu, Maharashtra, and Karnataka.
HI 8,8052	Its grain yield is 29.1 q/ha, matures in 109 days, and is suitable for timely sowing in rain-fed environments. It is also recommended for cultivation in Karnataka.

## 2.3. Harnessing the Key Technologies Applied to Crop Bio-Fortification

Reports imply that extra than 820 million human beings withinside the international are hungry and billion human beings are affected by micronutrient deficiencies (57,58). Most of the crop flowers can gather micronutrients; however, a few essential flowers lack the good enough quantities of such vitamins viz., Fe and Zn withinside the fit to be eaten elements (59), for instance, fundamental/staple vegetation which includes wheat, rice and maize incorporate low quantities of Fe and Zn (60). In the latest studies, it's far strongly said that micronutrient deficiencies boom susceptibility to many infectious diseases, which includes Covid-19 (61). Therefore, extra interest has been made to beautify such important vitamins withinside the crop flowers through bio-fortification, Figure 2 indicates it's a beneficial method to fight micronutrient deficiency. Different beneficial processes of bio-fortification are getting used to enhance the dietary cost of flowers, to triumph over dietary the troubles supplied in Figure 3, and additionally defined with the aid of using (62), with an observation that bio-fortification is a cost- powerful and sustainable agricultural method for growing the bioavailability of crucial elements/vitamins withinside the fit to be eaten elements of flowers and lowering malnutrition. Further, they have got additionally talked about that genetic bio-fortification primarily based totally on genetic engineering which includes growing or manipulating the expression of genes that have an effect on the law of steel homeostasis and provider proteins that serve to boom the micronutrient contents and extra productiveness thru CRISPR-Cas9 (bacterial Clustered Regularly Interspaced Short Palindromic Repeats) generation may be taken into consideration as a promising high- ability method or current and really superior GM generation for fixing the micronutrient deficiency hassle and this method changed into mentioned for the primary time with the aid of using (63) editing the germ line cells, Crispr-cas generation has the ability to expand transgenics without concerning transformation and tissue way of life flowers (64). (65) said that, throughout the globe, new genetic amendment methods (nGMs) processes, specifically genome editing, are utilized in fundamental and implemented studies. In parallel to conventional genetically changed generation a huge variety of nGM strategies are being advanced for the (genetic) amendment of organisms, which includes flowers, for studies functions or for the improvement of vegetation for agricultural functions. These nGMs also are noted as "new strategies" or "new breeding strategies" for enhancing centered traits (66).

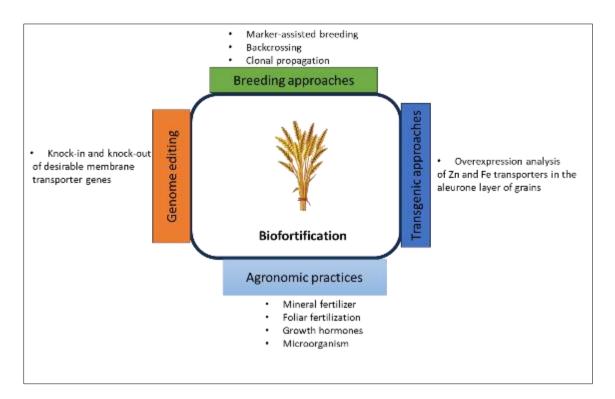


Figure 2 Schematic diagram of Biofortification strategies for micronutrient enhancement of food crops

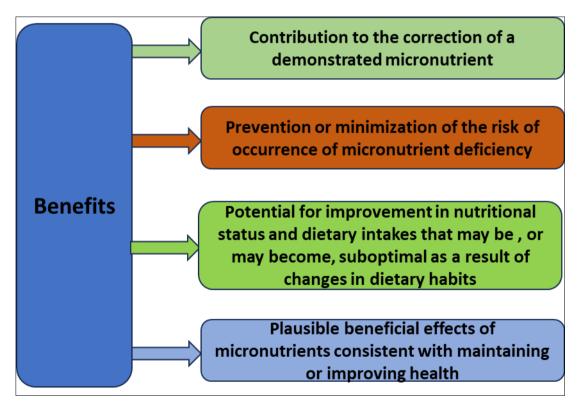


Figure 3 Schematic diagram of Biofortification for enhancing nutritional outcome

# 3. Conclusions

Wheat is taken into consideration one of the maximum economically essential cereal vegetation withinside the world. Its productiveness is excessive, however growing intake and converting weather shows the want for in addition development in its yield potential. Climate alternate is predicted to preserve posing biotic/abiotic stresses, and if

present day developments preserve, many elements of the planet turn into antagonistic to agriculture. On the opposite hand, due to the unexpectedly developing populace and the converting weather, call for wheat is predicted to develop quicker than the opposite predominant vegetation. Therefore, investments in exploring and harnessing current genetic assets which include bio-fortified wheat and facts concerning the position of cytokinins beneathneath every day and detrimental situations for growing the yield, grains containing sufficient vitamins and having a enough protection response, effect of morphological markers viz., live inexperienced tendencies, leaf tip necrosis, leaf attitude molecular markers, and different beneficial genetic facts if you want to produce sufficient meals grains which might be additionally wealthy withinside the required vitamins to make sure meals protection withinside the twenty first century is the want of the day.

Since cytokinins are the maximum essential endogenous materials moderating the physiological and molecular responses, thay have a key position at some point of of entirety of the lifestyles cycle of flora if you want to deliver a best yield. So, plant breeders should without delay goal the cytokinins to enhance centered tendencies with the aid of using utilising minimal input, as cytokinins are acknowledged to be a key motive force of seed yield and it is able to properly be the hormone that underpins the second one inexperienced revolution. The Green Revolution boosted crop yields at some point of the mid twentieth century with the aid of using introducing dwarf genotypes of wheat able to respond to a better dose of fertilization and sufficient irrigation without lodging. Now there's a want of a 2nd Green Revolution to fulfill out the call for of a unexpectedly developing populace. The Green Revolution became primarily based totally on vegetation conscious of excessive soil fertility however, now there's wanting to broaden the genotypes of wheat vegetation that could carry out higher beneathneath low input, low soil fertility, beneathneath harassed situations which include heat, terminal heat, drought, steel toxicity, and beneathneath biotic stresses as properly. By preserving the above statistics in mind, exploring genetic assets, harnessing the cytokinin key hormones, and making use of up-to-date molecular breeding approaches, plant breeders can broaden the advanced and solid genotypes so one can be capable of cater to the meals call for of the needy population.

# List of Abbreviations

- Cytokinin: CK, Zinc: Zn, Sulfur: S, Iron: Fe, Nitrogen: N, Potassium: P, Chlorine: Cl
- Cytokinin oxide/dehydrogenase: CKX.

# **Compliance with ethical standards**

## Acknowledgments

I greatly appreciate Dr. Rupak Chakraborty (Department of Agronomy, Horticulture and Plant Science, South Dakota State University) for his valuable suggestions.

# Disclosure of conflict of interest

No conflict of interest to be disclosed.

# Authors' Contributions

This Manuscript is fully written by Anindita Roy.

# References

- [1] Schnurbusch, Thorsten. "Wheat and barley biology: towards new frontiers." *Journal of integrative plant biology* 61, no. 3 (2019): 198-203..
- [2] Alzaayid, Dhiaa Thalij Jassim, and Rana Hashim Aloush. "Effect of cytokinin levels on some varieties of wheat on yield, growth and yield components." In *IOP Conference Series: Earth and Environmental Science*, vol. 910, no. 1, p. 012090. IOP Publishing, 2021.
- [3] Pingali, Prabhu L. "Green revolution: impacts, limits, and the path ahead." *Proceedings of the national academy of sciences* 109, no. 31 (2012): 12302-12308.
- [4] Reynolds, Matthew, and Hans Braun. "Benefits to low-input agriculture." *Nature Plants* 5, no. 7 (2019): 652-653.
- [5] Pingali, Prabhu. "Agricultural policy and nutrition outcomes–getting beyond the preoccupation with staple grains." *Food security* 7 (2015): 583-591..

- [6] Giraldo, Patricia, Elena Benavente, Francisco Manzano-Agugliaro, and Estela Gimenez. "Worldwide research trends on wheat and barley: A bibliometric comparative analysis." *Agronomy* 9, no. 7 (2019): 352.
- [7] Prasad, Ravindra. "Cytokinin and its key role to enrich the plant nutrients and growth under adverse conditionsan update." *Frontiers in Genetics* 13 (2022): 883924.
- [8] Nadolska-Orczyk, Anna, Izabela K. Rajchel, Wacław Orczyk, and Sebastian Gasparis. "Major genes determining yield-related traits in wheat and barley." *Theoretical and Applied Genetics* 130 (2017): 1081-1098.
- [9] Joshi, A. K., R. Chand, and B. Arun. "Relationship of plant height and days to maturity with resistance to spot blotch in wheat." *Euphytica* 123 (2002): 221-228.
- [10] Kumar, Uttam, Arun K. Joshi, Sundeep Kumar, Ramesh Chand, and Marion S. Röder. "Mapping of resistance to spot blotch disease caused by Bipolaris sorokiniana in spring wheat." *Theoretical and Applied Genetics* 118 (2009): 783-792.
- [11] Kumar, Uttam, Suneel Kumar, Ravindra Prasad, M. S. Roder, Sundeep Kumar, Ramesh Chand, Vinod Kumar Mishra, and Arun Kumar Joshi. "Genetic gain on resistance to spot blotch of wheat by developing lines with near immunity." (2019).
- [12] Lillemo, M., A. K. Joshi, R. Prasad, R. Chand, and R. Singh. "Association of Lr 34 and Lr 46 with spot blotch resistance in wheat." *Theor. Appl. Genet* 126 (2012): 711-719
- [13] Saari, E. E. "Leaf blight disease and associated soil-borne fungal pathogens of wheat in South and South East Asia." *Helminthosporium blights of wheat: spot blotch and tan spot* (1998): 37-51.
- [14] Prasad, R., L. C. Prasad, R. Chand, and A. K. Joshi. "Assessment of diversity for resistance to spot blotch disease and its association with certain phenotypic traits in barely." *Field crops research* 154 (2013): 195-200.
- [15] Gupta, P. K., Ramesh Chand, N. K. Vasistha, S. P. Pandey, U. Kumar, V. K. Mishra, and A. K. Joshi. "Spot blotch disease of wheat: the current status of research on genetics and breeding." *Plant pathology* 67, no. 3 (2018): 508-531.
- [16] de Viedma<sup>1</sup>, L. Q., and M. M. Kohli. "Spot blotch and tan spot of wheat in Paraguay." *Helminthosporium blights of wheat: Spot blotch and Tan spot* (1997): 126.
- [17] Golembiewski, R. C., JM, Jr Vargas, A. L. Jones, and A. R. Detweiler. "Detection of demethylation inhibitor (DMI) resistance in Sclerotinia homoeocarpa populations." (1995): 491-493.
- [18] Verma, Pankaj Kumar, Shikha Verma, Debasis Chakrabarty, and Nalini Pandey. "Biotechnological approaches to enhance zinc uptake and utilization efficiency in cereal crops." *Journal of Soil Science and Plant Nutrition* 21, no. 3 (2021): 2412-2424.
- [19] Myers, Samuel S., Antonella Zanobetti, Itai Kloog, Peter Huybers, Andrew DB Leakey, Arnold J. Bloom, Eli Carlisle et al. "Increasing CO2 threatens human nutrition." *Nature* 510, no. 7503 (2014): 139-142.
- [20] Moreno-Jiménez, Eduardo, César Plaza, Hugo Saiz, Rebeca Manzano, Maren Flagmeier, and Fernando T. Maestre. "Aridity and reduced soil micronutrient availability in global drylands." *Nature sustainability* 2, no. 5 (2019): 371-377.
- [21] Yadava, D. K., P. R. Choudhury, Firoz Hossain, and Dinesh Kumar. "Biofortified varieties: sustainable way to alleviate malnutrition." *Indian Council of Agricultural Research, New Delhi* (2017).
- [22] Ward, Elaine, Simon J. Foster, BART A. FRAAIJE, and H. ALASTAIR MCCARTNEY. "Plant pathogen diagnostics: immunological and nucleic acid-based approaches." *Annals of Applied Biology* 145, no. 1 (2004): 1-16.
- [23] Aggarwal, Rashmi, S. Gupta, S. Banerjee, and V. B. Singh. "Development of a SCAR marker for detection of Bipolaris sorokiniana causing spot blotch of wheat." *Canadian journal of microbiology* 57, no. 11 (2011): 934-942.
- [24] Pandey, Shree P., Sandeep Sharma, R. Chand, P. Shahi, and A. K. Joshi. "Clonal variability and its relevance in generation of new pathotypes in the spot blotch pathogen, Bipolaris sorokiniana." *Current microbiology* 56 (2008): 33-41.
- [25] Sun, X. F., D. X. Zhang, G. S. Gong, X. B. Qi, K. H. Ye, Y. Zhou, N. Liu, and X. L. Chang. "Spot blotch on volunteer wheat plants in Sichuan, China." *Journal of plant pathology* (2015): 173-176.
- [26] Duveiller, E. "Controlling foliar blights of wheat in the rice-wheat systems of Asia." *Plant disease* 88, no. 5 (2004): 552-556.
- [27] Sharma, Ram C., and Etienne Duveiller. "Effect of Helminthosporium leaf blight on performance of timely and late-seeded wheat under optimal and stressed levels of soil fertility and moisture." *Field Crops Research* 89, no. 2-3 (2004): 205-218.

- [28] Saari, E. E. "Leaf blight disease and associated soil-borne fungal pathogens of wheat in South and South East Asia." *Helminthosporium blights of wheat: spot blotch and tan spot* (1998): 37-51.
- [29] Regmi, A., J. Ladha, E. Pasuquin, H. Pathak, P. Hobbs, L. Shrestha, D. Gharti, and E. Duveiller. "The role of potassium in sustaining yields in a long-term rice-wheat experiment in the Indo-Gangetic Plains of Nepal." *Biology and Fertility of Soils* 36 (2002): 240-247.
- [30] Mohr, R. M., C. C. Bernier, D. N. Flaten, and G. J. Racz. "Effect of chloride fertilization on Bedford barley and Katepwa wheat." *Canadian journal of soil science* 75, no. 1 (1995): 15-24.
- [31] Krupinsky, J. M., and D. L. Tanaka. "Leaf spot diseases on winter wheat influenced by nitrogen, tillage, and having after a grass-alfalfa mixture in the conservation reserve program." *Plant Disease* 85, no. 7 (2001): 785-789.
- [32] Gami, S., J. Ladha, H. Pathak, M. Shah, E. Pasuquin, S. Pandey, P. Hobbs, D. Joshy, and R. Mishra. "Long-term changes in yield and soil fertility in a twenty-year rice-wheat experiment in Nepal." *Biology and Fertility of Soils* 34 (2001): 73-78.
- [33] Dos Santos, D. "Effet de la température et de la fertilisation N, P, K sur le degré d'attaque de génotypes de blé par Bipolaris sorokiniana." PhD diss., MS thesis, Université Catholique de Louvain, Belgium, 1998.
- [34] Bhandari, A. L., J. K. Ladha, H. Pathak, A. T. Padre, D. Dawe, and R. K. Gupta. "Yield and soil nutrient changes in a long-term rice-wheat rotation in India." *Soil Science Society of America Journal* 66, no. 1 (2002): 162-170.
- [35] Kabir, M. S., and I. Hossain. "Integrated management of Bipolaris leaf blight of wheat." (2000): 9-12.
- [36] Miller, Travis D. "Chloride fertilizer effects in winter wheat and interactions with foliar fungicides under severe leaf rust pressure." *Better Crops* 82, no. 1 (1998): 24-25.
- [37] Miller, Travis D., Kevin Tucker, and Doug Andrews. "Disease and yield response of winter wheat to chloride fertilizer and foliar fungicide in the Texas Blackland Prairie." *Texas Agr. Ext. Ser. htto://soilcrop. tamu. edu/publications/pubs/disease. pdf* (1999).
- [38] Szala, Karolina, Hanna Ogonowska, Boguslawa Lugowska, Barbara Zmijewska, Renata Wyszynska, Marta Dmochowska-Boguta, Waclaw Orczyk, and Anna Nadolska-Orczyk. "Different sets of TaCKX genes affect yield-related traits in wheat plants grown in a controlled environment and in field conditions." *BMC Plant Biology* 20 (2020): 1-13.
- [39] Szala, Karolina, Hanna Ogonowska, Boguslawa Lugowska, Barbara Zmijewska, Renata Wyszynska, Marta Dmochowska-Boguta, Waclaw Orczyk, and Anna Nadolska-Orczyk. "Different sets of TaCKX genes affect yield-related traits in wheat plants grown in a controlled environment and in field conditions." *BMC Plant Biology* 20 (2020): 1-13.
- [40] Jameson, Paula Elizabeth, and Jiancheng Song. "Cytokinin: a key driver of seed yield." *Journal of Experimental Botany* 67, no. 3 (2016): 593-606.
- [41] Ashikari, Motoyuki, Hitoshi Sakakibara, Shaoyang Lin, Toshio Yamamoto, Tomonori Takashi, Asuka Nishimura, Enrique R. Angeles, Qian Qian, Hidemi Kitano, and Makoto Matsuoka. "Cytokinin oxidase regulates rice grain production." *Science* 309, no. 5735 (2005): 741-745.
- [42] Zalewski, Wojciech, Petr Galuszka, Sebastian Gasparis, Wacław Orczyk, and Anna Nadolska-Orczyk. "Silencing of the HvCKX1 gene decreases the cytokinin oxidase/dehydrogenase level in barley and leads to higher plant productivity." *Journal of experimental botany* 61, no. 6 (2010): 1839-1851.
- [43] Jabłoński, Bartosz, Hanna Ogonowska, Karolina Szala, Andrzej Bajguz, Wacław Orczyk, and Anna Nadolska-Orczyk. "Silencing of TaCKX1 mediates expression of other TaCKX genes to increase yield parameters in wheat." *International Journal of Molecular Sciences* 21, no. 13 (2020): 4809.
- [44] Ogonowska, Hanna, Karolina Barchacka, Sebastian Gasparis, Bartosz Jablonski, Wacław Orczyk, Marta Dmochowska-Boguta, and Anna Nadolska-Orczyk. "Specificity of expression of TaCKX family genes in developing plants of wheat and their co-operation within and among organs." *PLoS One* 14, no. 4 (2019): e0214239.
- [45] Shanks, Carly M., Andreas Hecker, Chia-Yi Cheng, Luise Brand, Silvio Collani, Markus Schmid, G. Eric Schaller, Dierk Wanke, Klaus Harter, and Joseph J. Kieber. "Role of BASIC PENTACYSTEINE transcription factors in a subset of cytokinin signaling responses." *The Plant Journal* 95, no. 3 (2018): 458-473.
- [46] Yadava, D. K., P. R. Choudhury, Firoz Hossain, and Dinesh Kumar. "Biofortified varieties: sustainable way to alleviate malnutrition." *Indian Council of Agricultural Research, New Delhi* (2017).

- [47] Wang, Cheng, Jian Zeng, Yin Li, Wei Hu, Ling Chen, Yingjie Miao, Pengyi Deng et al. "Enrichment of provitamin A content in wheat (Triticum aestivum L.) by introduction of the bacterial carotenoid biosynthetic genes CrtB and CrtI." *Journal of Experimental Botany* 65, no. 9 (2014): 2545-2556.
- [48] Singh, Simrat Pal, Wilhelm Gruissem, and Navreet K. Bhullar. "Single genetic locus improvement of iron, zinc and β-carotene content in rice grains." *Scientific reports* 7, no. 1 (2017): 6883.
- [49] Qin, Xiaoqiong, Kathryn Fischer, Shu Yu, Jorge Dubcovsky, and Li Tian. "Distinct expression and function of carotenoid metabolic genes and homoeologs in developing wheat grains." *BMC plant biology* 16 (2016): 1-15.
- [50] Joshi, A. K., R. Chand, S. Kumar, and R. P. Singh. "Leaf tip necrosis: a phenotypic marker associated with resistance to spot blotch disease in wheat." *Crop Science* 44, no. 3 (2004): 792-796.
- [51] Joshi, A. K., R. Chand, and B. Arun. "Relationship of plant height and days to maturity with resistance to spot blotch in wheat." *Euphytica* 123 (2002): 221-228.
- [52] Prasad, R., L. C. Prasad, R. Chand, and A. K. Joshi. "Assessment of diversity for resistance to spot blotch disease and its association with certain phenotypic traits in barely." *Field crops research* 154 (2013): 195-200.
- [53] Schwarz, Ireen, Marie-Therese Scheirlinck, Elisabeth Otto, Isabel Bartrina, Ralf-Christian Schmidt, and Thomas Schmülling. "Cytokinin regulates the activity of the inflorescence meristem and components of seed yield in oilseed rape." *Journal of Experimental Botany* 71, no. 22 (2020): 7146-7159.
- [54] Connorton, James M., Eleanor R. Jones, Ildefonso Rodríguez-Ramiro, Susan Fairweather-Tait, Cristobal Uauy, and Janneke Balk. "Wheat vacuolar iron transporter TaVIT2 transports Fe and Mn and is effective for biofortification." *Plant Physiology* 174, no. 4 (2017): 2434-2444.
- [55] Lynch, Jonathan P. "Roots of the second green revolution." *Australian journal of Botany* 55, no. 5 (2007): 493-512.
- [56] Virk, Parminder S., Meike S. Andersson, Jairo Arcos, and Mahalingam Govindaraj. "Transition from targeted breeding to mainstreaming of biofortification traits in crop improvement programs." *Frontiers in Plant Science* 12 (2021): 703990..
- [57] Yuan, Yibing, Jill E. Cairns, Raman Babu, Zhuanfang Hao, and Xuecai Zhang. "Genome-wide association mapping and genomic prediction analyses reveal the genetic architecture of grain yield and flowering time under drought and heat stress conditions in maize." *Frontiers in plant science* 9 (2019): 426159.
- [58] Kane, Anne V., Duy M. Dinh, and Honorine D. Ward. "Childhood malnutrition and the intestinal microbiome." *Pediatric research* 77, no. 1 (2015): 256-262.
- [59] Boliko, Mbuli Charles. "FAO and the situation of food security and nutrition in the world." *Journal of nutritional science and vitaminology* 65, no. Supplement (2019): S4-S8..
- [60] Wakeel, Abdul, Muhammad Farooq, Khurram Bashir, and Levent Ozturk. "Micronutrient malnutrition and biofortification: recent advances and future perspectives." *Plant micronutrient use efficiency* (2018): 225-243.
- [61] Shariatipour, Nikwan, and Bahram Heidari. "Genetic-based biofortification of staple food crops to meet zinc and iron deficiency-related challenges." *Plant micronutrients: deficiency and toxicity management* (2020): 173-223.
- [62] Akhtar, Saeed, Jai K. Das, Tariq Ismail, Muqeet Wahid, Wisha Saeed, and Zulfiqar A. Bhutta. "Nutritional perspectives for the prevention and mitigation of COVID-19." *Nutrition reviews* 79, no. 3 (2021): 289-300.
- [63] Koç, Esra, and Belgizar Karayiğit. "Assessment of biofortification approaches used to improve micronutrientdense plants that are a sustainable solution to combat hidden hunger." *Journal of Soil Science and Plant Nutrition* 22, no. 1 (2022): 475-500.
- [64] Jinek, Martin, Krzysztof Chylinski, Ines Fonfara, Michael Hauer, Jennifer A. Doudna, and Emmanuelle Charpentier.
   "A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity." *science* 337, no. 6096 (2012): 816-821.
- [65] Malik, Kauser Abdulla, and Asma Maqbool. "Transgenic crops for biofortification." *Frontiers in Sustainable Food Systems* 4 (2020): 571402.
- [66] Eckerstorfer, Michael F., Margret Engelhard, Andreas Heissenberger, Samson Simon, and Hanka Teichmann. "Plants developed by new genetic modification techniques—comparison of existing regulatory frameworks in the EU and non-EU countries." *Frontiers in Bioengineering and Biotechnology* 7 (2019): 26.
- [67] Lusser, Maria, Claudia Parisi, Damien Plan, and Emilio Rodríguez-Cerezo. "Deployment of new biotechnologies in plant breeding." *Nature biotechnology* 30, no. 3 (2012): 231-239.