Towards nutrition security of India with biofortified cereal varieties

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Rice and wheat are the major staples contributing more than 75% to food-grain consumption, while maize, pearl millet and sorghum are important alternative cereals in India. Cereal biofortification is one of the promising approaches to alleviate micronutrient malnutrition. Here we present an overview of the efforts towards development of biofortified cereal varieties enhanced with iron, zinc, protein and provitamin-A using conventional breeding approaches, and the possibilities of scaling up and adoption to ease the burden of malnourishment.

Keywords: Biofortification, breeding, cereals, micronutrient malnutrition, nutrition security.

DEFICIENCIES of vitamins or minerals result in micronutrients malnutrition, often called hidden hunger as the symptoms are not apparent like protein–energy malnourishment. The Comprehensive National Nutritional Survey (CNNS) of India conducted on around one lakh children during 2016–18 revealed incidences of anaemia, zinc (Zn) and vitamin deficiencies[1].

The Government of India (GoI) implements multiple programmes to address micronutrient malnutrition such as National Nutrition Mission (POSHAN Abhiyaan), prophylactic supplementation of vitamin A, iron (Fe) and folic acid, and fortification of wheat flour, table salt and milk with vital micronutrients. A special emphasis on complete fortification of rice in the public distribution system (PDS) by 2024 has also been recently mooted by GoI (https://ffrc.fssai.gov.in/ffrc/home). While these serve as effective short-term strategies to increase micronutrient intake to bridge the nutritional gap, dietary diversification is considered as a long-term and sustainable strategy.

While increased agricultural production, economic opportunities and the National Food Security Act contributed to food security, nutrition security, especially micronutrient malnutrition requires further agricultural interventions. Though the production of micronutrients-rich non-staples such as vegetables, pulses, fruits and animal products has also increased, their affordability is less in comparison to staple cereals to people with low purchasing power. Thus, biofortification could be a suitable agricultural intervention to enrich micronutrient density in the commonly consumed cereals such as rice, wheat, etc. Biofortification refers to the genetic enhancement of key food crops with enhanced nutrients through agronomy, breeding and biotechnology strategies[2]. Biofortification is different from fortification, wherein nutrients are externally added to the food items.

Staple cereals have become a major food source during the lockdown period of COVID-19 pandemic in India because of their affordability by the low-income group, ready availability unlike perishable food items and also distribution through PDS. Thus, any incremental improvement in the nutritional status of staple cereals would have a direct impact in addressing the micronutrient malnutrition in the country.

The Consultative Group for International Agricultural Research (CGIAR) initiated a global challenge programme through HarvestPlus to develop nutrient-dense staple food crops across the world[3]. With the proof of concept provided by HarvestPlus, several organizations in India started research and development projects in the 2000s towards biofortified cereals funded through national and international agencies (http://www.icar-iirr.org/CRP/, https://data.gov.in/dataset-group-name/dbt-research-and-development).

The Indian Council of Agricultural Research (ICAR), New Delhi is playing a major role in ensuring food security for the nation by providing the research thrust for production of food grains (www.icar.org.in). Realizing the necessity of better nutrition in food grains, ICAR started several special programmes on development and popularization of biofortified crops and more than four million hectares is estimated to be under cultivation of biofortified crops in India[4]. The present review summarizes the efforts

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*For correspondence. (e-mail: CN.Neeraja@icar.gov.in) of ICAR in the development and release of biofortified cereal varieties, case studies of efficacy and scaling-up of biofortified varieties across the world, and policy implications for integration of biofortified cereal varieties in the country.

Identification of donor lines for high nutrient content

The primary criterion for the development of nutrient-rich or biofortified varieties is the availability of genetic variability for traits of interest in the germplasm. Using donors from the germplasm such as wild species, landraces and different genomes as in wheat, biofortified cereal varieties with enhanced nutrients can be developed deploying conventional breeding or marker-assisted selection (MAS) methods. For example, wide genetic variability exists for Zn and protein in polished rice. For Fe, though wide genetic variability exists in brown rice, the content is reduced drastically while polishing (milling) the rice[5]. Conventional maize has inferior endosperm protein composition because of low essential amino acids content such as lysine and tryptophan. The discovery of maize mutant *opaque2* (*o2*) with enhanced levels of essential amino acids resulted in the development of diverse, open-pollinated varieties and hybrids in quality protein maize (QPM) background[6]. Natural genetic variation in β-carotene hydroxylase 1 (*crtRB1*) gene is now used as a source for the development of provitamin-A-rich maize through MAS[7,8]. In wheat, wide genetic variability exists for Fe, Zn and protein, and in pearl millet and sorghum large genetic variability for Fe and Zn has been reported, which can be exploited for breeding of biofortified varieties[9,10]. Simultaneously, anti-nutritional factors affecting the bioavailability of minerals like phytates are also being characterized during the development of biofortified cereals[11].

Development of biofortified cereal varieties

For acceptance and adoption by farmers, biofortified varieties should be high-yielding with the desirable quality parameters. Since most of the donor genotypes for nutrient traits are poor yielders, conscious breeding efforts are needed to combine high yield and high nutrient content while developing biofortified cereal varieties. For the phenotyping of biofortified genetic material, a high-throughput estimation system like X-ray fluorescence spectroscopy (XRF) is needed for quick identification of promising lines from breeding materials with increased nutrient content. The HarvestPlus initiative provided XRF equipment to several national and international laboratories in India to facilitate rapid estimation of Fe and Zn. In addition to the strong and active conventional breeding programmes, biotechnological strategies like MAS are also being deployed in the development of biofortified cereals, e.g. maize and wheat[6,7,12].

Evaluation and release of biofortified cereal varieties

ICAR adopts systematic evaluation of breeding materials of various crops through All India Coordinated Research Project (AICRP) networks across the country to identify consistent genotypes for yield along with other traits for the commercial release of varieties (https://www.icar.org.in/content/aicrps-network-projects). This approach based on multi-location testing facilitates rapid generation of breeding lines by developers and identification of appropriate high-yielding varieties, which are then notified as new varieties by the Central Sub-Committee on Crop Standards, Notification and Release of Varieties (CSCCSNRV) for commercial cultivation. The biofortified cereal breeding lines are being evaluated under AICRP through a separate biofortified trial in rice (http://www.aicrip-intranet.in/), integrated with quality trials in wheat (https://www.iiwbr.org/about-aicrps/) and QPM trials in maize (https://iimr.icar.gov.in). For pearl millet, minimum Fe and Zn content was made mandatory for varietal release in India[10]. Since 2013, several biofortified cereal varieties have been released by ICAR through evaluation under AICRP[13,14]. In addition to facilitating the release of biofortified varieties, various initiatives of ICAR for the development and release of bioforitifed cereals through the National Agricultural Research System (NARS) along with international institutions and private organizations to achieve nutritional security of the nation are summarized in this review.

Rice

Rice is the major staple for two-thirds of the Indian population. However, polished rice, the most preferred form for consumption is a poor source of nutrients[15]. The baseline derived from Zn content in polished (milled) rice of popular varieties ranges from <12 to 14 mg kg^{-1}, and even up to 35 mg kg^{-1} has been reported in the rice germplasm[16]. The baseline of protein in polished grains of different varieties widely grown by the farmers is about 7–8% and the range of grain protein content in *indica* germplasm is more than 15% (refs 17, 18). Several donors in rice germplasm have been identified with high Zn and protein content, but ~40% of nutrients is lost during polishing. Conventional breeding efforts deploying the existing genetic variability could increase Zn and protein in polished rice leading to the release of nine biofortified rice varieties through the All India Coordinated Rice Improvement Project (AICRIP) (Table 1). For the biofortified rice varieties to be released under AICRIP in India, either their Zn content should be 24 mg kg^{-1} or their protein content should be ≥10% in polished rice; the yield should be on par with

Table 1. Released biofortified rice varieties with high zinc and protein

Variety	Trait(s) improved		Year of release	Developer
	Zinc (ppm)	Protein (%)		
CR Dhan 310	–	10.3	2016	ICAR-NRRI, Cuttack
DRR Dhan 45	22.6	–	2016	ICAR-IIRR, Hyderabad
DRR Dhan 48	24.0	–	2018	ICAR-IIRR, Hyderabad
DRR Dhan 49	25.2	–	2018	ICAR-IIRR, Hyderabad
Zinco Rice MS	27.4	–	2018	IGKV, Raipur
CR Dhan 311	20.1	10.3	2018	ICAR-NRRI, Cuttack
CR Dhan 315	24.9	–	2020	ICAR-NRRI, Cuttack
CR Dhan 411	–	10.1	2021	ICAR-NRRI, Cuttack
DRR Dhan 63	24.2		2021	ICAR-IIRR, Hyderabad

ICAR-NRRI, ICAR-National Rice Research Institute; ICAR-IIRR, ICAR-Indian Institute of Rice Research; IGKV, Indira Gandhi Krishi Vishwavidyalaya.

yield-check genotype along with the desired cooking quality.

Wheat

Wheat is another major staple food crop with a major role in addressing food security of India. The baseline of nutrients in adapted varieties of wheat is about 32–35 mg kg^{-1} of Fe, 30–32 mg kg^{-1} of Zn and 10.5–11.5% of protein. The benchmark levels for nutrients were set as 45 mg kg^{-1} of Fe, 40 mg kg^{-1} of Zn and 13% of protein[13]. Studies indicated wide variability in landraces and secondary gene pool, viz. two-fold variations in Fe and Zn content among hexaploid wheat genotypes and fourfold in diploid/durum/dicoccum accessions[9,19–21]. A quantitative trait locus (QTL) (*Gpc-B1*) associated with higher protein content was identified in *Triticum dicoccoides*, and transferred into wheat for higher grain protein and mineral content in India[12]. The introgression of resistance of stripe rust genes along with a combination of higher grain protein is also being attempted. Through the AICRP on Wheat and Barley, more than 25 biofortified varieties comprising both bread and durum wheat with either high Fe or Zn or protein, or their combinations, have been released in India (Table 2). In durum wheat, additional trait of yellow pigment content is also taken into consideration.

Maize

Maize can significantly alleviate malnutrition in India with its promising protein quality and provitamin-A, although it is not a major staple food in the country. Traditional maize contains low lysine (1.5–2.0%) and tryptophan (0.3–0.4%) in endosperm protein, while the target level is 3.5–4.0% lysine and 0.8–1.0% tryptophan[13]. A recessive gene *o2* enhances lysine and tryptophan by nearly two-fold[22]. Since the release of the first soft endosperm-based nutritious maize composites in 1970, the first hard-endosperm QPM composite in the late 1990s and the first QPM hybrid in 2000s, several QPM hybrids have been released so far for commercial cultivation in various agro-ecologies of the country[23]. Research efforts have also led the development of four QPM versions of elite commercial hybrids through MAS[6]. Traditional yellow maize contains low levels of provitamin-A (<2.5 mg kg^{-1}) compared to the global target of 15 mg kg^{-1} (ref. 8). Using the favourable allele of *crtRB1*, provitamin-A of parental lines was increased to 15 mg kg^{-1} in freshly harvested grains. Provitamin-A-rich version of QPM hybrids have been developed through MAS (Table 3). Fourteen hybrids of maize which are rich in provitamin-A or with a combination of high lysine, tryptophan and provitamin-A have been released for commercial cultivation in India.

Pearl millet

Biofortified pearl millet is a success story in India with its first released variety ‘Dhanashakti’ for high Fe with the research programme supported by HarvestPlus, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru and AICRP on Pearl Millet. The benchmark levels for optimum Fe (>42 mg kg^{-1}) and Zn (>32 mg kg^{-1}) content were fixed for varietal release in pearl millet through AICRP, and several biofortified varieties have also been released[13] (Table 4). New sources of Fe and Zn content (other than *iniadi*) in the germplasm collection are also being explored for genetic diversification for high Fe and Zn content[24]. The development of biofortified pearl millet hybrids/varieties invariably includes high yield, downy mildew resistance and drought tolerance required for ready adoption by the farmers.

Sorghum

Sorghum is an important staple crop for the resource-poor farmers in the drylands with its better resilience to adverse climatic conditions like heat and drought[25]. Wide variability for grain Fe (12–68 mg kg^{-1}) and Zn (11–44 mg kg^{-1}) was

Table 2. Released biofortified wheat varieties with high iron, zinc and protein

Variety	Trait(s) improved			Year of release	Developer
	Iron (ppm)	Zinc (ppm)	Protein (%)		
WB 02	40.0	42.0	–	2017	ICAR-IIWBR, Karnal
HPBW 01	40.0	40.6	–	2017	PAU, Ludhiana
HI 8759	41.1	42.8	12.0	2017	ICAR-IARI, Regional Station (RS), Indore
HI 1605	43.0	–	13.0	2017	ICAR-IARI, RS, Indore
HD 3171	–	47.1	–	2017	ICAR-IARI, New Delhi
HI 8777 (durum)	48.7	43.6	–	2018	ICAR-IARI, RS, Indore
MACS 4028 (durum)	46.1	40.3	14.7	2018	ARI, Pune
PBW 752	–	–	12.4	2018	PAU, Ludhiana
PBW 757	–	42.3	–	2018	PAU, Ludhiana
DBW 187	43.1	–	–	2018	ICAR-IIWBR, Karnal
DBW 173	40.7	–	12.5	2018	ICAR-IIWBR, Karnal
UAS 375	–	–	13.8	2018	UAS, Dharwad
DDW 47 (durum)	40.1	–	12.7	2020	ICAR-IIWBR, Karnal
PPW 771	–	41.4	–	2020	PAU, Ludhiana
HI 8802 (durum)	–	–	13.0	2020	ICAR-IARI, RS, Indore
HI 8805 (durum)	40.4	–	12.8	2020	ICAR-IARI, RS, Indore
HD 3249	42.5	–	–	2020	ICAR-IARI, New Delhi
MACS 4058 (durum)	39.5	37.8	14.7	2020	ARI, Pune
HD 3298	43.1	–	12.1	2020	ICAR-IARI, New Delhi
HI 1633	41.6	41.1	12.4	2020	ICAR-IARI, RS, Indore
DBW 303	–	–	12.1	2020	ICAR-IIWBR, Karnal
DDW 48 (durum)	–	–	12.1	2020	ICAR-IIWBR, Karnal
DBW 332	–	40.6	12.2	2021	ICAR-IIWBR, Karnal
DBW 327	–	40.6	–	2021	ICAR-IIWBR, Karnal
HI1636	–	40.4	–	2021	ICAR-IARI, RS, Indore
HI 8823 (durum)	–	40.1	12.1	2021	ICAR-IARI, RS, Indore
HUW838	–	41.8	–	2021	BHU, Varanasi
MP(JW)1358	40.6	–	12.1	2021	ZARS, JNKVV, Powarkheda

ICAR-IIWBR, ICAR-Indian Institute of Wheat and Barley Research; PAU, Punjab Agricultural University; ICAR-IARI, ICAR-Indian Agricultural Research Institute; ARI, Agharkar Research Institute; UAS, University of Agricultural Sciences; BHU, Banaras Hindu University; JNKVV, Jawahar Lal Nehru Krishi Viswavidyalaya; ZARS, Zonal Agricultural Research Station.

observed among the public-bred cultivars, parental lines and some selected germplasm accessions along with a positive correlation between grain Fe and Zn[26–28]. Based on available variability and stability and the baseline content of Fe as 25–28 mg kg^{-1} and Zn as 15–18 mg kg^{-1}, the benchmark levels are being proposed for varietal release under AICRP. Donor parents for Fe and Zn have been identified among exotic germplasms, and the poor agronomic background and photosensitive nature of these donors act as major hindrances. Being naturally enriched with more nutrients than rice and wheat, sorghum and millets value chain is also being strengthened for enhanced consumption of these nutricereals through various efforts by the Indian Institute of Millets Research, Hyderabad (https://millets.res.in; https://www.nutrihubiimr.com/).

Bioefficacy studies

Bioavailability of nutrients becomes critical for successful adoption of the biofortified cereal varieties. Efficacy trials are necessary to convince policy makers about the possibility of integration of biofortified varieties into the food system through the laws of the land, and also farmers and consumers for their adoption. Among biofortified cereals, efficacy trials were demonstrated for vitamin-A orange maize and high Zn maize in African countries[29]. For rice and wheat, only a few efficacy trials were conducted using mostly agronomically or post-harvest biofortified products of rice and wheat as a proof of concept in Bangladesh and Pakistan[30–33]. The efficacy studies of Zn-biofortified wheat have shown reduction of maternal and child morbidity in India[34]. Four independent studies showed the impact of consumption of biofortified pearl millet on Fe in the target populations of several states in the country[35–38].

Scaling up biofortified cereal varieties

The introduction of biofortified cereal varieties into the existing cropping systems of India logically should be the replacement of existing popular cereal varieties, for which the biofortified varieties should be nearly similar in yield, quality, duration and agronomic practices. About 70 biofortified cereals have been released in the country for rice, wheat, maize and pearl millet, which are gradually gaining farmers' acceptance[14]. Upscaling the cultivation of these varieties through enhanced quality seed production and

Table 3. Released biofortified maize hybrids with high lysine, tryptophan and provitamin-A

	Trait(s) improved				
Hybrids	Lysine (%)	Tryptophan (%)	Provitamin-A (ppm)	Year of release	Developer
Vivek QPM 9	4.19	0.83			
Pusa HM4 Improved	3.62	0.91	–	2017	ICAR-IARI, New Delhi
Pusa HM8 Improved	4.18	1.06	–	2017	ICAR-IARI, New Delhi
Pusa HM9 Improved	2.97	0.68	–	2017	ICAR-IARI, New Delhi
Pusa Vivek QPM9 Improved	2.67	0.74	8.15	2017	ICAR-IARI, New Delhi
Pusa Vivek Hybrid-27 Improved	–	–	5.49	2020	ICAR-IARI, New Delhi
Pusa HQPM-5 Improved	4.25	0.94	6.77	2020	ICAR-IARI, New Delhi
Pusa HQPM-7 Improved	4.19	0.93	7.10	2020	ICAR-IARI, New Delhi
LQMH-1	3.03	0.73	–	2020	ICAR-IIMR, Ludhiana
LQMH-2	3.04	0.66	–	2020	ICAR-IIMR, Ludhiana
LQMH-3	3.48	0.77	–	2020	ICAR-IIMR, Ludhiana
Malviya Swarna Makka 1	3.89	0.97	–	2021	BHU, Varanasi
Pusa HQPM-1 Improved	4.59	0.85	7.02	2021	ICAR-IARI, New Delhi and CCSHAU, Hisar
Pusa Biofortified Maize Hybrid 1	3.37	0.72	6.6	2021	ICAR-IARI, New Delhi

Other QPM cultivars released in India – composite: Shakti (1971), Rattan (1971), Protina (1971) and Shakti-1 (1997); Hhybrid: Shaktiman-1 (2001), Shaktiman-2 (2004), HQPM-1 (2005), Shaktiman-3 (2006), Shaktiman-4 (2006), HQPM-5 (2007), HQPM-7 (2008), Vivek QPM-9 (2008), HQPM-4 (2010), Pratap QPM Hybrid-1 (2013) and Shaktiman-5 (2013).

ICAR-IIMR, ICAR-Indian Institute of Maize Research; CCSHAU, CCS Haryana Agricultural University.

Table 4. Released biofortified pearl millet open-pollinated varieties and hybrids with high iron and zinc

	Trait(s) improved			
Hybrids/varieties	Fe (ppm)	Zn (ppm)	Year of release	Developer
HHB 299 (hybrid)	73.0	41.0	2017	CCSHAU, Hisar
AHB 1200 Fe (hybrid)	73.0	–	2017	VNMKV, Parbhani
AHB 1269 Fe (hybrid)	91.0	43.0	2018	VNMKV, Parbhani
ABV 04 (OPV)	70.0	63.0	2018	ANGRAU, Ananthapuramu
Phule Mahasakthi (hybrid)	87.0	41.0	2018	MPKV, Dhule
RHB 233 (hybrid)	83.0	46.0	2019	SKNAU, Jobner
RHB 234 (hybrid)	84.0	41.0	2019	SKNAU, Jobner
HHB 311 (hybrid)	83.0	–	2020	CCSHAU, Hisar
HHB 67 Improved 2 (hybrid) with 15.5% protein	54.8	39.6	2021	CCSHAU, Hisar

VNMKV, Vasantrao Naik Marathwada Krishi Vidyapeeth; ANGRAU, Acharya NG Ranga Agricultural University; MPKV, Mahatma Phule Krishi Vidyapeeth; SKNAU, Sri Karan Narendra Agricultural University.

supply, licensing and popularization among farmers without any change in the cropping pattern, area or management practices would strengthen the adoption. The integration of these biofortified crops into the food system can be further strengthened through learning from similar experiences of the other countries, where biofortified crops have already been adopted[39]. Distribution of free, small, trial seed packs, informative and attractive flyers and posters, community radio programmes and social media could assist in the popularization of biofortified cereals. Creation of demand and premium price for biofortified grains are the other driving forces for enhanced adoption of biofortified cultivars by the farmers. Studies on willingness to pay for the biofortified cereals showed the importance of extensive and intensive awareness and promotion of biofortified crops[40].

Case study of biofortified pearl millet varieties in India

Collective efforts of HarvestPlus, ICRISAT and ICAR since 2011 resulted in the release of the first-iron rich pearl millet variety in 2014, followed by many other varieties/hybrids. Involvement of private sector has helped in seed production and dissemination of the varieties/hybrids. The biofortified pearl millet varieties have reached the regular seed system as breeder seeds and seed companies as truthfully labelled seeds. The commercial sale of the seeds has been taken up through standardized size packs with attractive primary packaging and mandatory labelling according to the Seeds Act (1996). The demand was created by demonstrations to farmers, field days and promotions at points of sale. The personnel of private seed companies have been trained in nutrition messaging for biofortified pearl millet. Around 20,000 ha area was reported to be under cultivation of biofortified pearl millet, mostly in Maharashtra and Rajasthan[24].

Multiple ministries of the Indian Government are implementing several food-grain procurement and distribution programmes targeting different sections of the needy population. The integration of biofortified cereals in these programmes can be a part of practical and cost-effective strategies to deliver the essential micronutrients like Zn, Fe and protein to vulnerable communities. Government policy for procurement and supply of biofortified cereal grains is essential for their popularization. Initially, biofortified cereals can be procured by states and distributed to various welfare schemes. This would facilitate the integration of biofortified cereals in the PDS of the state as a first step. The procurement can be initially taken up in the states identified with 184 high-priority districts and 200 high-burden districts for malnutrition (https://niti.gov.in/). Some states have taken the lead in the introduction of nutricereals like sorghum and millets in the PDS (sorghum and finger millet in Karnataka), or in social welfare schemes like Anganwadi Services and Integrated Child Development Services (ICDS) (under the Ministry of Women and Child Development, GoI) on a pilot scale (Odisha, Telangana, Madhya Pradesh), and the mid-day meal scheme (under the Ministry of Education, GoI) (Karnataka, Odisha, Maharashtra, Madhya Pradesh, Andhra Pradesh) at the regional level.

Convergence of various schemes like the National Food Security Mission (NFSM), Rashtriya Krishi Vikas Yojana (RKVY), ICDS and mid-day meal with the participation of the Ministries of Agriculture, Women and Child Development, Education, Rural Development, Panchayati Raj, Food Processing Industries, Consumer Affairs, Food and Public Distribution, Tribal Affairs and Commerce, supported by strong extension and public awareness is essential to take the benefits of biofortification to the next level. With the availability of biofortified cereal varieties released during last five years, the Government has mandated inclusion of these nutrient-rich varieties in the frontline demonstrations organized under NFSM.

Way forward for biofortified cereals to enhance human nutrition

Cereals being the major staple food are ideal targets for biofortification. According to a recent report, there was disproportionate increase in the price of non-cereal food, but that of cereals like rice and wheat was stable during and after lockdown due to the COVID-19 pandemic[41]. Potential impact of Zn supplementation on COVID-19 pathogenesis was suggested to be beneficial for most of the population, especially those with suboptimal Zn status[42]. Most of released biofortified cereal varieties of rice, wheat and pearl millet are high in Zn. Thus, in addition to the several programmes taken up by the government, integration of biofortified varieties into the food system could be one of the significant steps to boost public health. Preliminary studies at the ICMR-National Institute of Nutrition, Hyderabad on the bioavailability of Zn in the biofortified rice variety, DRR Dhan 45 indicated it to be twice that of the control variety, IR64. Bioavailability of carotenoids is proportional to the grain carotenoid content, and biofortified maize (Pro A Hybrid 122 and QPM + Pro-A Hybrid 119) at usual consumption levels can contribute 60–70% recommended dietary allowances of vitamin-A[43]. Coordinated efforts from policy makers and researchers can translate the benefits of the biofortified cereals to address the problem of micronutrient malnutrition in the country.

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