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Strategizing pigeonpea for enhancing health-benefitting traits: A path to nutritional advancements

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ABSTRACT

Nutritional security is the key objective of India's 2030 Vision and UN Sustainable Development Goal 3. Although great progress has achieved towards food security, it is vitally important to solve protein- energy malnutrition and micronutrient deficiencies for millions of people worldwide providing by providing nutrient-rich foods a long-term solution. Pigeonpea is a major daily diet of developing and undeveloped nations covering Asian and African households, and increasing its protein and micronutrient (iron and zinc content) is a feasible approach. Thus, this review focusses on strategizing how pigeonpea should provide nutritional assurance in the coming decade. we primarily summarizes the dietary profile, health advantages, and anti-nutritional factors that hinder pigeonpea. Furthermore, current progress through conventional breeding and molecular tools was comprehensively discussed, while providing strategies to amalgamate advances in transgenics, omics and rapid generation advancement platforms to enhance health-benefitting traits and tackling the anti-nutritional factors contributing potentially towards the nutritional security of pigeonpea food.

1. Introduction

The world population reached 8 billion milestone in November 2022, which presages multiple opportunities and challenges along its way. Though the prospective side displays positive human development, the other side holds the rising effects of malnutrition, hidden hunger, and diverse ailments. Current factsheets of malnutrition, explicit that the world is far from being nutritionally resilient. This imbalanced nourishing syndrome could be broadly categorized into two areas-one with stunting, wasting, and deficiencies and the other with overweight, obesity, and diet-driven illness. The deficiencies of protein and micronutrients holds a concerning contribution to these syndromes.

Protein-energy malnutrition (PEM) has been a hidden epidemic all these years with its spread all over the globe, affecting predominantly children under the age of five and adults over 65 years. The reports show alarming numbers of 149 million children under the age of 5 affected by stunting, 45 million affected by wasting, and 39 million by overweight [1]. Though this issue is around the globe, its prevalence is high around Asian and African regions [2]. Another vast spread and pressing ailment is the micronutrient deficiency. Iron deficiency tops the chart with nearly 1.7 billion people under its effect, majorly children and pregnant women of low and middle-income countries [3].

Addressing this issue projected an immediate global challenge and the United Nations member states in 2015 formed the consortium to share a road map for 17 sustainable development goals (SDG) of which SDG3 emphasizes on nutrition, well-being, child, and maternal mortality by 2030 [4]. Over the years, while the world lurched with COVID-19, conflict, and climate change, there has been a setback in its progress. Navigating these nutritive issues thus requires a multi-sectoral approach of which enhancing the availability of nutrition-rich foods from plants to palate drives towards a sustainable solution.

Importance of availing nutritive foods goes without saying, while it requires a network of macro- and micro-nutrients to blend perfectly in our food plate. Pulses have always been a compatible nutritional partner known for their protein and micronutrient richness. Among the pulses, Pigeonpea [*Cajanus cajan* (L.) Millspaugh] forms a sustainable and economic solution to the low input, resource-poor regions. This Nutri-rich pulse significantly spread over undernourished Asian and African

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regions for its protein-richness, thus, well known as “poor man's meat” [5,6]. It complements the consumer with starch, vitamins, and minerals added to their diet forming the fitting answer in attaining nutritional security. Simultaneously, it's also regarded as a “sustainable savior” for its food, feed, ameliorative, and medicinal properties [6]. From yesteryears, protein balance is maintained by blending the cereals and pulses in the diet due to their complementing amino acid constituents. Mutual quality compensation is closest to the ideal value when the ratio by weight of cereals to legumes is roughly 70:30 [7].

Despite the tremendous potential, its nutritional profile remained underutilized over the years. This sustainable crop could be a potential “change agent” in addressing food and nutritional security. Previous reviews brief regarding the seed quality content in pigeonpea, it's biological assessment techniques, correlation to seed morphological characteristics, progress in breeding for high protein lines while extending up to molecular strategies for enhancing seed protein content [8–10]. There lacks any report combining the macronutrient along with micronutrient and anti-nutritional aspects in pigeonpea. Furthermore, the recent advancements in breeding techniques are yet to be tapped in this crop for the nutritional benefit. Thus, this review highlights the nutritive potential of pigeonpea combining all the quality aspects, providing future strategies and prospects of partnering novel omics, transgenics and rapid generation advancement *via* correlating to other crops. Adding these novel tools to the current breeding progress would be an adjunct in hastening the quality-rich pigeonpea.

2. Pigeonpea seed - a smart packet of nutrition

Pigeonpea seeds are highly nutritious with a balanced amount of protein, fat, carbohydrate, and fiber (**Table-1**). They are also known for their superiority over other pulses in biological value [11]. In general, pigeonpea seeds comprise 85 percent cotyledons, 14 percent seed coats, and less than 1 percent embryo [12]. A significant portion of cotyledons and seed coats hold higher carbohydrates over proteins, while the embryo holds protein [11].

Pigeonpea seeds are notable for their protein share (nearly 22 %), their digestibility and amino acid profile which determines their quality. The protein fractions are classified into four broad categories namely - albumin, globulin (which is further divided into vicilin and legumin fraction), glutelin, and prolamin [13]. Globulin is reported to be the prime protein in the cotyledons accounting for nearly 60 % of the total protein, while prolamin shares the least fraction. Among the amino acid distribution, in analogy with other legumes, pigeonpea seed contains high amounts of lysine, leucine, aspartic acid, glutamic acid, and arginine while being deficient in Sulphur-containing amino acids (methionine and cysteine) [14]. Albumin storage proteins are noted to be high in methionine and cysteine over legumin fraction of globulins. While vicilin proteins are concentrated with methionine and lack cysteine [15]. Consuming a cereal-pulse diet has complemented this imbalance but still, there awaits need for major breeding and biotechnology interventions to develop persistent solutions.

Pigeonpea seeds are rich source of ascorbic acid, folic acid, niacin and Pantothenic acid. The mineral composition of pigeonpea includes phosphorus (367 mg/100g), magnesium (183 mg/100g), iron (5.37 mg/100g), calcium (139 mg/100g), zinc (2.76 mg/100g), manganese and potassium, however low in sodium. High levels of vitamins and minerals render pigeonpea an efficient source to establish global nutritional security [16].

Table 1
Nutritional composition of pigeonpea [14].

Nutrients	Composition
Moisture	11.27
Protein	17.95
a. Albumin (%)	10.20
b. Globulin (%)	59.90
c. Glutelin (%)	17.40
d. Prolamin (%)	3.00
Fat	2.77
Carbohydrate	57.45
Fibre	6.98
Ash	3.58

3. Pigeonpea - a capsulated nutri-medicinal serving

Over the years, pigeonpea has been well regarded for its significant vital elements, including protein, vitamins, minerals, beneficial phytochemicals, and bioactive molecules. Every inch of the plant plays its part in contributing to humankind as a potential medicinal and nutritional source. From the root to shoot, their extracts own anti-bacterial touch with potential wound-healing properties. Leaves contribute to traditional medicine in treating jaundice, smallpox, chicken pox, measles, and sickle cell anemia by maintaining the red blood cell count [6,17].

Seeds are the prime products of the plant, with several utilities alongside the feed. The well-known facts of protein abundance assist individuals' principal growth and development. It also promotes the healing properties and cell regeneration in the body. Complementarily, the seeds also possess a bountiful of vitamins and minerals within. The vitamins such as folate, riboflavin, niacin, and ascorbic acid have a significant role. Folate deficiency creates neural tube defects in unborn children which could be cured with pigeonpea seeds. Vitamin B_2 and B_3 in the seeds promote carbohydrate metabolism and maintain energy levels, preventing lipid deposition in the body [16]. Vitamin C adds to immunity by enhancing the platelet count in the blood. The major mineral shared in the seed is potassium which acts as a vasodilator widening the blood vessels’ muscular walls. As well, the potassium presence counteracts the sodium deposition and thus, blood pressure. These benefits, present pigeonpea as an apt serve for tackling coronary ailments [17].

The seeds possess an added fibre contributing to digestive health which reduces constipation, cramping, and diarrhea. Seed fibre has the property to stabilize the glucose levels in diabetic patients and improve the metabolism rate hence, controlling obesity. The raffinose oligosaccharide composition in the seed contributes to the pre-biotic potential which helps promote the function of gastro-intestinal microbes. They inturn improve the high-density lipoprotein, and curb the low-density lipoprotein [18,19].

The presence of abundant alkaloids and antioxidants adds to the medicinal value of the seeds. Antioxidants such as pinostrobin, cajanin, stilbene acid, vitexin, and orientin are accounted for their anti-cancer properties. Furthermore, they add the hypo-cholesterolemic effect by regulating the cholesterol saturation in the bile juice [20]. Alkaloids of pigeonpea such as *cajanol* extracted from roots have been implicated in inducing stress response and apoptosis in human breast cancer cells [21]. It is an effective herbal remedy for the treatment and prevention of inflammation or associated ailments [22]. Anti-microbial Cajanus lactone is a potential anti-bacterial agent against gram-positive microorganisms [23]. The presence of tannins, flavonoids, and alkaloids in pigeonpea extract has clinically relevant antifungal activity [24]. Gel formulation from pigeonpea extract has wound closure properties, comparable with a band-aid [25]. Pigeonpea also has a hepato-protective function which improves liver and kidney health.

Although the Nutri-medicinal profile of the pigeonpea is profound, the utilization statistics at the consumers' end are limited. Thus, enhancing the nutritional potential within the plant forms a deep-rooted justification. These solutions call for navigating the avenues with integrated breeding strategies.

4. A hitch in nutri-progress: pigeonpea anti-nutritional factors

One major hindrance in expanding the pigeonpea to global plate is the presence of its anti-nutritional factors. The quality of the protein foods is usually ranked based on their protein digestibility, available proportions of requisite amino acids, and nitrogen absorption [26]. Pigeonpea has a considerable proportion of these factors, yet its concentration is minimal compared to other legumes. All these anti-nutritional elements are found to be concentrated primarily around the seed coat with an added thermo-labile nature which has been an advantage in pigeonpea consumption [27–29]. Major antinutrients are oxalate, phytate, tannins, lectins, phenols, saponins, alkaloids, cyanogenic glycosides, and enzyme inhibitors (protease inhibitor, amylase inhibitor, and trypsin inhibitor). Among these, phytic acid, tannins and lectins hold the major share. The concentration of these factors in the currently cultivated pigeonpea appears to be way below the lethal dose minimum aiding in expanding their consumption. Yet their sparse concentrations have considerable effect on the bioavailability of nutrients majorly proteins [30,31]. Phytic acid content in the seed is known to obstruct the iron absorption in the body [32]. Tannins interfere with the protein absorption ability [33]. In pigeonpea, the phytic acid, tannin levels range from 11.7 mg g^{-1} to 3.8–17.1 mg g^{-1} respectively [34,35]. Lectins act as hemagglutinins, coagulating the undigested nitrogen and carbohydrates in the intestine reducing the ingestion ability [36]. Protease inhibitors inhibit the activity of proteolytic enzymes in mammals' GI tracts affecting the protein digestion and absorption [37,38]. Saponin levels in chickpeas, mungbean, and pigeonpea, range from 0.05 to 0.23 % [39]. Their side effects include bloating in ruminants, reduced nutrient absorption, decreased liver cholesterol, overall growth rate, and reduced intestinal absorption of nutrients by binding saponins to small intestine cells [40].

5. Fostering nutrient-rich pigeonpea: strategies and success stories

As India becomes self-sufficient in food security, the next prime focus is nutritional security. India houses 40 % of the population on vegetarian diets, and pulses become their go-to source of nutrients [41]. Pigeonpea consumed as a regular dal, has the upper hand on nutritional demand met by its consumers. The present-day transforming health consciousness has brought a global shift in dietary choices from animal protein to non-meat, vegan or vegetarian and ethical food systems. Diving deep into plant-based diets, protein, and micronutrients-based products top the chart. The advent of innovation has captured the global protein market valued at USD 38 billion (2019) and is expected to grow at a rate of 10.5 % from 2021 to 2028 [42]. The legume-based protein segment is gaining wider acceptance due to its adoption in vegan diets and its ability to reduce cardiovascular disease, diabetes, cell damage, and high blood pressure. Parallelly, the global biofortified market is also growing, valued at 129.85 million$ in 2020 and projected to reach 9.4 % growth i.e., 243.85 million$ by 2027 [43].

To meet the growing nutritional market, breeding for nutrient-rich

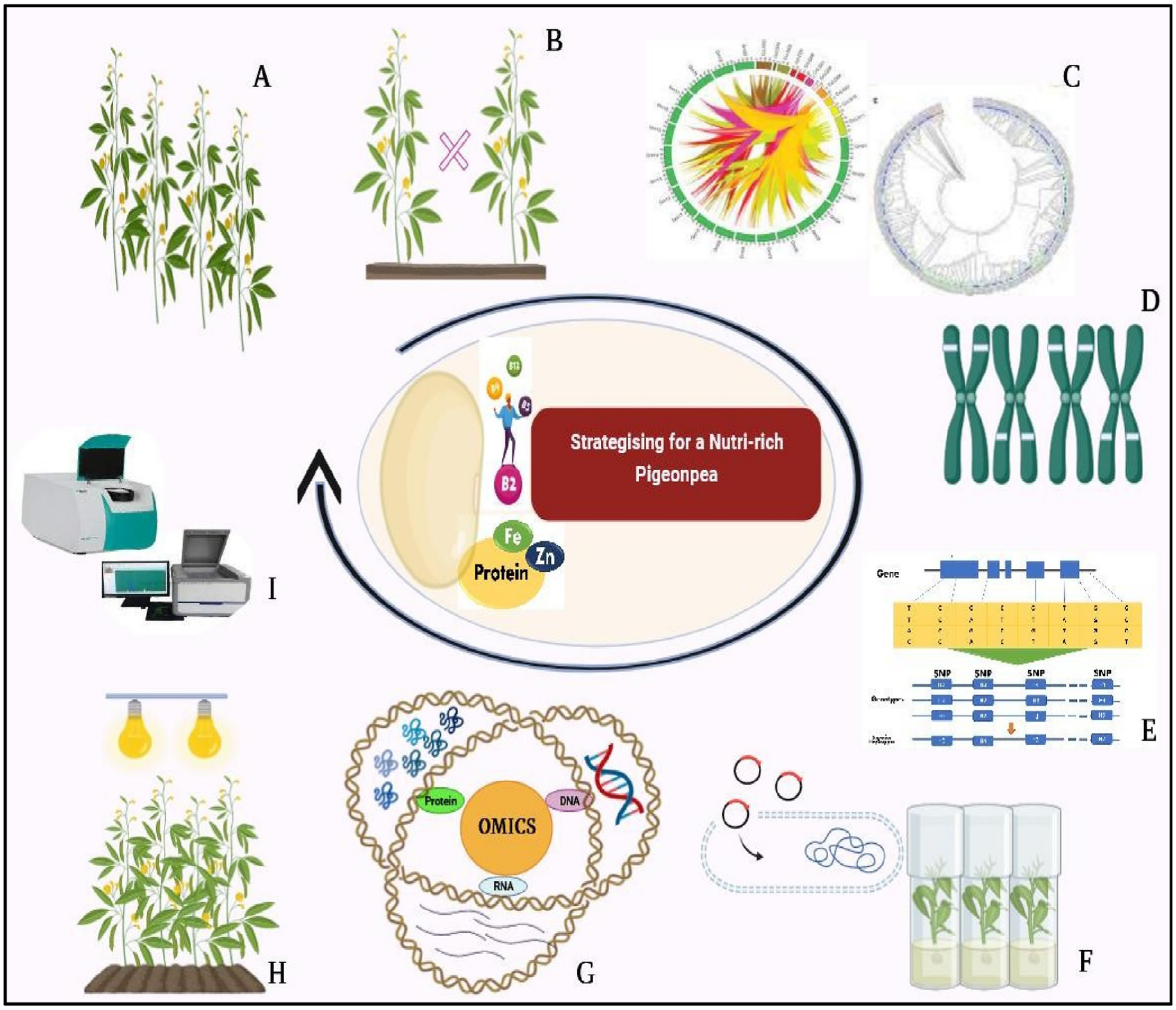

Fig. 1. Strategies for enhancing the health-benefitting traits in Pigeonpea: **A**. Germplasm exploration; **B**. Development of High protein lines (HPL) by hybridization; **C**. Availability of reference genome and Whole genome Resequencing data; **D**. QTL identification in HPLs for SPC; **E**. Identification of superior haplotypes for Haplotype based breeding; **F**. Trangenic approaches; **G**. Omics based advances; **H**. Rapid Generation Advancement Technique; **I**. Phenomic solution with XRF (iron and zinc) and NIRS (SPC).

crops is the prerequisite. A modernized breeding approach with an amalgam of breeding, transgenics, omics and rapid generation advancement is apt for development (**Figure-1**). Harvest plus leads globally in developing and disseminating nutrient-rich crops. The current serving of soya and pea as the source of protein will soon fall short to meet the nutritional demands of the global population, thus pushing the native pulses on forefront. Pigeonpea being the highly produced pulse after, soya, pea, chickpea, and lentil finds its potential to be in the nutritional market.

5.1. Crop nutrition through conventional breeding tools

5.1.1. Breeding for high protein lines

The negative trend of soya protein due to allergens and pea protein not being sufficient to meet global demand has widened the scope for pigeonpea as a protein-rich source. Pigeonpea has good variability for protein content ranging from 14 % to 28 % seed protein content (SPC) in germplasm and wild accessions. Wild protein sources *from Cajanus scarabaeoides, Cajanus albicans* and *Cajanus sericeus* has also been reported [44]. Further, through breeding intervention, High protein lines (HPLs) with SPC of about 25 % were developed at ICRISAT, Patancheru by crossing (wild x cultivated) *C. scarabaeoids* and *C. cajan* cultivated [45].

The SPC in pigeonpea is polygenic in nature. Both additive, as well as non-additive gene action, governs the trait [46]. A study comprising 23 genotypes, majorly involved in developing mapping populations like IL, MAGIC, NAM, and RILs showed high heritability, low GCV, and low genetic gain indicating poor response to selection. It also reported the negative correlation of the trait with seed weight i.e. selection for increased seed weight would result in reduced SPC [45]. On the contrary, selection for increased seed yield would result in decreased SPC [46]. The above results set the necessity to further unravel the genetic basis of the trait. Accordingly, a study based on five F_2 populations (ICP 11605 × ICP 14209, ICP 8863 × ICP 11605, HPL 24 × ICP 11605, ICP 8863 × ICPL 87119, and ICP 5529 × ICP 11605) was conducted to identify the genomic regions associated with the trait. Fourteen major effect QTLs (out of forty-eight QTLs) explaining 23.5 % PV was found to be located on CcLG02, CcLG03, CcLG06, and CcLG11 chromosomes in pigeonpea [46]. The genomic regions identified for SPC paved the way for haplotype-based breeding in pigeonpea at ICRISAT, Patancheru.

In 2006, the Pigeonpea Genomics Initiative (PGI) supported by the Indian Council of Agricultural Research (ICAR) under the Indo-US Agriculture Knowledge Initiative, the first set of molecular markers, mapping populations, and transcriptome assemblies were generated in pigeonpea. A milestone for pigeonpea genomics in 2012 was establishing two consortia - International Initiative for Pigeonpea Genomics (IIPG), led by International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and second led by National Research Centre on Plant Biotechnology (NRCPB) delivered two draft genome assemblies for Asha (ICPL 87119) [47]. This helped in developing high-density genotyping platforms such as genotyping by sequencing (GBS) and 'Axiom®Cajanus SNP Array' in pigeonpea [48]. In 2017, the whole genome resequencing of 292 accessions comprising breeding lines, landraces, and wild species, characterized genome-wide variations in pigeonpea [49].

The availability of whole genome resequencing data in pigeonpea [49] formed the basis for haplotype-based breeding. Identifying haplotypes followed by scouting the superior haplotypes are the pre-requisite of haplotype-based breeding. A haplotype is a combination of alleles for different polymorphisms (SNPs, insertions/deletions) present on the identical chromosomes, which are inherited together with a minimum chance of contemporary recombination [50]. Any individual might have two haplotypes for a stretch of chromosomal DNA, whereas a population might have several haplotypes for the same stretch of a chromosome. The whole genome re-sequencing (WGRS) based association approach would help in the identification of marker-trait association (MTAs) at higher resolution as well as the number of haplotypes identified for MTAs for SPC in pigeonpea. The genomics regions (CcLG02, CcLG03, CcLG06, and CcLG11 chromosomes) identified from previous F_2 population can be utilized to identify haplotypes [46]. Further identification of superior haplotypes can be done using Haplotype and phenotype analysis (haplo-pheno analysis). If the average performance of the group of individuals containing a particular haplotype is significantly higher than the average performance of group of individuals containing the next haplotype, such haplotypes are called superior haplotypes. The genotypes with superior haplotypes can be identified and deployed in breeding. The intricacies of haplotype identification in pigeonpea will soon be published.

5.1.2. Breeding for higher zinc and iron content

Currently, there is growing concern to address nutrient malnutrition through different interventions. Typically, these interventions are categorized into four major groups: pharmaceutical supplementation, industrial fortification, dietary diversification, and biofortification [51]. Biofortification has been developed for alleviating malnutrition problems and its strategy involves developing crop varieties with superior micronutrient qualities. It includes increasing nutrient levels in the edible parts of crops as well as their bioavailability. There is a need to develop biofortified pigeonpea genotypes to increase the quantity, bioavailability and improve their macro- and micro-nutrient profiles.

A range of 25–45 mg/kg of iron and 26–48 mg/kg of zinc is found in cultivated pigeonpea (ICRISAT unpublished data, 2020). The recommended dietary allowance (RDA) of 13 mg per day (children) and 17 mg per day (adult) for iron and 7 mg per day (children) and 12 mg per day (adult) for zinc is necessary. But in India, the pigeonpea consumed as dal or whole seed on an average contributes to a meagre of 14.93 mg, which is less than the RDA [52]. Thus, efforts to enhance iron and zinc content in pigeonpea are underway.

5.2. A tap into transgenics

5.2.1. Harnessing transgenics to enhance seed quality

Conventional plant breeding methods have yet to attain success in enhancing the content of these amino acids without compromising agronomic and food-processing properties [53]. Understanding metabolic pathways associated with increasing essential amino acid (EAA) accumulation in model plants (*Arabidopsis* and tobacco) was a significant trigger for translating this knowledge into increasing EAA levels in legumes. The following table describes different biotechnological approaches to improve essential amino acids in various crops (Table-2). These genes could be further utilized in pigeonpea transgenics to attempt in enhancing the SAA content.

Table 2
Biotechnological approaches for improving essential amino acids in various crops.

S.No	Amino acid	Gene name	Technique	Crop	Reference
1.	Cysteine	CsyE	Translational fusion	Potato	[54]
2.		MhSultr3; 1a	Over-expression	Apple *(Malus domestica)*	[55]
3.		GmOASTL4	Over-expression	Tobacco (*Nicotiana tabacum)*	[56]
4.		AtCGS	Over-expression	Alfalfa (*Medicago sativa* L.)	[57]
5.	Methionine	T-AtCGS	Over-expression	Transgenic tobacco plants	[58]
6.		PrLeg	Transformation	Potato	[59]
7.		Gm2S-1	Over-expression	Soybean (*Glycine max*)	[60]

Plant nutrient and mineral content could be achieved by improving soil mineral uptake in roots or mineral storage within edible plants. As iron and zinc are not synthesized in the plants, they are acquired from the soil rhizosphere and immediate environment [61]. Zinc deficiency in soil affects plant growth, yield in pigeonpea [62].

There are three biotechnological approaches fortifying plants

- Enhancing nutrient storage in plants by introducing appropriate storage proteins (Eg: Ferritin for Fe storage)
- Enhancing uptake and transport of nutrients by over-expression of genes
- Improving bioavailability

Previous studies examined plant ferritins from soybean, tobacco, and lettuce and observed its synthesis is also strictly controlled by the iron status of the cells. Genetically engineered soybean ferritin cDNA was first introduced into tobacco using *Agrobacterium tumefaciens*-mediated transformation [63,64]. Further, soybean ferritin gene *SoyferH1* was overexpressed in rice using the rice endosperm-specific, 1.3-kb *OsGluB1* promoter, which resulted in a 3-fold increase of high iron content (38.1 ± 4.5 μg/g DW) than normal seeds (11.2 ± 0.9 μg/g DW) [61,65]. Constitutive over-expression of *AtNAS1* increased in 6-fold higher iron content in rice (*Oryza sativa* ssp. *Japonica* cv.) [66]. They concluded that AtNAS1 combined with endosperm-specific ferritin expression is an effective method for increasing Fe concentration in polished rice seeds. Contrary to this expressing the *SoyferH1* gene under the control of two endosperm-specific promoters, *OsGlb1*, and *OsGluB1* did not significantly increase up to the expected iron concentration [67].

5.3. Omics-guided crop enhancement in pioneering nutrient-rich pigeonpea varieties

5.3.1. Leveraging omics tools for unlocking nutrient potential

Omics is a collection of modern-day technologies to analyze an extensive and complete data set of a specific class or type of biological molecule in a cell, tissue, organ, or whole organism [68]. These technologies are cutting-edge tools for studying how organisms respond to genetic and environmental changes [69]. They provide a holistic approach in studying systems biology by generating a database with a comprehensive view of the organism's genes, proteins, and metabolites [69,70]. The Omics system comprises four major areas: genomics, transcriptomics, proteomics, and metabolomics [70].

Transcriptomic studies deal with the quantification of the expression of genes in a tissue at a particular stage [71,72]. A comparative transcriptomic analysis investigated the relationship between the β-subunit of soybean seed storage protein and sulphur-containing amino acid composition to improve sulphur-containing amino acids in legumes. The genes involved in cysteine, methionine, and glutathione anabolism were up regulated. The study suggests that an internal regulatory mechanism within the seed might be responsible for seed protein composition rebalance, and sulphur assimilation might be deeply involved in β subunit accumulation [73].

Metabolomics is the comprehensive study of changes in metabolites (<1500 Da) to understand physiology and biochemistry better [74]. In legumes, metabolomic studies focused on identifying primary and secondary stress-responsive metabolites involved in adaptation and sensing mechanisms that contribute to improved nutrition and protein content [75,76]. Metabolomics was used in a few studies to improve sulphur-containing amino acids in soybean [77,78]. They have identified and isolated genes from soybean seeds, such as Gm2S-l protein which contains high levels of methionine and cystine [79]. The omics studies contribute to a better understanding of the genetic mechanisms underlying sulphur-containing amino acid metabolism in legumes and provides insights for molecular marker-assisted selection breeding to improve pigeonpea nutritional quality. Future research combining omics approaches with genome editing tools such as the RNAi system, CRISPR/Cas9, TALENs, and ZFNs could lead to the development of agronomically sustainable plants with improved protein quality [71].

5.4. Catalyzing nutritional progress in pigeonpea: rapid generation advancement

Rapid generation advancement (RGA) is a powerful technology fastening the generation advancement for nutritional progress in pigeonpea. Owing to the crops' photosensitive nature and longer duration, advancing a generation is quite challenging. Traditionally one season per year is the possibility. With the intervention of RGA and single pod descent method two to four seasons are possible annually. It is a combination of modified photoperiod, light intensity, the wavelength of light, nutrient application, and timely crop management resulting in early flowering and podding. The recent standardization of RGA protocol for pigeonpea has leveraged two-four seasons/year [80]. In a high-end RGA facility, about 25000 plants can be advanced at a time, increasing the breeding efficiency as well as genetic gain.

5.5. Bridging the phenotype-genotype loop

The era of genomics in the past few decades came up with revolutionized breeding, offering umpteen opportunities to answer the present-day challenges. However, attaining the bigger picture of nutritional security asks for comprehensive actions to dissect the quality of products further in multiple environments. This challenge needs the looping of genotype assessments in real-world situations. Classical phenotyping methods for seed quality evaluations majorly included low throughput methodologies which necessitate the destruction of the entire tissue for specific results. Additionally, these conventional methods are laborious, time-consuming, and at lower resolutions. These setbacks channelized the pathway for solutions through novel phenomics tools.

Phenomics is the branch of science that allows for non-destructive, multi-dimensional imaging at higher resolutions. It aids attaining precise, accurate results at manifold spatial and temporal resolutions to address the traditional phenotyping bottlenecks. Though the initial attempts to market the high throughput phenotyping platforms (HTTP) occurred nearly a decade ago based on artificial intelligence and robotics platforms, the advancements in the grain quality parameters have been relatively minimal [81]. The major focus sustained with estimating the grain protein content among the macronutrients (protein) and iron (Fe) and zinc (Zn) content within the micronutrient classifications in limited crops.

5.5.1. Seed protein content estimation in pigeonpea

The protein content analysis in pigeonpea still hovers around the chemical-based methods such as the Lowry method, selenium digestion method, and Bradford method while the recent advancement of other legumes and cereals into the Near-infrared (NIR) technology could well be explored. NIR technology works on the principle of lamberts-beer law where the sample upon interacting with the near-infrared wavelength light absorbs, captures, and transmits the radiation. The intensity absorbed could be correlated with the protein content of the sample [82]. This advancement is spreading widely among various legumes while efforts in pigeonpea are underway at ICRISAT. These efforts need to be further validated and standardized for the specific protocol to be used in future breeding.

5.5.2. Micronutrient analysis in pigeonpea

The nutritional evaluation in legumes traditionally used several digestion-based techniques such as atomic absorption spectroscopy (AAS) and inductively coupled plasma mass spectroscopy (ICP–MS). These techniques were off-late replaced with the novel and sophisticated x-ray fluorescence (XRF) spectroscopy technique in pigeonpea. XRF spectroscopy works on the principle of identification and quantification of the elemental source when the source is sufficiently excited with the xrays of right wavelength. The detectors work by capturing the intensity of the dispersed radiation from the seed sample and correlating it with its concentrations to develop a working spectrum for the sample [83]. This technique found success in the pigeonpea due to its precision, accuracy, and ease over traditional procedures.

Caution at harvesting and sampling is a crucial step in pigeonpea. The pods are manually harvested at the maturity stage and the seeds were threshed carefully without any contact with metal containers to avoid contamination, mainly Aluminum. The higher the aluminum, the more misleading will be the reading for iron and zinc. The cleaned seeds are collected in cloth bags and about 30g of seeds of each genotype are drawn for iron and zinc estimation. The iron and zinc content in pigeonpea seeds are measured with Oxford X-supreme 8000 model X-ray Fluorescence analyzer (XRF).

Integrating the phenomics tools into the breeding platforms would require a holistic approach from researchers of multi-disciplines to work along. Further, they must be complemented by joint trials at global locations to attain a non-linear standardized model with greater precision in fewer years. This calls for an integrated quality analysis to develop a standardized model, enhanced utilization, and reliable results.

5.6. *Tackling the food safety concerns: strategizing the seed toxic effects*

Through the years, the anti-nutritional factors (ANFs) have been taken care of with the aid of soaking, processing, and heating strategies due to their detrimental effects on the body. Among the other legumes, pigeonpea encompasses the advantage of minimal toxins. Report suggests a decrease of 50 % of phytic acid concentration upon germination in pigeonpea [84]. While nearly a 54 %, 39 % and 33 % reduction during fermentation, autoclaving and roasting respectively [85]. Tannin range reduced up to 48 %, 52–59 %, 94–98 %, 58.4 % through soaking, germination, dehulling and cooking of pigeonpea seeds respectively [86, 87]. Lectins showed a profound 72 % reduction upon soaking treatment. Heating and acidification were the given long-spread solutions for tackling a few of these factors, however excessive heating also created nutritional imbalance affecting the heat-labile methionine in the seed. Thus, an optimized trade-off is imperative for the balance.

Among all the factors, several protease inhibitors in pigeonpea persist even during the heating process. These factors have also been regarded for their contribution to *H. armigera* resistance by interacting with their gut proteinases in wild species [88]. Furthermore, the seed coat accommodating higher levels of toxins also has a major portion of protein bodies attached which is lost in the process of dehulling. Soaking in distilled water and mixed salt solutions have also been reported to address these toxins [89].

To develop an optimized trade-off between the health effects and the utilities, durable solutions are the need of the hour which include identification and selection of lines with lower quantities of these toxicities [90]. Considerable diversity has been reported with low anti-nutritional factors showing the potential for further breeding [90,91]. Breeding for lower ANFs would include marking of the genes governing them and dissecting the environmental action over it. Reports suggest the effect of temperature and maturity period over the trypsin and chymotrypsin inhibitors which require further validation [92]. Further, conventional breeding techniques could be utilized to bring the lower ANF governing genes into the breeding pipeline. Additionally, gene editing platforms would also provide new avenues for eliminating the genes with optimized protocols in the near future.

6. Conclusion and future prospects

From the green revolution to current-day agriculture, breeding has evolved from time to time. The past 6 decades focused on food security rigorously, however, the upcoming decades' focus is on the nutritional security. Breeding for protein/iron/zinc biofortified crops is the next research niche. Advances in omics platforms add to precision, efficiency, and genetic gain. Pigeonpea being the highest produced and consumed pulse in India, gives ample opportunity to leverage nutritional security if introduced through Public Distribution System. Haplotype-based breeding for enhancing Seed Protein/iron/zinc content and further progression with proteomic and metabolomics studies to understand the molecular basis of the trait of interest is the future line of work. However, standardizing both NIRS and ED XRF protocols for the estimation of protein/iron/zinc content in pigeonpea is the follow-up from phenomics envisaging the vision 2030 UN SDG3.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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CRediT authorship contribution statement

Jwala Pranati: Conceptualization, Writing – original draft, Writing – review & editing. **Vaishnavi Chilakamarri:** Conceptualization, Writing – original draft, Writing – review & editing. **Ashwini Kalyan:** Conceptualization, Writing – original draft, Writing – review & editing. **H.B. Shruthi:** Conceptualization, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing. **Naresh Bomma:** Methodology, Resources, Writing – review & editing. **Kalenahalli Yogendra:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing. **Prakash Gangashetty:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Visualization, Writing – review & editing.

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