J Appl Genetics (2015) 56:151–161
DOI 10.1007/s13353-014-0268-z

PLANT GENETICS • REVIEW

Genetics- and genomics-based interventions for nutritional enhancement of grain legume crops: status and outlook

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Received: 27 August 2014 / Revised: 22 December 2014 / Accepted: 23 December 2014 / Published online: 16 January 2015

Abstract Meeting the food demands and ensuring nutritional security of the ever increasing global population in the face of degrading natural resource base and impending climate change is the biggest challenge of the twenty first century. The consequences of mineral/micronutrient deficiencies or the hidden hunger in the developing world are indeed alarming and need urgent attention. In addressing the problems associated with mineral/micronutrient deficiency, grain legumes as an integral component of the farming systems in the developing world have to play a crucial role. For resource-poor populations, a strategy based on selecting and/or developing grain legume cultivars with grains denser in micronutrients, by biofortification, seems the most appropriate and attractive approach to address the problem. This is evident from the on-going global research efforts on biofortification to provide nutrient-dense grains for use by the poorest of the poor in the developing countries. Towards this end, rapidly growing genomics technologies hold promise to hasten the progress of breeding nutritious legume crops. In conjunction with the myriad of expansions in genomics, advances in other 'omics' technologies particularly plant ionomics or ionome profiling open up novel opportunities to comprehensively examine the elemental composition and mineral networks of an organism in a rapid and cost-effective manner. These emerging technologies would effectively guide the scientific community to enrich the edible parts of grain legumes with bio-available minerals and enhancers/promoters. We believe that the application of these new-generation tools in turn would provide crop-based solutions to hidden hunger worldwide for achieving global nutritional security.

Keywords Biofortification · Genetic diversity · Genomics · Hidden hunger · Legumes · Malnutrition · QTL

Background

The world population is increasing at an alarming rate. The current global population of over seven billion is estimated to cross nine billion by 2050 (Evans 2009; Godfray et al. 2010). The current global population has already reached a point generally considered as the sustainable level (http://www.worldpopulationbalance.org/3_times_sustainable). The distressingly increasing population growth would likely lead towards a remarkably enhanced proportion of people that suffer from protein-calorie undernourishment and nutrient scarcity, more particularly the micronutrient deficiencies (MNDs). The minerals that are indispensable to human health include a wide range of macronutrients [sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), phosphorus (P) and chlorine (Cl)] and micro-nutrients or trace elements [iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), iodine (I), fluorine (F), selenium (Se), molybdenum (Mo), cobalt (Co) and boron (B)] (see Bouis and Welch 2010). In relation to human-health, other terms like micro-nutrient

Communicated by: Andrzej Górny

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malnutrition (MNM) or *hidden hunger* are also used frequently to indicate the intensity of these dietary deficiencies. Currently, more than half of the world population encounters the critical problem of MNM (Bouis 1999, 2000; WHO 2000; Nestel et al. 2006; http://www.fao.org/docrep/x0245e/x0245e01.htm). In particular, the severity is noteworthy in the case of infants, kindergarten children and pregnant women (WHO 2000; Graham et al. 2001; Tulchinsky 2010).

In addition to exerting profound pressure on human health, MNM is likely to have reflective repercussions in the world's economy (Graham et al. 2001; Dwivedi et al. 2012). More importantly, the poorest regions in the developing world are likely to be hit most hard by the burgeoning prevalence of MNM. Even at the present time, a large fraction of the undernourished population inhabits the most disadvantageous regions of the world, especially Southeast Asia and sub-Saharan Africa (Ramakrishnan 2002). For example, the average intake of Ca by the population in the developing world is less than half of that by the populations in the developed nations (Nordin 2000). Apart from vitamin A, I, Fe and Zn deficiencies, which are noted as serious health concerns worldwide in world health report (2000), the deficiencies of other nutrients like folate are also placing global human health at stake (Ramakrishnan 2002; Mayer et al. 2008).

According to Tulchinsky (2010), it is estimated that over 30 % of the global population suffers from Fe deficiency which renders them vulnerable to a range of infectious diseases including malaria (http://www.who.int/nutrition/topics/ida/en/). Similarly, nearly two billion are afflicted with inadequate iodine nutrition. The global scenario describing the proportions of people deficient in other micronutrients also indicates increasing trends towards worldwide occurrence of infectious diseases (http://www.wcs-heal.org/global-challenges/public-health-issues-and-costs/malnutrition/micronutrient-deficiency). Concerning the optimum requirements of essential elements, the recommended dietary allowances (RDAs) pertaining to various proteins/amino acids, vitamins, minerals and trace elements were reviewed comprehensively in the 10th edition (http://www.nap.edu/catalog/1349.html) by the National Research Council (NRC) and Food and Nutrition Board of the United States (see Grusak and Cakmak 2005).

Taking into account the current scenario of global nutritional insecurity, here we attempt to underscore the prospective role of grain legume crops in augmenting nutritional security to global population together with an update on the emerging trends, and scope of genetics- and genomics-based approaches for enriching the grains of food legumes with an emphasis on micronutrients.

Strategies to combat global micronutrient deficiency

The increasing severity of micronutrient-related deficiencies and their grave economic and health implications have received attention from the scientific community worldwide. Therefore, given the global significance of MNM, a millennium summit of the United Nations was held in 2000 that led to the establishment of eight specific goals popularly known as millennium development goals (MDGs). All eight MDGs were set to achieve the relevant specific targets by 2015. Of the eight goals, three goals were exclusively related to MNM that focus on reducing child mortality, eradicating extreme poverty and hunger, and improving maternal health (http://www.un.org/millenniumgoals/bkgd.shtml; see Mayer et al. 2008).

Several prospective remedies have been proposed in order to address the issue of MNM. The potential options to gain a nutritionally-balanced food involve exploring dietary diversity, fortifying processed food, food supplementation and medical food. Examples of the industrial fortification include fortification of common salt with iodine, wheat flour with folates and vegetable oils with vitamins A and D. Similarly, medical food is given under the supervision of physicians, while supplementation of food involves a range of products that contain dietary supplements like vitamins and minerals (Powell 2007; Mayer et al. 2008). However, the modified food derived from the above-mentioned means often remains out of reach of the resource poor and underprivileged as they either lack easy access to the markets/governmental agencies/NGOs or possess insufficient resources/limited income to obtain industrially-processed food (Bouis and Welch 2010; Dwivedi et al. 2012; Hoekenga 2014). In this context, crop-based biofortification offers a more efficient, cost-effective and sustainable means that relies on enriching micronutrient-density in the edible parts of the crops. Conventional breeding and genetic engineering approaches are being applied to enhance the nutritional quality of important staple/specialty crops in various biofortification schemes worldwide (Hoekenga 2014). White and Broadley (2009) advocated that attention should be directed towards not only to enrich the plant parts with the bioavailable nutrients, but also to manage the levels of various promoters and anti-nutritional factors. The bioavailability of mineral/micronutrients is commonly assessed using *Caco-2* cells. Derived from the human colon carcinoma, the astounding property of *Caco-2* cells to undergo differentiation, offers a standard in vitro system to facilitate the transport study of Fe and other mineral nutrients (Sanchez et al. 1996; Ismail 1999; Glahn 2009). A recent study in lentil showed significant potential for biofortification given the variability of Fe concentration and relative bioavailability, as demonstrated with *Caco-2* bioassay (DellaValle et al. 2013). Similarly, analysis of field pea low phytic acid (*lpa*) mutants (1-150-81 and 1-2347-144) with *Caco-2* cell culture method revealed up to 1.9-fold increase in the Fe bioavailability of mutants compared to the normal phytate genotypes (Liu et al. 2014).

Grain legumes for reducing MNM

Grain legumes, owing to their high protein and mineral/micronutrient contents, play an increasingly important role in alleviating MMN especially in the developing world where the dietary needs of people are met principally by the cereal based diets (Graham et al. 2001). The carbohydrate-rich diets supply inadequate quantity of the nutritionally balanced food, thus exacerbating nutritional insecurity (Carvalho and Vasconcelos 2013). Further, legume crops contain a much higher amount of micronutrients compared to cereals (White and Broadley 2009). Therefore, given the enormous socio-economic implications of legume crops in resource poor and marginalized regions, grain legumes have emerged as the most likely candidate crops for biofortification through applying conventional or biotechnological interventions. The remarkable nutritional value of grain legumes was underscored by Iqbal et al. (2006) through exploring amino acid profiles and elemental composition (macronutrients and trace elements) in four major grain legumes viz. chickpea, cowpea, lentil and green pea. The preference for biofortifying legume crops stems from the anatomy of seed structure which is comprised of a thick seed coat covering a well-developed embryo and cotyledons, thereby providing a great scope for "*micronutrient sub-compartmentalization*" (Blair et al. 2013). This is in contrast to the cereals in which a less developed embryo and endosperm are usually surrounded by a thin aleurone layer. In addition, the milling process also takes bran out of the cereals, thus further reducing their nutritional worth. In line with the above-stated view, grain legume such as common bean, which is generally consumed as a whole grain, offers better prospects for implementing crop-biofortification (Islam et al. 2002).

Scientific approaches to resolve the issue of MNM

Enhancing mineral/micronutrient density in the edible portions of food crops could be facilitated by various means. Some of these potential ways are discussed here:

Exploiting the genetic diversity

Accessing the exploitable natural variation available in the crop gene pool for the mineral/micronutrient-of-interest is the simplest way to discover the genotypes that might act as potential donors in the downstream breeding schemes (Dwivedi et al. 2012). Extensive examination of genetic variability was carried out in grain legumes to determine the extent of exploitable range of mineral contents. For example, Beebe et al. (2000) investigated the mineral contents (B, Ca, Cu, Fe, K, Mg, Mn, Na, P, S and Zn) in a comprehensive collection, composed of 1150 accessions (119 wild and 1031 cultivated), and documented the presence of substantial genetic variability for the minerals including micronutrients studied. The Fe content ranged from 34 to 89 ppm (mg/kg grain) in the cultivated collection, while the Zn content varied from 21 to 54 ppm (Beebe et al. 2000). In addition, the associations among important mineral traits like significant positive correlations of S content with Fe and Zn contents open up new avenues for simultaneous improvement of multiple mineral traits. In a similar study, Islam et al. (2002) surveyed the common bean core-collection comprising 1072 accessions at the International Center for Tropical Agriculture (CIAT) genebank, and analysed the elemental composition relative to five nutrient elements (Ca, P, S, Fe and Zn) using inductively coupled plasma-atomic emission spectrometry (ICP-AES). They reported a considerable range in Fe (34.6 to 91.9 mg/kg) and Zn (20.7 to 59.4 mg/kg) concentrations in the germplasm studied. In a following study, Islam et al. (2004) examined 426 accessions (mostly Andean-type) from common bean core-collection; and they observed genetic variation in Fe and Zn content in the introgressed (G1) as well as non-introgressed (G2) groups. The G1 and G2 showed average Fe concentrations of 59.0 and 55.7 mg/kg, respectively; while the average Zn contents were found to be 35.0 and 32.2 mg/kg, respectively. Ultimately, the above-stated studies led to the identification of two promising genotypes, i.e. G14519 and G21242 (http://www.biokemi.org/biozoom/issues/525/articles/2397) with higher mineral/micronutrient contents; and in subsequent research these genotypes were used for further improvement through selection-hybridization-selection scheme.

Substantial natural variation was observed in lentil collection from South-Eastern Turkey comprising 39 landraces and seven cultivars (Karaköy et al. 2012). Similarly, Kumar et al. (2014) observed a wide range of genetic variation for Fe (40–61 mg/kg) and Zn (38–103 mg/kg) contents among 41 lentil genotypes evaluated at three locations, indicating scope for biofortification. A random amplified polymorphic DNA (RAPD)-based diversity analysis was performed on 16 mungbean genotypes, which showed marked variation for Fe and Zn contents (Taunk et al. 2012). Baloch et al. (2014) analysed faba bean germplasm collection (129 landraces and four cultivars) from Turkey, and based on the experimental results, they reported the existence of noticeable variation for seed mineral contents in the sample studied. According to Nair et al. (2013), a reasonable amount of genetic variability for mineral concentrations (0.03–0.06 g kg^{-1} for Fe, and 0.02–0.04 g kg^{-1} for Zn) in mungbean expands the scope of mineral enrichment through efficiently capturing the existing variation. Evaluation of elemental composition was also performed across cowpea cultivars and some other under-researched orphan legume crops including pigeonpea, lima bean grown in Nigeria (Aletor and Aladetimi 1989). A recent study analysed the protein and mineral composition in cowpea using advanced breeding lines (F_6) from six different crosses (Santos and Boiteux 2013). Likewise, while interrogating a panel of 240 germplasm lines in pigeonpea, the best line exhibited almost four times greater grain Zn density than that observed in the line with lowest Zn content. Furthermore, the genotypes with contrasting Zn content were selected to generate an F_2 mapping population comprising 265 individuals (Basavarajeshwari et al. 2014). More recently, Ray et al. (2014) examined the range of variation in micronutrient content across different cultivars in four major pulse crops, i.e. lentil (18), field pea (17), common bean (10) and chickpea (8); and substantial genetic variability was reported for selected micronutrients, indicating opportunity for enhancing nutrient densities in crops through breeding. Similarly, a set of 94 chickpea accessions (23 desi and 71 kabuli types) was screened for Fe and Zn concentrations at two different locations in Canada. Noticeably, the highest average values for Fe (60.1 ppm) and Zn (48.3 ppm) concentrations were observed for a kabuli genotype (CDC Verano) (Diapari et al. 2014).

In conjunction with the mineral contents, emphasis has also been directed towards examining the natural variation relating to inhibitors or promoters that control the absorption of important mineral/micronutrients by the human intestine (White and Broadley 2009). Gupta et al. (2013) recently surveyed folate concentrations across different lentil cultivars, and compared their experimental findings with the results reported for other pulse crops such as pea and chickpea.

Introducing these nutrient-rich novel genotypes to breeding schemes for progressively recovering the superior segregants/recombinants with enhanced nutritional value stands to be the most preferred conventional approach for accelerated nutritional enhancement (Hirschi 2009). The scientific and technological advances in the field of genomics have indeed revealed impressive collections of genomics tools for leguminous crops (Bohra et al. 2014a, b; Varshney et al. 2015). To this end, the declining cost per data points and ever-increasing throughput of next generation sequencing (NGS) platforms paved the way for rapid and cost-effective development of whole genome sequences in various crops. The biotechnological interventions, in particular the omics tools, are an important part of 'HarvestPlus' (Andersson et al. 2014), a global initiative to improve the nutritional status of people (http://www.harvestplus.org/; see Pfeiffer and McClafferty 2007).

As a result of these constantly evolving sequencing systems, draft genome sequences have become available not only for the model species like *Medicago*, and *Lotus* but also for the lesser researched grain legumes like pigeonpea, chickpea, lupin, and more recently mung bean (see Bohra et al. 2014b). Furthermore, the newly introduced NGS assays like genotyping-by-sequencing (GBS) and whole genome resequencing (WGRS) would assist breeders/biotechnologists in the evaluation and exploitation of genome-scale genetic diversity (Poland and Rife 2012). For instance, a genome-wide analysis of the domestication patterns was performed across 17 wild and 14 cultivated soybeans through resequencing at approximately 5× depth (Lam et al. 2010). As a result of genome-wide linkage disequilibrium (LD) analysis, the authors observed long LD blocks on chromosome 10 (associated with nutritional quality) with substantial dissimilarity in nucleotide variation between the wild and cultivated pools. Based on the observation, they proposed the diverse ways whereby selection operated between cultivated and wild soybeans. The whole genome genotyping/sequencing assays are of immense importance particularly relative to mining of the beneficial alleles in a panel of diverse accessions.

Detection of QTLs controlling the mineral concentration/content

As is evident from the continuous distribution pattern, the quantitative nature of mineral content renders these traits amenable to the molecular dissection using quantitative genetics approaches. For instance, Beebe et al. (2000) reported normal distribution patterns for segregation of Fe and Zn contents in a recombinant inbred line (RIL) population derived from the cross G21657×G21078. Likewise, continuous distribution was also reported in common bean by Blair et al. (2009a) while examining Fe and Zn concentrations in a RIL-based population (DOR364×G19833). To locate the precise position of important gene(s)/quantitative trait loci (QTL) in the genome constitutes a *sine qua non* to genomics-assisted biofortification program. A robust DNA marker tightly associated with mineral content/concentration in the edible plant parts will noticeably speed up the progress in developing biofortified crops.

Basically, two QTL mapping methods viz., family-based linkage mapping and association analysis (also known as LD mapping) are implemented to pinpoint the genomic location of gene(s)/QTLs that have measurable effects on the phenotype (Mackay and Powell 2007; Mitchell-Olds 2010; Würschum 2012; Bohra 2013). LD analysis offers procedural advantages over family-based QTL mapping as the former does not require a mapping population (Bergelson and Roux 2010; Korte and Farlow 2013). More importantly, LD analysis enables to assign causative locus to an exceptionally narrow genomic region, i.e. high-resolution mapping (Cavanagh et al. 2008; Rafalski 2010; Würschum 2012; Huang and Han 2014). However, in contrast to linkage mapping, association analysis generally lacks the power to detect rare alleles (Bergelson and Roux 2010). To a large extent, both linkage and LD analyses act in a complementary mode (Mitchell-Olds 2010; Huang and Han 2014). Several attempts have been reported in legumes that aimed to map the QTLs influencing the mineral/micronutrient concentration/content (Table 1).

QTL analysis was undertaken in two model legumes, i.e. *Medicago truncatula* and *Lotus japonicus* to detect the QTLs responsible for enhanced mineral concentrations in seeds. In *Medicago*, a total of 46 significant QTLs influencing mineral concentration were discovered in recombinant inbreds derived from the cross Jemalong-6×DZA 315.16 (Sankaran et al. 2009). Likewise, more than 100 QTLs were recovered from a RIL population (Miyakojima MG-20×Gifu B-129) in *Lotus* (Klein and Grusak 2009). In the case of soybean, a joint linkage map was constructed from three different RIL populations, viz. Williams 82×NKS19-90, Williams 82×DSR-173 and Williams 82×Vinton 81. This joint linkage map was further used to find out the QTLs of interest. As a result, 40 QTLs were declared significant that affected almost 18 different traits related to seed mineral concentrations, total nitrogen: total sulfur (N: S) ratio in seed, cysteine and methionine concentrations (Ramamurthy et al. 2014). Earlier, significant QTLs controlling Ca content in soybean seeds were identified from $F_{2:3}$ and $F_{2:4}$ families (Zhang et al. (2009). Individually, the four QTLs viz. Ca1, Ca2, Ca3 and Ca4 contributed 10.7, 16.3, 14.9, and 9.7 %, respectively to the observed phenotypic variance (PV) (Table 1). The potential of these QTLs in MAS was further confirmed through QTL mapping in two additional mapping populations. Besides validating previously reported QTLs, Orazaly et al. (2014) recently discovered novel QTLs for seed Ca accumulation (designated as Ca5) on soybean chromosome 18.

In common bean, Guzmán-Maldonado et al. (2003) reported QTLs for Fe, Zn and Ca contents in an F_2 population (Table 1). They discovered two QTLs for Ca content; two QTLs for Fe content and one QTL for Zn content, accounting up to 25, 25 and 15 %, respectively of the overall PV. Common clusters of QTLs for Fe/Zn concentrations were observed on bean chromosome 6 and chromosome 11. It is important to note that common bean has also served as an excellent system to understand the inheritance of Fe concentration (Hoekenga 2014). Likewise, Cichy et al. (2009) analysed the variability for seed Fe, Zn, P and phytic acid (PA) concentrations in a RIL population, belonging to the Andean gene pool. Interestingly, in addition to individual QTLs, co-localization of QTLs was noticed for Fe and Zn contents in three linkage groups (LGs), i.e. B1, B6 and B11, which collectively explained up to 40 % PV. In parallel, major QTLs for P were also mapped on six different LGs, accounting for 17–55 % of the total PV. In common bean, Blair et al. (2009a) used a RIL population (DOR 364×G 19833) for QTL discovery, and identified a total of 26 QTLs associated with Fe and Zn content. They employed ICP and atomic absorption spectrometry (AAS) for the estimation of Fe and Zn concentration in common bean seeds. Most of these QTLs were clustered on LG B11, which explained up to 48 % of the total PV for Fe and Zn concentrations. Therefore, the above studies highlighted the potential of this particular LG (B 11) in nutritional genomics of common bean.

Apart from the domesticated forms, enormous potential (often under-estimated) of common bean wild relatives was examined using advanced backcross (AB)-QTL approach. Originally proposed by Tanksley and Nelson (1996), AB-QTL scheme aims to extract the beneficial exotic QTLs, and to incorporate these QTLs into the elite genetic background within a single mapping scheme. Blair and Izquierdo (2012) generated 138 $BC_2F_{3:5}$ individuals through crossing a wild common bean genotype G10022 (higher Fe content) with an elite cultivar Cerinza. The QTLs associated with Fe and Zn contents were mapped on different chromosomes viz., b01, b04 b06, b07, b08, b10 and b11. The QTLs for Fe concentration (Fe7.1) and Fe content (Fe_cont8.1) accounted for 8 and 12 % of the entire PV. On the other hand, the four QTLs were found to be associated with Zn content, i.e. Zn_cont2.1, Zn_cont5.1, Zn_cont5.2, Zn_cont7.1, which individually governed 12, 13, 12 and 10 % variability, respectively. More importantly, common bean AB-QTL population led to the recovery of some introgression lines, which showed higher mineral content (Blair and Izquierdo 2012). Blair et al. (2013) have also reported recovery of nutritionally superior Andean lines from the two backcross populations viz., CAL 96×G14519 and CAL 143×G14519.

As in the case of common bean, QTLs related to minerals were also identified in other grain legumes. Sompong et al. (2012) mapped QTLs having low to moderate effects on levels of phytic acid P (PAP), total P (TP), and inorganic P (IP) in F_2 and $F_{2:3}$ populations both developed from the cross V1725BG×AusTRCF321925. Consequently, seven QTLs were located on five LGs and the PVs were found in the range of 3.43 to 11.24 %. Earlier, Sompong et al. (2010) reported the presence of significant variation for PA content in mungbean seeds by analysing a set of 250 diverse accessions. Also, they investigated the inheritance patterns that underlie the seed phytate content in mungbean F_2 population (V1658BBR×V1141BG), and the results revealed two independent loci interacting with each in a duplicate-type mode (9:7). Similarly, the PAP locus was mapped to LG V in pea albeit in a qualitative fashion, and more importantly, the PAP locus was placed within the genomic region that also harboured a strong QTL (PV=60 %) for Fe bioavailability and eight SNP makers. Co-occurrence of the single nucleotide polymorphism (SNP) markers with both PAP content and Fe bioavailability will enable selection of nutritionally-superior pea genotypes with low PAP content and high Fe bioavailability (Shunmugam et al. 2014).

By applying QTL mapping to a RIL population, Ates et al. (2014) mapped the genetic determinants within the lentil genome that influence the uptake of important micronutrients such as Mn and Zn, and discovered a total of 121 QTLs controlling the micronutrient uptake (Table 1). Apart from linkage analysis, association mapping has also been undertaken in these grain legumes to elucidate the genetic architecture of nutrient

Table 1 Nutritionally important QTLs identified in model and crop legumes

Crop	Trait	Mapping population	Type of population	QTL/nearest marker	PV explained by the QTL	Reference
Common bean	Seed coat Fe	Cerinza×G10022	Backcross	PV-gaat1	8.76	Blair et al. 2013
	Seed coat Zn	Cerinza×G10022	Backcross	PV35	27.4	Blair et al. 2013
	Seed coat Ca	Xana×Cornell 49242	RIL	Ca1 (McatEtc46), Ca7 (P gene), Ca9 (McagEac7)	–	Casañas et al. 2013
	Seed coat Mg	Xana×Cornell 49242	RIL	Mg7xc (P gene)	–	Casañas et al. 2013
	Seed mineral QTL	G21242×G21078	RIL	SSR/RAPD	–	Blair et al. 2011
	Seed Fe content	(Cerinza×(Cerinza×G10022)	Advanced backcross	Fe_cont8.1	12	Blair and Izquierdo 2012
	Seed Zn content	(Cerinza×(Cerinza×G10022)	Advanced backcross	Zn_cont5.1	13	Blair and Izquierdo 2012
	Seed P concentration	AND696×G19833	RIL	ATA4	32	Cichy et al. 2009
	Seed phytic acid %	AND696×G19833	RIL	PVctt1	18	Cichy et al. 2009
	Seed Fe concentration	AND696×G19833	RIL	AGAT05	36	Cichy et al. 2009
	Seed Zn concentration	AND696×G19833	RIL	fin	34	Cichy et al. 2009
	Seed coat Fe and Zn	G21657×G21078	RIL	–	–	Beebe et al. 2000
	Ca content	Bayo Baranda×G 22837	$F_{2:3}$	AFLP	25	Guzmán-Maldonado et al. 2003
	Fe content	Bayo Baranda×G 22837	$F_{2:3}$	AFLP	25	Guzmán-Maldonado et al. 2003
	Zn concentration	Bayo Baranda×G 22837	$F_{2:3}$	AFLP	15	Guzmán-Maldonado et al. 2003
	Fe reductase activity	DOR364×G19833	RIL	Two major QTLs	–	Blair et al. 2010
	Zn concentration	DOR364×G19833	RIL	Zn-ICPb11.2 (K126G)	29	Blair et al. 2009a
	Fe concentration	DOR364×G19833	RIL	Fe-ICPb11.1 (BMd33)	47	Blair et al. 2009a
	Net phytate content (mg/seed)	G2333×G19839	RIL	BM209–BMd40	33	Blair et al. 2009b
	Net P content (mg seed–1)	G2333×G19839	RIL	E070.9–BM46	43	Blair et al. 2009b
Soybean	Seed mineral, cysteine, and methionine concentrations	Williams 82×NKS19-90, Williams 82×DSR-173, Williams 82×Vinton 81	RIL	–	–	Ramamurthy et al. 2014
	Seed Ca content	SS-516×Camp	$F_{2:3}$	SSR (Sat_174-Satt354) (Sat_290-Satt115)	16.3	Zhang et al. 2009
	Seed Ca content	PI 408052 C×PI 407818 B	$F_{2:4}$	SSR	13	Orazaly et al. 2014
		KS4303sp×PI 407818 B	$F_{2:4}$	SSR	–	Orazaly et al. 2014
Medicago truncatula	Seed mineral concentrations	Jemalong-6×DZA 315.16	RIL	46 QTL	–	Sankaran et al. 2009
Lotus japonicus	Seed nutrients	Miyakojima MG-20×Gifu B-129	RIL	103 QTLs (55 markers)	–	Klein and Grusak 2009
	Ferric reductase activity	Miyakojima MG-20×Gifu B-129	RIL	One major QTL	30	Klein et al. 2012
Mung bean	Seed PAP	V1725BG×AusTRCF321925	F_2	SSR	11	Sompong et al. 2012
Lentil	Mn and Zn	CDC-Redberry×ILL7502	RIL	AFLP, SSR, SNP	–	Ates et al. 2014
	Fe uptake	ILL 8006–BM (Barimasur-4)×CDC Milestone	RIL	AFLP/SSR/SNP	–	Aldemir et al. 2014
Field pea	Fe bioavailability	1-2347-144×CDC Meadow	RIL	PAP locus, SNP	60	Shunmugam et al. 2014

accumulation. A genome-wide association analysis was recently performed in chickpea through scanning genomes of 94 diverse accessions with 1129 single nucleotide polymorphism (SNP) markers. Consequently, eight SNPs were found contributing towards Fe and Zn accumulation with individual R^2 or PV values varying between 1 and 7 % (Diapari et al. 2014).

Engineering the relevant biosynthetic pathways using genetic modification

When the sufficient genetic variation is not available or accessible in the crop gene pool, scientists may resort to the technologies that introduce foreign gene(s) through applying a suite of modern biotechnology techniques collectively termed as genetic modification (GM) (Mayer et al. 2008). At the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), efforts were made to enhance the level of β-carotene by introducing maize *phytoene synthase* 1 (*psy1*) gene in groundnut (Bhatnagar et al. 2011). Similarly, transgenic events carrying *Zea mays phytoene synthase* (*Zmpsy*) *1* and *Le lycopene* β-*cyclase* (LCYB) were also developed. In order to engineer the β-carotene biosynthesis pathway, *phytoene synthase* (*psy*) gene from daffodil (*Narcissus pseudonarcissus*) driven by the endosperm-specific promoter was introduced into rice. In addition, *carotene desaturase* (*Crtl*) gene from the bacterium *Erwinia uredovora* was transferred under the control of constitutive 35S promoter which ultimately led to the development of first generation of golden rice (GR 1) (Beyer et al. 2002; http://www.goldenrice.org/Content2-How/how1_sci.php). Significant improvement has been witnessed in the area of carotene biofortification that aims to enrich vitamin A content in plants (Al-Babili and Beyer 2005).

Understanding the genetic architecture and nutritional physiology via mutation approach

Like linkage and LD analyses that associate the observed phenotypic variability to genetic polymorphisms, mutation analysis also intends to uncover the genetic determinants through moving from phenotype (trait-of-interest) to genotype (causative locus) (Schneeber and Weigel 2011; Korte and Farlow 2013). From a nutritional physiology point of view, two mutants viz., bronze (*brz*) and degenerated leaflets (*dgl*) were developed in pea, which accumulated greater quantities of Fe due to defective Fe uptake and hence, demonstrated altered endogenous Fe homeostatic activity (see Wang et al. 2003). Interestingly, the above mutants are serving as a model for developing strategies for improving Fe content not only in closely related legumes, but also in cereals (Grusak 2000). Similarly, given the role of phytate in mineral bioavailability, a recessive monogenic *lpa* mutant (*lpa*-280-10) was isolated from ethyl methane sulfonate (EMS)-mutagenized population derived from common bean line ‘905’. It is important to mention that the *lpa*-280-10 showed almost 90 % reduction in the PA content compared to the progenitor (Campion et al. 2009). More importantly, as frequently observed in the case of other *lpa* mutants, *lpa*-280-10 did not exert any harmful effect on agronomic performance, and additionally this line also exhibited reduced content of raffinosaccharides (up to 25 % reduction). EMS-mutagenesis also permitted the isolation of *lpa* mutants in other grain legumes like field pea (Warkentin et al. 2012) and soybean (Wilcox et al. 2000), and the isolated mutants showed considerably reduced amount of the PA in seeds (see Shunmugam et al. 2014).

Thus, these mutant stocks serve as a global genetic resource for gene discovery together with providing a robust platform for community-oriented research. In recent years, the advances in mutation-detection systems such as targeting induced local lesions in genomes (TILLING) have enabled assigning of molecular function to the candidate gene(s) in large scale (Parry et al. 2009). Also, the multiplexing arrangements using NGS have rendered this mutation-detection assay (NGS-TILLING) more amenable to the users in terms of time, throughput and cost-effectiveness (Marroni et al. 2011; Tsai et al. 2011). The relevance of the rapidly evolving methods particularly ionomics-based profiling that bestow the benefits of fast detection of the mutants, are discussed briefly in the following section.

Plant ionomics: emerging technology to enable comprehensive understanding of mineral transport and gene networks

The new generation phenomics platforms enable the acquisition of precise and accurate phenotyping data within a short period of time, though these assays demand technical skills and a consistent scaling-up in sophisticated analytical tools to derive meaningful inferences from the multi-dimensional data (Cobb et al. 2013). By nature, the new-generation phenomics platforms are usually automated and reasonably less-strenuous, thereby facilitating large-scale phenotypic screening with remarkable accuracy.

With regard to the examination of mineral/micronutrients and their complex networks, ionomics or ionome profiling has emerged as an attractive area that facilitates the genome-scale understanding of dynamics of elemental accumulation in the living systems (Baxter 2010). Salt et al. (2008) have defined ionome as “*The elemental composition of a living system constituting the inorganic sector*”. In the context to ionomics, several techniques driven by the electronic or nuclear property of the element are currently being used to investigate elemental composition in different organisms (Singh et al. 2013). These techniques include AAS, ion beam analysis (IBA), X-ray fluorescence spectroscopy (XRF), ICP mass spectroscopy (ICP-MS) and neutron activation analysis (NAA) among others (Pfeiffer and McClafferty 2007; Djingove et al. 2013; Singh et al. 2013). Recently, the use of ICP-MS allowed comprehensive profiling of ionome in several organisms (see Baxter 2010). In order to better understand the mineral dynamics, Singh et al. (2013) advocated using the combination of sophisticated techniques like laser capture micro-dissection (LCM) and synchrotron-based X-ray fluorescence microscopy (SXRF). In the case of legumes, successful application of ion profiling has been demonstrated in the characterization of mutants in model legume species *L. japonicus* (Chen et al. 2009). By targeting 15 elements, Chen et al. (2009) screened ~2000 EMS-mutagenized lines with ICP-MS, and recovered 31 mutants with elemental profiles significantly deviating from the wild type (MG-20). The ionome datasets relating to various organisms are available at http://www.ionomicshub.org/home/PiiMS.

Perspectives

By virtue of their high protein and mineral contents, nutritional breeding of legumes remains crucial to the improvement of the nutritional status of resource-poor people. Several international collaborative efforts are underway to enrich mineral including micronutrient contents in the edible parts of grain legume crops. Since the nutritional security of the global population depends on the bioavailability of the enhanced nutrient, enriching the crops with mineral nutrients alone will not serve the purpose. To understand the complex mineral network, emphasis needs to be given on implementing a system biology approach for detailed dissection of the biosynthetic pathways that underlie nutrient synthesis, nutrient accumulation and their subsequent transportation to the relevant plant parts. Towards this end, newer technologies especially emerging in the area of ionomics and metabolomics would assist greatly in enhancing knowledge on the mineral/metabolite dynamics, and relevant gene networks (Salt et al. 2008; Baxter 2009, 2010; Fernie and Schauer 2009). Moreover, steadily diminishing cost coupled with increasing access to the new-generation sequencing and genotyping systems generate new prospects to identify the novel genes/QTLs responsible for enhanced mineral content/concentration. In particular, these whole genome oriented strategies allow access to the immense wealth of novel genetic variation that resides within the crop gene pool (Glaszmann et al. 2010).

The GM and other technologies that collectively constitute the reverse genetics approach are particularly suitable when adequate variability does not exist either within the cross-compatible gene pools or carried by such wild ancestors that are reproductively isolated from the domesticated forms with strong hybridization barriers. Further, the GM-based food obtained by altering the mineral/micronutrient biosynthetic pathways is likely to fall within the ambit of intellectual property laws and regulatory/biosafety issues (Delmer 2005; Hirschi 2009; Parry et al. 2009; Mba et al. 2012). Additionally, the quantitative nature (polygenic control) of the majority of the agriculturally important traits advocates the utilization of natural variation over GM technologies (Huang and Han 2014). Therefore, as recommended by Hirschi (2009), the optimization of experimental strategies is imperative ahead of proceeding for the development of nutrient-rich crop genotypes.

Primarily, focus must be on those improvement strategies which rely on harnessing the natural variation available in crop gene pools, and transgenic or GM-based strategy should be adopted only when the improvement is beyond the reach of breeding technologies (Hirschi 2009). The same point-of-view was put forth by various other researchers, which especially concerns implementation of biotechnological interventions in the developing world (Delmer 2005; Pfeiffer and McClafferty 2007). In addition, Hirschi (2009) also suggested taking into concern the concomitant alterations that are manifested in a plant's response to the various stresses while genetically manipulating the anti-nutrient content in food crops. For instance, during investigating the role of calcium oxalate (an anti-nutrient) in model legume *M. truncatula*, Korth et al. (2006) noted an increased incidence of insects in particular the chewing type (*Spodoptera exigua* Hübner) on calcium oxalate-defective mutants (*cod* lines: *cod*5 and *cod*6).

In summary, the tremendous advances in plant nutritional genomics offer readily accessible and cost-effective crop-based solutions to the increasing problems of global nutritional insecurity. We anticipate that these new-generation genomics tools will supplement the conventional nutritional breeding to a greater extent, which would be reflected in the faster availability of the highly nutrient-dense cultivars of grain legumes.

Acknowledgments AB, SK, AKP, US, DS and NPS acknowledge support from Indian Council of Agricultural Research (ICAR), New Delhi, India.

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