Website : www.asthafoundation.in
Frontiers in Crop Improvement
Vol 9 (2) : 85-90 (July-2021) Print ISSN : 2393-8234 Online ISSN : 2454-6011
Astha Foundation, Meerut (U.P.) India

Nutritional Enhancement in Legumes Using Recent Plant Breeding and Biotechnological Approaches

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Abstract

The biggest challenge of the twenty first century is to meet the global demands for food and nutritional security. With the increasing global population, the constant decrease in natural resources and the effects of climate change has made this challenge really difficult. Perhaps the problem of hidden hunger or malnutrition due to micronutrient deficiencies is quite profound globally and needs immediate attention. With respect to micronutrient deficiencies grain legumes that are an integral part of human diets as wells as farming systems play a critical role in addressing the deficiencies associated with nutrients. Focussing more on the rural nutritional security where there are lack of resources selection of nutrient rich grain legumes or developing cultivars which are of good quality and highly enriched with nutritional characteristics is a remarkable approach to deal with the issue of malnutrition. The ongoing research in the field of bio fortification will definitely prove to be an efficient tool in providing nutrient dense legumes to the poorest of the poor. Genomics along with the multi "omics" approaches and next generation genomic sequencing will help the researchers to enrich the edible grain legumes with major bioavailable compounds. Pulses and legumes are important source of plant proteins, dietary fibre, carbohydrates, many bioactive compounds and secondary metabolites.Improving the nutrient content of legumes with the help of genomics, transgenic and recent trends in plant breeding approaches will be a revolutionary step to combat malnutrition. The below literature highlights the various methods used for the quality enhancement of major legumes.

Keywords : *Bio fortification, genomics, next generation sequencing, bioactive compounds, secondary metabolites.*

Introduction

Quality of an edible crop refers to the fitness of the purpose for which it is used. In case of seeds purpose for which the seed meal is used is taken into consideration e.g. as human and animal food, producing value added products etc., but the ultimate use of quality in the case of food crops is the nutritional quality. As the situation of malnutrition is a prevailing condition globally it is very important to enrich our diets with the desired amount of nutrients and also make sure that each of us should have the access to nutrient rich diet only then the condition of malnutrition can be managed. Each year almost 20 million babies are born underweight. It is estimated that 1/3rd of all women in their reproductive age are anaemic and therefore women are also vulnerable to this horrific condition of malnutrition. The cases of malnutrition is more prevalent in infants, children and pregnant women. One of the cause of malnutrition that can be quoted is globalization since now the lifestyle of people have shifted to more usage of packed "ready to use" food items and junk foods which contain a high amount of sugar, salt, fat and most importantly they are nutrient deficient. They have lower amounts of nutrients like protein, fibre, minerals and vitamins. The consumption of such food items ultimately leads to malnutrition and other side effects of improper nutrition. However many of the food items have been fortified to enhance nutrition but they are quite expensive and not affordable by the rural households which are among the majority target groups of malnutrition. Therefore it is very necessary to consume the naturally produced balanced diet. When we talk of the naturally available nutrients then the consumption of pulses and legumes rank in the list.

Cropping systems that include legumes not only improves household food but also enhance the nutritional requirements and improves income security too (1) In addition to enhancing nutritional availability growing legumes also have certain extra benefits like soil nitrogen fixation, adding organic matter into the soil which leads to boosting soil fertility, apart from all these as already mentioned above legume crops increase and intensify small landholder's incomes.

A large part of traditional diet constitutes of pulses all around the world. The word "pulse" was derived from the Latin word "puls" which means a thick slurry. Pulses belong to the legume family, it is likely that all pulses are

Received : July-2021; Revised : July-2021; Accepted : July-2021

legumes but all legumes are not pulses for eg,pulses include chickpeas, lentils, peas etc. which are legumes but other legumes like soybeans, peanuts etc. these are not pulses. Grain legumes have complemented cereals not only in human diet but also for sustainability of agro-systems all over the world. The legumes are called “poor man’s meat” due to their protein content and therefore they are very popular among the poor. The major legumes that have contributed to nutritional and food security in major parts of the world are- Chickpea, Soybean, groundnut, lentil, pigeon pea, faba bean, cowpea and common bean. However this literature will focus on chickpeas, soybeans, mungbeans and pigeonpeas.

Nutrition profile of major Legumes : Legumes are plants that belong to family Leguminosae that produce pods. Leguminosae is a large family that consists of 18000 species of plants. There are more than 1000 species of legumes that are grown worldwide out of which only 20 are cultivated for consumption. However there is an increasing trend of cultivating pulses due to their high protein content, low calorie content and glycaemic index (2). Therefore a diet comprising of pulses as well as cereals is in huge demand these days. The nutritional profile of major legumes and pulses is described individually.

Chickpea : Chickpea is regarded as a good source of carbohydrates and protein which together constitute almost 80% of the total dry seed mass. Chickpea is good for human health as it is cholesterol free and a good source of dietary fibre, vitamins and minerals. (3). The carbohydrate content in chickpea in much more higher than other pulses. It contains all mono, di as well as poly saccharides. The dietary fibre in chickpea is 18-22g/100g of raw chickpea seed. Among all the pulses it is reported that chickpeas have a higher protein bioavailability.

Soybean : Soybean proteins exist as storage proteins in the form of globulins. Soy protein contains low sulphur amino acids. (4). Soybean is also a good source of vitamins and minerals however the vitamin E content depends upon the variety. Bioactive compounds like isoflavones are present in soybean in the form of Daidzen, genistein and glyciten (0.1-0.4% dry weight). Apart from this there are certain anti nutritional factors present in soy protein like saponin. Kunitz inhibitor and Bowman-Birk inhibitor are two types of trypsin inhibitor found in soybean that affect growth adversely and also causes pancreatic hypertrophy. Soybean is also an important oilseed crop as it contains about 20% of oil.

Mungbean : Mungbean is a good source of protein and most number of proteins are storage proteins like globulin (60%) and albumin (25%). (4). Due to the presence of starch in mungbeans it is used to prepare noodles. However unlike soybean, oil content in mungbean is quite low. Bioactive compounds like phenol and flavonoids are found in significant quantity.

Pigeon pea : Among the cultivated legumes pigeon pea has reported lower glycaemic index. (4). Studies on Pigeon pea have reported that most abundant poly unsaturated fatty acid (PUFA) is Linoleic acid. Pigeon pea also contains anti nutritional compounds like phytolectins, polyphenols and trypsin inhibitors.

Since legumes especially pulses and oilseeds are such an important part of human diet due to their nutrition profile and consumption of these by the human population will eventually lead to reduction in malnutrition. Therefore it becomes very necessary to improve legumes in terms of their quality, as growing quality rich pulses and oilseeds will directly affect human health and lifestyle. Many quality traits like micronutrient content (iron, zinc, and calcium), protein content, bioactive compounds, oil content etc. are yet to be explored effectively. Discovery of target specific molecular markers for quality traits has already been done and further studies of exploring different genes is in progress (1). The major plant breeding as well as molecular approaches involved for the enhancement of quality in legumes are enlisted in the figure (Fig.-1) below :

Genomics : An integrated genomic approach that involved QTL mapping, association analysis and differential gene expression profiling has been deployed as an efficient strategy for rapid identification of quality component traits. In a study conducted by (5), they used a similar integrated technique including GWAS to depict functional markers that corresponds to regulate seed Fe and Zn concentrations. The SNP based QTL mapping mapped eight genomic regions containing QTLs that govern Fe and Zn concentrations. Around 24620 SNPs were discovered from genotyping and 13 Fe and Zn gene orthologs were genotyped in a population of 92 chickpea accessions of desi and kabuli types. With the help of GWAS 11 trait associated SNPs that were closely linked to QTLs were analysed. Functional markers from 16 candidate genes and 8 major QTLs regulating Fe and Zn concentrations in seed were identified from this strategy which proves to be a good source for marker assisted breeding for developing iron and zinc bio fortified chickpea crops.

A similar study was performed in soybean seeds by (6) where the genetic loci that were involved in the metabolic pathways of accumulation of minerals in seeds were identified. QTL mapping was performed in 184 RILs (recombinant inbred lines) and 219 cultivars of soybean. Using linkage mapping 35 QTL regulating mineral concentrations were identified. 20 promising lines having

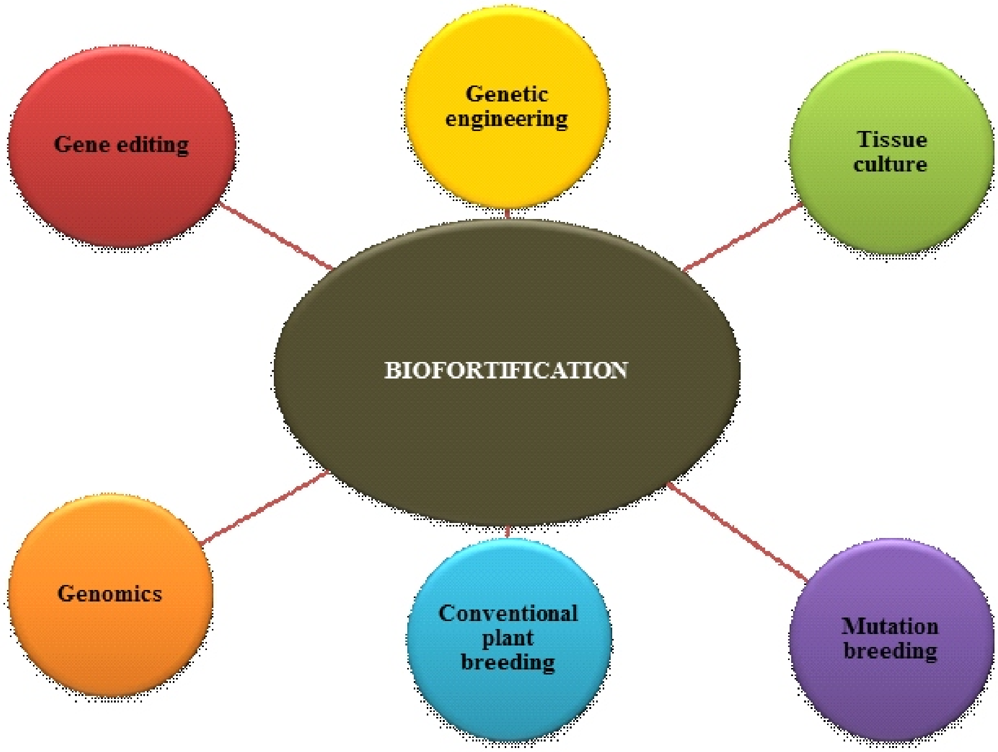

Fig.-1 : Flow chart of different methods used for biofortification.

regions of mineral QTL out of which 3 QTL for seed weight were mapped in RILs 2 QTL for seed weight were co-localized with seed Ca, Zn and P concentration QTL which flanked in the same region. These results again provide the foundation for bio fortification through marker-assisted breeding.

Mungbean is one of the highest protein containing legume among all, but it lacks micronutrient content like Zn and Fe. Mungbean biofortification focusses on reducing this micronutrient malnutrition. With this objective it was reported in a study conducted by (7) where they identified QTLs for seed Zn and Fe content in RILs (recombinant inbred lines) population. It was observed that RILs showed high level of transgressive segregation and genetic variation.17 QTLs 2 for iron and 15 for zinc were mapped on four linkage groups in mungbean. The genomic regions qZn-4-3 and qFe-4-1 on chromosome no. 4 between the markers PVBR82-BM210 and qZn-11-2 and qFe-11-1 on chromosome 11 between markers BM141-BM184 were located on the same chromosomal regions for Zn and Fe concentration which suggested that they can be pleiotropic QTLs or closely linked. The SSR markers that are associated with QTLs for high zinc and iron content can be used to introgress these traits with the help of marker assisted breeding to enhance micronutrient deficiency in mungbean.

Genetic Engineering : Recent advances in transgenic breeding by integrating an alien DNA into crops has become one of the biggest breakthrough in the field of plant breeding. However there are a number of methods used for genetic transformation but out of which the most common ones are the use of *Agrobacterium tumifaciens* mediated or by particle bombardment method. One such research study was performed by (8) where they transformed pigeon pea by introducing *dhdpr-1* gene with a goal to enhance the lysine content in seed protein. This was done with the help of phaseolin or an *Arabidopsis* 2S2 promoter which was developed with the help of *Agrobacterium* transformation and particle bombardment method. The transgenics showed a 2 to 6 fold increased DHDPR-1 activity as compared to the wild type. This gave 1.6-8.5 times increases lysine content in the transgenics. Apart from this the LKR enzyme (lysine ketoglutarate reductase) that is responsible for lysine degradation was also increased in the transgenics during the later stages of seed development and as a result of this lysine increased but also resulted in increased content of threonine (9, 10).

Golden rice is one of the best examples of a biofortified transgenics that was engineered to produce higher levels of â carotene. This was developed by using two encasement of the genes phytoene synthase(Psy) and carotene desaturase (Crtl). "New golden rice" has been reported recently which contains two bicistronic recombinant genes Psy-2A-Crtl(PAC) and Psy-IRES-Crtl(PIC). Out of which the PAC gene proved to be more potent and has enhanced the expression of the two genes

Table-1 : Wild protein donors of Pigeon pea; Source-ICRISAT.

Wild species	Protein %	100-seed weight (g)	Seed colour	Plant type
C. scarabaeoides	28.4	2.3	Dark	Trailing
C. sericeous	29.4	1.9	Dark	Erect
C. albicans	30.5	2.8	Dark	Creeper

responsible for â carotene production. (11) In case of legumes Soybean is considered as world's important crop as it is a good source of oil and protein. In a study conducted by (12) where they transformed soybean plants using Agrobacterium mediated transformation and also the PAC gene discussed above to develop high content of â carotene in soybean. Moreover it was reported that it produced exceeding levels of â carotene in soybean Tran's formants as it produced in rice endosperm using the same gene. The carotenoid biosynthetic pathway was altered so that the recombinant PAC gene could be expressed by taking use of two distinct promoters' â conglycinin for seed expression and CaMV-35S for over expression. The results reported that the seed colour was changed in â PAC gene from yellow to orange while there was no change in 35S-PAC lines. The four orange seeds were selected as â-PAC transgenic lines.

Mutation Breeding : Mutation breeding technique has an important role in crops to induce genetic variability. Chemical mutagens prove to be much potent in terms of inducing mutations as compared to the physical ones. Barshile *et al.* 2009 reported the use effect of mutagens on the protein content and nitrate reducatase activity (NRA) in chickpea. This concept was used by (13) where biochemical analysis was undertaken in 11 chickpea germplasm, viable mutants and 200 micro mutants to estimate the seed protein content. The mutagen used was EMS (ethyl methane sulphonate) 0.05%, 0.10% and 0.15% and sodium azide (0.01%, 0.02% and 0.03%) The seed protein estimation was performed in the M_2 and M_3 generation. This study emphasized that there was increase as well as decrease in the protein content due to mutation. The mutants that were found to have increased seed protein content provide us with valuable germplasm of nutri rich lines of chickpea.

Mutation breeding was also reported in Pigeon pea by (13) where he induced mutagenesis in two pigeon pea genotypes with the help of mutagen EMS and sodium azide. Biometrical analysis was carried out in M_3 generation. 11 viable mutants were screened on the basis of traits like water soluble protein content, carbohydrate content and percentage of nitrogen content from the two genotypes. It was observed that the highest soluble protein was found in early maturing mutant in case of the first genotype where as in the second genotype highest soluble protein was observed in the mutant with three seeded pods. The carbohydrate content was found to be maximum in the dwarf mutants and small pod mutants. The results clearly showed that mutation breeding helped in increasing the protein content.

A similar study that involved QTL mapping along with mutation breeding was illustrated by (13) where a large population of soybean was mutagenized with the help of fast neutrons. The mutagenized population was subjected to screening for total oil and protein content where ten mutants were selected. The seeds of these selected mutants were analysed for oil by GC-MS, gene mapping techniques and protein profiling by SDS-PAGE technology. The oil profile of the mutants with higher protein showed the presence of five fatty acids (palmitic, stearic, oleic, linoleic and linolenic acids) at different proportions in the mutants. The protein profile of the mutants selected exhibited variations in the storage and anti-nutritional factor proteins.

These above investigations indicate that mutation breeding can be considered an important tool for the nutritional improvement of legumes.

Gene Editing : Soybean one of the essential legume crops and a rich source of vegetable oils. Soybean oil with less oleic acid content results in monounsaturated fatty acid which helps in reducing health problems to a large extent. It was proved by researchers that soybean genome contains two identical copies of FAD_{2}-1 and FAD_2-2 that codes for the microsomal 6-desaturase enzyme. (14) attempted a sequence specific mutation of FAD_2-2 gene in soybean with the help of CRISPR-Cas9 technology. The guide RNA sequence was developed which targeted sites of FAD_2-2 loci in soybean. The binary vector that was constructed was successfully transformed into soybean cotyledon with the help of *Agrobacterium tumafaciens*. The results showed high frequent mutation efficiency as 21% out of all transgenic soybean plants were subjected to targeted mutagenesis. Not only this has the fatty acid profiled of the mutant seeds revealed that oleic acid content was increased up to 65.58% whereas the linoleic acid was 16.08%. This study sums up that CRISPR-Cas9 gene editing can help in producing quality rich crops.

Another important secondary metabolite that is present in legumes is isoflavones. It is present in almost all leguminous plants and plays a critical role in plant environment interactions and are also beneficial to human health in different ways. The enzyme that is responsible for isoflavone synthesis is Isoflavone synthase. This enzyme has a common substrate of flavanone-3-

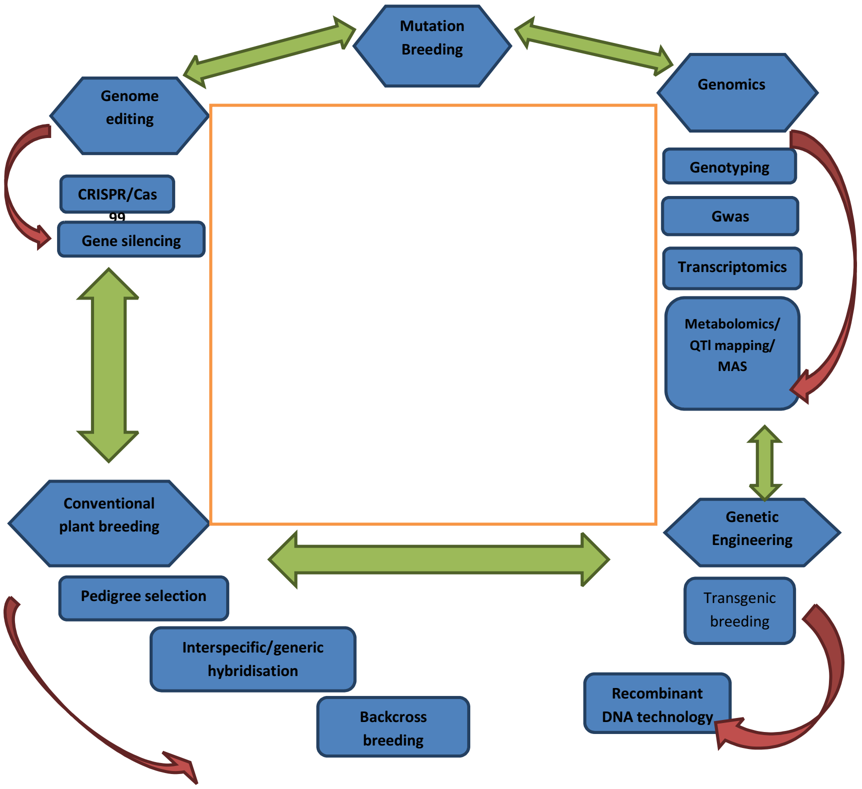

Fig (1)- Illustration of all the interrelated methods responsible for the quality enhancement of legume crops (Green arrows indicate the primary methods and the red arrows indicate the secondary methods)

hydroxylase (F_3H). In a study by (14) gene editing technology CRISPR/Cas9 was demonstrated to target *GMF3H & GMF3H2* in soybean hairy roots and plants. Metabolomics analysis of T_0 mutants revealed improvement in isoflavone content. The mutants had twice the leaf isoflavone content and an additional improvement was seen in the reduction in the soybean mosaic virus coat protein by one-third after infection. These results suggested that enhanced isoflavone content also improves resistance to soybean mosaic virus.

Conventional Breeding Practices

Interspecific hybridisation is one such method in conventional plant breeding where there is exchange of genetic material between two different species. This method was incorporated by (15) with an aim to improve the amino acid and protein content in the mungbean genotypes by crossing mungbean genotypes with mash bean and then screening the recombinant genotypes for quality traits. The majority of the recombinant genotypes exhibited improvement for all amino acids over the parental genotypes. Based on the performance of the recombinant genotypes five were observed as prominent and elite lines that possessed excellent nutritional quality.

Even introgressing desirable traits from the wild species is also an ideal method in conventional plant breeding which was demonstrated by the researchers of ICRISAT where they decided to use wild relatives of pigeon pea to develop high yielding protein rich genotypes of pigeon pea, the reason being that there a ease in crossing the wild ones with the cultivated type (16). The three wild relatives used as protein donors were *C. scarabaeoides, C. sericeous & C. albicans*. The table below describes briefly the three protein donors of pigeon pea.

Before hybridisation the genotypes were screened for protein content and those who exhibited high protein content were used in hybridisation. Pedigree selection was carried out in the F_2 generation keeping in mind protein content to be the criteria of selection. In F_5 generation lines with 28% protein content were observed and subsequently in F_6- F_8 generation lines with 28-32 % protein content were also observed this was a positive sign. The results revealed that the lines had significantly higher protein content than the checks and had high globulin factor (63.5 to 66.2). These lines were also superior as they had greater sulphur containing amino acids. It was suggested that if these lines are multiplied properly they can overcome the problem of rural nutrition.

Conclusions

Nutritional breeding for grain legume crops remains crucial for improving the nutritional security of rural population due to their high protein and mineral content. Several global research efforts are undergoing to enhance the micronutrients present in the grain legumes. More emphasis is required to develop a systems biology approach to dissect the metabolic pathways responsible for nutrient synthesis, accumulation and their transportation to different plant parts. It is very important that all the resources essential for the quality enhancement of crops should be complemented by an international and multidisciplinary approach that can drive the crop improvement in a positive direction and also the research outcomes will directly reach the beneficiary effectively. Enhancing the nutrient profile of legumes by reducing the anti-nutritional factors can be achieved by genetic manipulation by using the natural intra or interspecific variation or by engineering the metabolic pathways. Legumes research entering into the field of genomics accompanying marker assisted breeding and biotechnological approaches gives a new momentum for further application of technologies to boost global production of legumes and pulses.

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