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BIOFORTIFICATION IN PULSES-A NUTRIENTCRAFTING BLESSING IN CURRENT SCENARIO

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BIOFORTIFICATION IN PULSES:

A NUTRIENTCRAFTING BLESSING IN CURRENT SCENARIO

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ABSTRACT

With the extensive use of advanced technologies in agriculture, much anticipated increase in crop productivity and yield have tremendously improved .But even sufficient amount of meal is not able to combat the problems like malnutrition or hidden hunger , which is the main reason initializing various health problems worldwide. This led to the revolutionary idea of **Bio-fortification**, when the same amount of food on table can provide much better nutrition , with the help of various techniques .Thus bio-fortification is increase in nutrient content in a given crop by means of the biotechnology and other techniques. Pulses or grain legumes consists of a group of crops , which are major source of proteins, carbohydrates and minerals. Hence major bio-fortifications applications in pulses cultivation can help combat the problems of suppressed nutrition or hidden hunger especially in Asian and African countries . This article is focused on the techniques, applications and the advances of bio-fortifications in pulses in current scenario .Dr Howarth Bouis is the pioneer of Biofortification under worldwide HARVEST PLUS programme to improve the nutritional accessibility and to ensure global food security. This shift from calorie dense to nutrient balanced diet has become a high priority research area in pulse crops and several improved varieties have been developed and released for consumption.

INTRODUCTION

Pulses are grain legumes with dry seeds as the edible part .Few of them are *cajanus cajan , cicer-arietinum , lens esculenta , pisum sativum*, which were considered as primary grain pulses recognised by United Nations Food and Agriculture Organisation (January 2011).

Belonging to Fabaceae family , they are rich source of plant proteins. Also complex carbohydrates present in them lowers the glycemic index , thus they are absorbed slowly in blood reducing chances of diabetes. In addition to this *phytochemicals ,saponins and tannins* present in pulses have anti carcinogenic effects. International year of pulses in 2016 was declared by FAO to promote its nutritional benefits and inclusion of this super food in major diet regime. Pulses are good source of vitamins like *folate* and their high iron content help in reducing the chances of anemia. Even with all these benefits global problems of malnutrition and hidden hunger cannot be solved just by regular consumption of pulses. But **Bio-fortification can solve this problem to a greater extent.** Many Asian, African and South American countries are emphasizing on increase in nutritional value of pulses in an extensive and cost effective manner.

Pulses are easily cultivable and cheaper ,hence they can be easily availed to economically weaker population. In India Pulses are the major source of protein for poor people and those who follow vegetarian diet only. Thus assimilation of vitamins and minerals like iron , zinc, selenium into the body becomes a major concern. Hence by use of conventional methods for breeding or by genetics or genome based methods and metabolic engineering bio-fortification could enable a leap forward in the concerned area. Bio-fortification helps researchers to provide public health benefits with no side effects and it could be easily supplied and conveyed to rural population when direct supplementation is a lot difficult. Bio-fortification incorporates selected nutrient into pulses or other crops, reducing the requirement of consumption of multiple foods.

Studies of Dr.**Howarth Bouis** at , **International food policy research institute (IFPRI)**, indicated the prime importance of better mineral , vitamin diet rather than high calorie diet for better growth and health. This led to beginning of **HARVEST PLUS** , **a key initiative for bio-fortification**. screening of thousands of varieties of crop seeds in seed banks for higher nutrition content is done with the help of marker assisted selection. Then these varieties are cultivated in target region to be sold in local markets as indicated in figure below. **Major concern** is identification of suitable trait in a pulse variety and development of a new bio-fortified variety by use of different techniques. Not only nutrient retention but its bioavailability is an important factor involved in this process.

This whole process is completed in **three phases**:-

1. **Discovery,**
2. **Development &**
3. **Dissemination**.

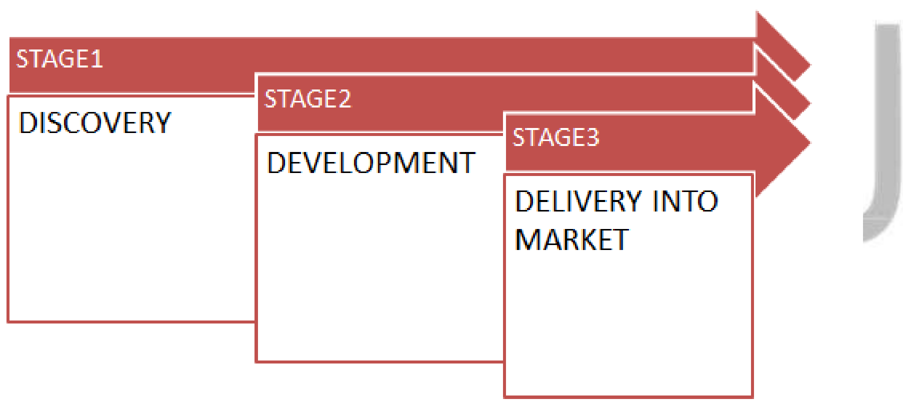


Fig 1 . Stages in pulses biofortification

Discovery stage involves defining desired nutrient level and associated traits along with a system to measure phenotypes or plant performance associated with that trait.

The **development stage** includes target identification, **1**) Setting of nutrient target level and **2**) Germplasm screening and **3**) Discovery of required genes. Second the development stage indicates the level of nutrition retained in target crops and finally the dissemination stage includes the marketing and the consumption of bio-fortified crops in local regions. Although transgenic approach has been much emphasized but conventional breeding of pulses bio-fortification has much greater successs rate and higher stability. But when a particular micronutrient has no occurence in pulses naturally then transgenic approach is much reliable option for bio-fortification. Transgenic approaches can also be used to alter metabolic pathways to increase or redistribute the micronutrient concentration in the edible seeds of pulses. The government organizations of various countries are accepting this technique . This **HARVEST PLUS** programme for development of biofortified crops has been initiated by **Consultative group on international Agricultural research (CGIAR)** and funded by *Bill and Melinda Gates foundation* , United States and by European Union.

Dissemination stage involves official release of biofortified pulse crops in the market and to ensure its acceptability in local markets. The Biofortification priority index (BPI) contributes to closing any sort of information gaps regarding process by generating country –crop-micronutrient specific indices, ranking countries globally , according to the suitability for investment in biofortification interventions. This BPI (Biofortification priority index) is calculated from data available from various sources like Food and Agriculture Organization of the United Nations(FAO), World Health Organization (WHO) and United States Department of Agriculture (USDA).

REVIEW OF LITERATURE

BIOFORTIFICATION: Evidence and lesson learned linking agriculture and nutrition ; by Howarth Bouis , jan low Margeret Mc evan et. al. This publication from second International conference on nutrition focused on the importance of biofortification of crops as an effective means to combat global malnutrition and hidden hunger. Effective strategies were discussed to increase the nutritional content and their bioavailability. Much effort have been placed on techniques for implementing biofortification and breeding and further release into the market of different countries. Various Transgenic projects have been funded by BILL AND MELINDA GATES FOUNDATION.

Increase in bioaccessibility of iron and zinc under different environmental conditions was studied. Finally market development of biofortified crops with efffective strategies , so that a bond between agriculture and nutrition is enhanced .

BIOFORTIFICATION Of pulses and legumes to enhance nutrition by: Shishir kumar and Geetanjali pandey: This article describes the nutrient profile of various pulses and legumes with proteins ,carbohydrates, lipids vitamins ,minerals ,isoflavones, saponins as their main content. To increase the bioavailability of proteins a hybrid variety BC1F2 of mung bean and black gram was obtained with high methionine content , through interspecific breeding. Decreasing the content of antinutrients like phytate to increases the bioavailability of iron was achieved by recombinant DNA technology.

GENOMIC interventions for biofortification of food crops by : Abhishek Bohra et.al. This article emphasizes utilization of new genetic engineering techniques like Genome wide association studies (GWAS) and genomic selection as means of achieving biofortification in pulse crops.

Improving nutrition through biofortification : A review of evidence from Harvest plus ,2003 through 2016 by Howarth Bouis ,Amy Saltzman : Both Howarth Bouis and Amy Saltzman received Global world food prize ,2016 for implementing and scaling up of biofortification of crops with an achievable target to reach one billion people by 2030 .A crop development framework was established and use of transgenic and conventional breeding approaches led to breeding crop improvement. Germplasm screening and development of molecular markers and continuous field trials led to the development of advanced biofortified crops . To shorten the time consumed and faster delivery into the market higher micronutrient variety or fast track varieties are selected. Different case studies in different countries after market release were examined for future references . Regular supply , policy framework and market demand are the three main driving forces to keep research work on biofortification flourishing.

TYPES OF NUTRIENTS PRESENT IN PULSES

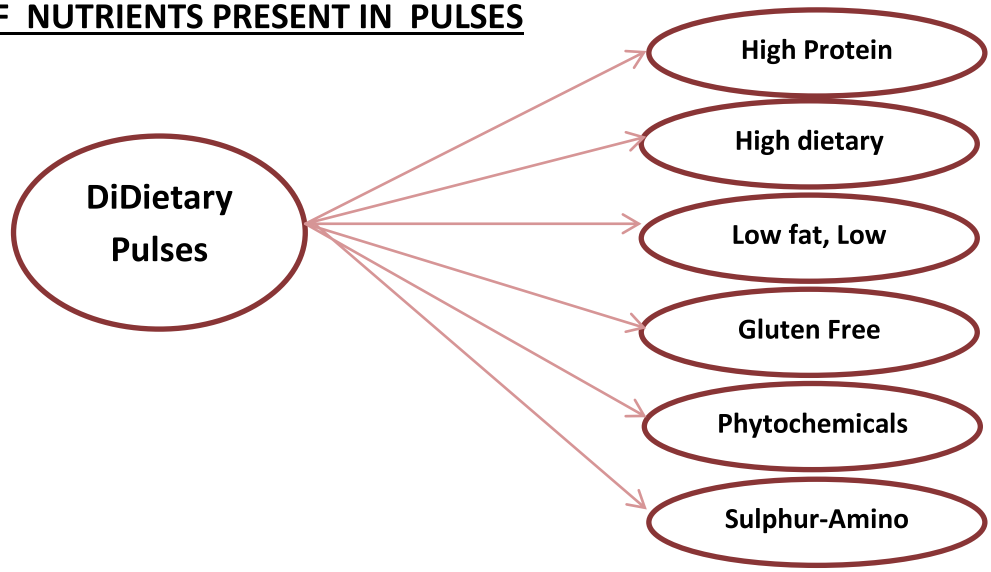


Fig2 Nutritional Composition Of Pulses

NUTRITIONAL COMPOSITION OF PULSES

Pulses are the major source of plant based proteins. They have improved benefits of high protein quality, fibers , carbohydrates and a complimentary essential amino acid profile. With high dose of both soluble and insoluble fiber , they help in losing excess weight ,maintaining lower glycemic index , feeling fuller for a greater time period.

Pulses are gluten free and highly beneficial in autoimmune disorders .Regular Bengal gram contains 17grams per 100 grams of protein ,5.3 grams per 100 grams of fat and high content of minerals and vitamins like riboflavin and thiamine. Although methionine and cysteine are low in content ,it is rich in lysine .The nutritional value can be increased by fermentation and germination. They have high thiamine folate , and healthy fats.

Lentils have 22.3 grams per 100 grams of protein content and 1.7 grams per 100 grams of fat content .

Black gram pulses have 24 grams per 100 grams of proteins .Peas (*Pisum sativum)* contains 22percent proteins mainly albumin and globulins like vicilin, legumin, and convicilin. This provides them high emulsifying and foaming texture.

Vicia faba or faba beans main protein content include albumins and globulins. They have low starch contents but certain antinutrients like tannins ,vicin are also present.

Pigeon peas *(Cajanus cajan*) have high level of proteins and amino acids like methionine, lysine and tryptophan. Carbohydrate content is almost 57 percent. It also contains calcium ,magnesium ,iron and fatty acids. Other components include tannins ,saponins ,resins, reducing sugar and terpenoids along with 2'2' methyl cajanone,2'- hydroxy genistein ,isoflavanones ,cajanin. Components with antibacterial activity like hexadecanoic acids , alpha amyrin,beta sitosterol, pinostrobin ,longistylin A and C are also present. Components with antiplasmodic activity like cajaninstilbene acid,pinostrobin, vitexin, and orientin are also present in significant amount.

Green grams have 31 percent protein content with high lysine and low methionine content. Calcium , phosphorous, and iron present in significant quantity along with linoleic acids , tocopherols, and antioxidants.

BIOFORTIFICATON: TECHNIQUES INVOLVED IN NUTRITION ENHANCEMENT

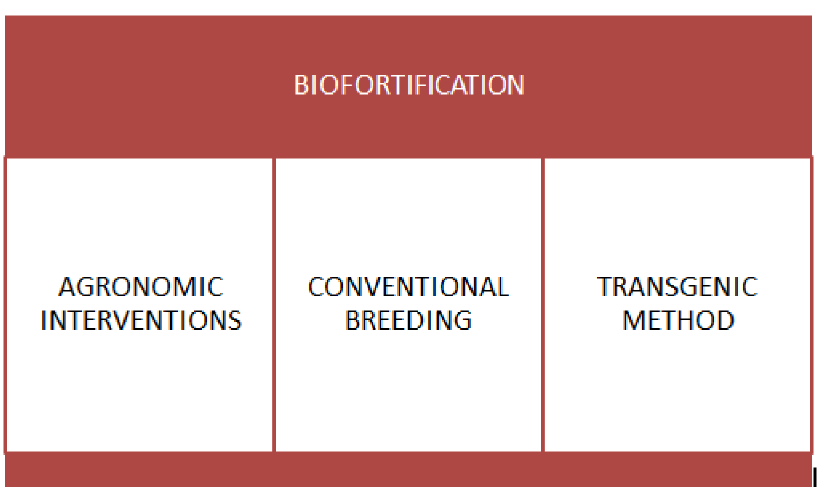


Fig 3. types of techniques involved in pulses biofortification.

Important micronutrients like Iron ,Zinc, Selenium, Iodine, their inclusion and enhancement in the nutritional profile of pulses .

Bio-fortification pathways are mainly categorized into three distinct types :-

1. Transgenic methods
2. Conventional breeding
3. Agronomic interventions

TYPES OF BIOFORTIFICATION

1. TRANSGENIC METHODS

This method has been successfully utilized for development for biofortified Soybean(Glycine max), common beans (Phaseolus vulgaris), lupines (Lupinus angustifolius) and many other pulse crops.

This process might require high investment in the initial stage but it becomes more reliable and sustainable method once a transgenic variety is developed and marketed. This method is still under progress for pulses as far as key micronutrients like iron , zinc , folate are concerned . With the use of advanced technologies like CRISPR/CAS9, (clustered regularly interspaced short palindromic repeats), ZFN(zinc finger nucleases) and TALENs(transcription activator like effector nucleases) various constraints in genomic technologies have been overcome to enhance the nutritional profile of pulses. Also by use of these gene editing technologies desired pathways from bacteria can also be incorporated for exploitation of metabolic engineering.

Availability of fully sequenced genome of pulse crops and have made it possible to use this method for micronutrient enrichment ,restructuring of metabolic pathways, multigene transfer and removal of antinutrients from edible tissues. Although single gene interventions does not affect the yield of target micronutrients in plants ,hence combined approaches are used in which genes encoding the enzyme pathways are modified and overexpressed. Combinatorial nuclear transformation method is also used.

1.1 DIFFERENT TRANSGENIC APPROACHES FOR COMMON BEANS

By introduction of a transgene encoding methionine rich storage albumin from Brazil nuts into common beans (Phaseolus vulgaris), methionine and cysteine enhancement was attempted succesfully.(F.J.L Aragao et .al.1999). This procedure was done by Particle bombardment method and transient expression of 2S - albumin gene from Brazil nuts was observed during the early embryonic stages .Use of biolistic methods have provided successful transgenic lines which was confirmed by western blot and ELISA. Bean plants were transformed by a chimeric construct having double 35SCaMV promoter and AMV enhancer sequence. Almost 23% of increase in methionine content was observed.

1.2 BIO-FORTIFICATION OF LUPINS (*Lupinus angustifolius*)

Almost 90% of increase in methionine content was observed in transgenic Lupin variety when sunflower seed albumin SSA, was stably transformed and expressed with a single tandem insertion of the transferred DNA. *Agrobacterium tumefaciens* mediated transformation with plasmid pBSF16 was done. A three gene construct containing *bar, ssa* and *uid A* genes was made with these genes coding for phosphinithricin, sunflower seed albumin ,and beta- glucourinidase (GUS) respectively. The chimeric *ssa* gene was excised with EcoR1 and inserted into EcoR1 site of binary vector pTAB16 to create pBSF16 . This multigene construct was then transfered to lupins. SDS –PAGE and immunoblotting technique confirmed the homozygosity of *ssa* gene in transgenic lines . A substantial amount of change in methionine and cysteine level was observed ,with increase in methionine content by 94 % and reduction in cysteine content by 12%. (Molvig L ,Tabe , Moore AE,USA 1997)

1.3 BENEFITS OF TRANSGENIC APPROACH

This method overcome various limitations like limited genetic variations in plant varieties and it is independent of their evolutionary and taxonomic status. Metabolic engineering can be effectively exploited for nutrient profile improvement in pulses. Thus transgenic pulses like lupins and common beans hold potential to reduce micronutrient malnutrition in consumers . Similar genetic modifications have been utilized in lentils for bio-fortifications in iron and zinc content in lentil pulses.(Dil Thavarajah et al 2009) . Low phytic acid lines have been potentially developed in pulses to improve mineral absorption by reducing phytate concentration. In common beans these *lpa* mutant lines have been developed to improve iron level .(Panzeri et al.) It was observed that defective *mrp1* gene for multidrug resistance associated protein (MRP), leads to *lpa1* mutation in common beans downregulating phytic acid pathway and increasing iron bioavailability.

1.4 CONSTRAINTS OF TRANSGENIC APPROACHES

Due to lesser acceptance in local markets transgenic varieties is available in limited range. Also initial stages in the development of a transgenic variety is expensive and requires many years of research for accomplishment.

2. CONVENTIONAL BREEDING METHOD FOR PULSES BIO-FORTIFICATION

Conventional breeding in one of the most cost effective and reliable method to increase the micronutrients concentration in pulses .Due to lesser acceptance of genetically modified pulse crops and very less success in transgenic approaches plant breeding has been under extensive application in current scenario. parental lines with better nutrient profile is effectively screened and utilized for the development of molecular markers for marker assisted selection in pulses. Due to availability of wide gene pool and recombinant inbred lines conventional breeding is successful in pulses .

2.1 EXAMPLES OF BIOFORTIFIED PULSES BY PLANT BREEDING

In an attempt for bio-fortification of iron and zinc content ,HARVEST PLUS program have utilized genetic diversity available in gene banks for lentils.

(The International center for agriculture research in dry areas).(2015). various lentil varieties like *Barimasur* , *ILL7723,Khajurah, L4704 ,Pusa vaibhav* with high iron and zinc content have been launched in market in countries like Nepal ,Bangladesh ,Ethiopia.

In India ,a variety developed by GB Pant university and HARVEST PLUS, *Pant Lobia* with high iron density in cow pea is another example of biofortified pulses through plant breeding. In study conducted by consultative group on international agricultural research (CGIAR), it was observed during iron and zinc bio-fortification in *Phaseolus vulgaris* , that increase in concentration of one mineral can enhance the range of other micronutrient , thus almost doubling their bioavailability

Recombinant inbred lines of common beans were used for 80% increase in iron concentration and 50% increase in zinc concentration was observed. Various iron and zinc biofortified varieties of common beans have been launched in Rwanda, Congo and many Asian countries .

2.2 CONSTRAINTS IN PLANT BREEDING METHOD FOR BIO-FORTIFICATION

Sometimes the genetic variability in gene pool for various pulses is not sufficient in terms of micronutrient concentration. Also this is a much time taking process as development of desired nutrient profile in a crop require many generations of cultivation. This method cannot be utilized for highly specific traits inclusion in a new variety. Sometimes the presence and the effects of different antinutrients (like phytate, tannins, oxalate, hemaglutinis) cannot be effectively screened as they hamper the process of micronutrient bioavailability.(Pfeiffer WH et al 2007, Bouis HE ,2000).

3. AGRONOMIC INTERVENTIONS

Agronomic bio-fortification involves method of direct physical application of micronutrients mostly in form of chemical fertilizers to improve the nutritional profile of various pulses. This is accomplished mainly by three ways:- addition of mineral fertilizers to growth medium like soil, direct applications to leaves called as foliar application and by microbial intervention. This is cost effective and hassle free method with specific applications of required micronutrients.

Plant breeding and agronomic method for bio-fortification can be complementary to each other. Addition or spray of fertilizers have primary role in root and shoot development, thereby increasing the surface area for mineral absorption. On the other hand pulses can absorb many soluble compounds and gases through leaves ,hence it is the most effective and fastest way of increasing nutrients density in pulses.

3.1 APPLICATION OF MINERAL FERTILIZERS TO SOIL

Soil being the growth medium can play a crucial role in uptake of desired micronutrients when mineral fertilizers are applied for bio-fortification. The mobility of nutrients from soil into plant cell and their solubility are major factors affecting this process. Important micronutrients like selenium, iodine and zinc can be easily added in form of mineral fertilizers, as they have high mobility .

3.1.1 SELENIUM BIO-FORTIFICATION

Selenium can be increased in concentration by direct application of fertilizers along with sodium selenate. This is due to greater mobility of selenite ions which increases their bioavailability in pulses like chick pea and common beans.

3.1.2 ZINC BIO-FORTIFICATION

Zinc is applied in form of Zinc fertilizers in cow peas and common beans to increase its concentration. The bioavailability of zinc is in direct correlation with iron concentration and it plays a major role in protein synthesis. It was stated that maximum zinc concentration was obtained in chick peas when 25kg per hectare of zinc sulphate was applied to the soil.(Hidoto et al). Besides this, seeds with high zinc concentration have greater biotic and abiotic stress resistance. Other sources of zinc are ZnO and zinc oxysulphate. Their assimilation in plants varies in pulses due to difference in zinc sensitivity. Application of zinc sulphate heptahydrate improves uptake and bioavailability of zinc ,iron and phosphorous along with increased nodulation and high yield in chickpea.(Shivay et al2014). Also various Mung beans varieties like *Samrat* and *Narendra moong 1* showed greater yield in response to zinc application. Lentils and Field peas show greater increase in zinc concentration due to soil application. But their uptake is controlled by environmental conditions and genotypic variations among different varieties of pulses. Maqsood et al in Canada proved that when 5kg per hectare of zinc soil application was done on Melfort soil association ,almost 72 mg of zinc per kilogram of small red lentil was obtained.

3.1.3 IRON BIO-FORTIFICATION

Due to lesser mobility iron bio-fortification is bit difficult as compared to zinc and selenium. But direct application of ferrous sulphate increased almost 29.4 % of iron content in chick peas and cow peas.

3.2 FOLIAR APPLICATION METHOD

3.2.1 FOLIAR APPLICATION FOR BIO-FORTIFICATION

When the translocation of minerals to the edible parts of the plants is difficult or when their bioavailability in the soil is limited then direct application of mineral fertilizers to the leaves is another efficient method to increase its concentration. In pulses various cases have proved this method to be more successful when used in combination with soil application. Since most of the pulses are grown in dryland or rainfed conditions ,foliar applications is of greater benefit for them. mostly zinc ,iron or urea is applied in spray form. Direct applications of foliar nutrients make their absorption in leaf epidermal layer much easier .They are then translocated to other parts of the plants through phloem. Addition of lime in the spray helps in greater translocation .(Zhang et al 2010) .Few examples are discussed below:-

- **Cicer arietinum (Chick Pea)** :Bio-fortification was performed by foliar application of Zn -EDTA (Zinc ethylene diamine tetra acetic acid) at different stages (at active vegetative stage ,flowering and grain filling stages) .*(Poblaciones MJ et al ,2014)*

- **Pisum sativum (Sweet Pea)** :Bio-fortification of sweet pea was conducted by foliar application of zinc sulphate 0.25% or 0.5% (w/v) before flowering and early grain stage in combination with the soil application of zinc sulphate.*(Poblaciones MJ, RengelZ ,2016)*

- **Phaseolus Vulgaris (Common Beans)**: Biofortified by zinc foliar spray along with humic acid or chitosan during reproductive growth phase in Egypt *(E A Ibrahim et al 2015)*

- Urea solution when applied through leaves ,have reported increase in yield and chlorophyll content in chickpea *(Verma et al*)

3.2.2 FACTORS AFFECTING FOLIAR BIO-FORTIFICATION

When mineral nutrients are directly applied on the leaves their transport in plant cells is not uniform. Downward transport of nutrients from leaves to stem takes place via phloem only. So the variations in rate of transport is affected by types of fertilizers, time of application, stages of growth of plants during application and concentration of solution applied. Mostly chelates are considered as a better form eg Zn-EDTA.(Pearson et al 1995).

3.3 MICROBIAL INTERVENTIONS

Also called as PGP (plant growth promoting organisms) ,various microorganisms like Pseudomonas monteilii , Acenatobactor tandoii, Brevibacterium antiquum ,Enetrobacter ludwigi can be utilized as an effective tool for bio-fortification. These plant growth promoting microbes commonly found in soil play an extensive role in plant growth by producing secondary metabolites which are required by plants. They also play an important role in uptake of mineral nutrients especially Zinc and iron. Siderophores ,organic acids and exopolysaccharides produced by PGP increases the bioavailability of minerals at root soil interface and enhances further translocation. Various PGP have been studied for role of bio-fortification in chickpeas and pigeon pea at ICRISAT (Patencheru) Subramaniam Gopalakrishnan et al. The seeds of chick pea and pigeon pea were treated with bacterial strains for almost 40 minutes and then immmediately sown . Until flowering stage plants were inoculated with these bacteria . After harvesting seeds were observed to have greater yield , greater pod yield , seed weight and pod number with greater increase in micronutrient level . Almost 12% increase in iron content , 5% increase in Zn ,8% increase in copper ,39% increase in Mn and upto 11% increase in calcium level was observed.

MECHANISM OF MICROBIAL ACTION IN BIO-FORTIFICATION : COMPLEX INTERACTION BETWEEN PLANTS AND MICROBES HELPS IN BIO-FORTIFICATION

Mechanism involves Auxin (Indole acetic acid), siderophores , phosphorous solubilization ,antibiosis, competition to antagonize phytopathogen, antifungal metabolites and fluorescent pigments. Siderophores are low molecular weight iron binding compounds which bind with Fe^{3+} and converts it to easily absorbable form to be utilized by plants.

These bacterial strains are well adapted in pH 5 to 13 and survive temperature conditions ideal for both chick pea and pigeon pea .Siderophores are believed to increase mineral mobility by acting as solubilizing agent for iron under iron limitation conditions. Also root system traits are enhanced with increase in root length ,weight and volume thus increasing the level of mineral uptake by plants. Mycorrhizal association is also shown to improve iron, selenium and zinc concentration in various legumes and pulses.

OTHER METHODS FOR PULSES BIOFORTIFICATION

To improve Zn concentration in *Moong Beans (Vigna radiata)* another method called seed priming and seed coating was also used improving seed capacity .Seed priming is a technique in which germination starts but radical does not emerge(Nawaz et al 2013). This induces early growth and better crop standing especially in legumes and pulses (Musa et al 2001) . In two types of *Moong Beans* genotypes (NM-92 and NM-2006) combination method of osmopriming of seeds ,soil application and foliar application was used using zinc sulphate hepta hydrate ($ZnSO_4.H_2O$) in **Nuclear Institute of Agriculture and Biology (NIAB) PAKISTAN.**

CHALLENGES IN BIO-FORTIFICATION OF PULSES

Although process of pulses bio-fortification have been successful in many areas due to adaptation of all above mentioned strategies but there are few challenges hampering the process .

Naturally occurring antinutrients present in the plants reduces the bioavailability of micronutrients. Biel et al conformed presence of phytic acid which reduces bioavailability of iron and zinc in legumes and pulses. Other inhibitors present are saponins , lathyrogens , protease inhibitors and alpha amylase inhibitors , present in legumes, presence of polyphenols also hamper iron uptake in black beans seed coat.

Narrow genetic variations in plant gene pool and longer time required for research and cultivation pose another challenge to bio-fortification. Pulse cultivation has been considered to be cashless crop and many farmer have switched to other crops due to poor marketing strategy and lesser acceptability of transgenic pulses . Bioitic and abiotic stress also affect yield and alter the nutritional profile of harvested pulses.

Post harvesting processes also affect the nutritional profile ,as milling and polishing removes various micronutrients To overcome these problems collaboration between plant breeders , nutritionist ,genetic engineers and marketing specialist is important .Expansion of germplasm conservation for better characterization and screening of pulses varieties is required for both conventional and genetic engineering method. Single loci multinutrient trait helps in qualitative improvement of pulses.

;-METHODOLOGY FOR TRANSGENIC APPROACH FOR BIOFORTIFICATION IN PULSE

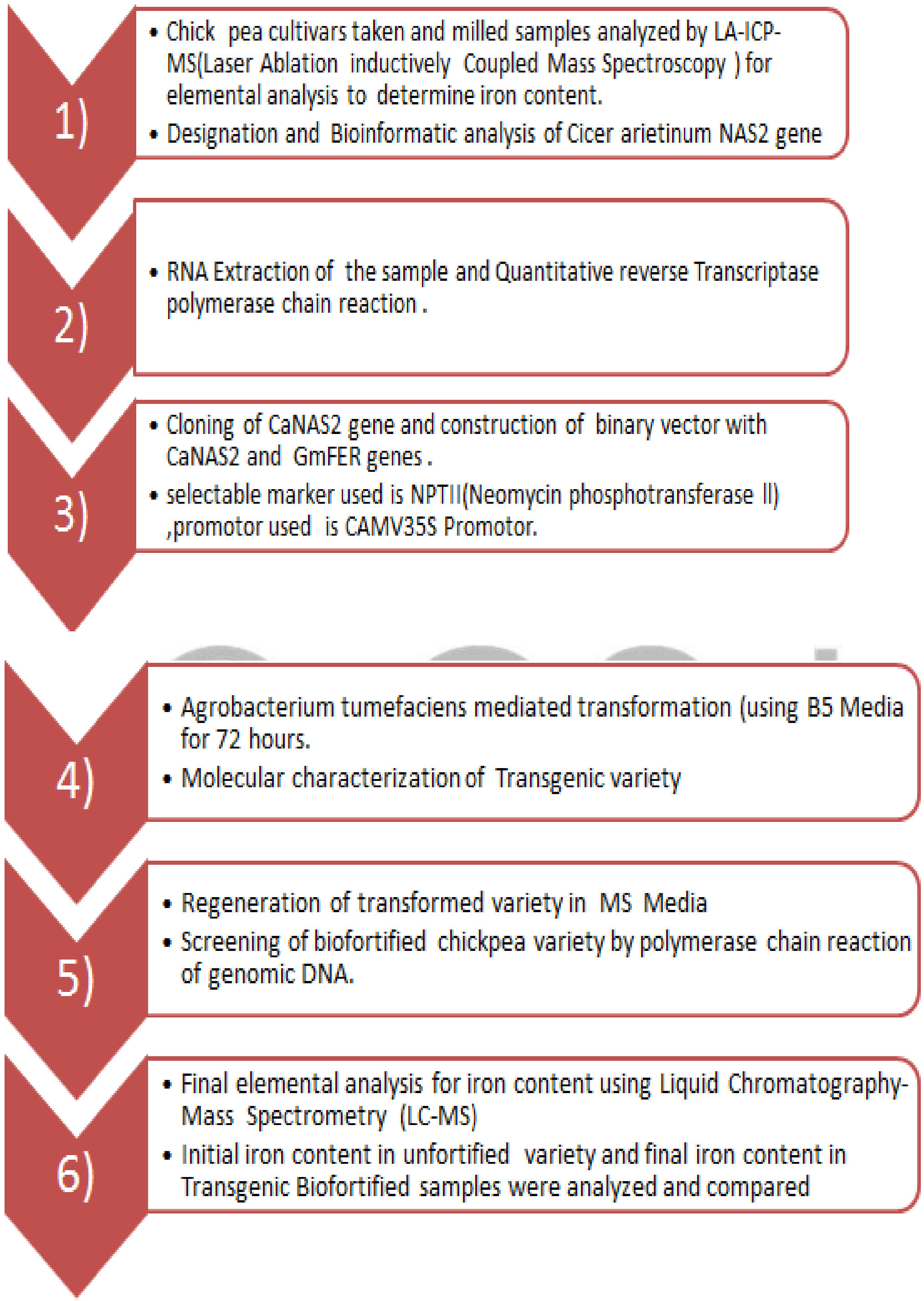
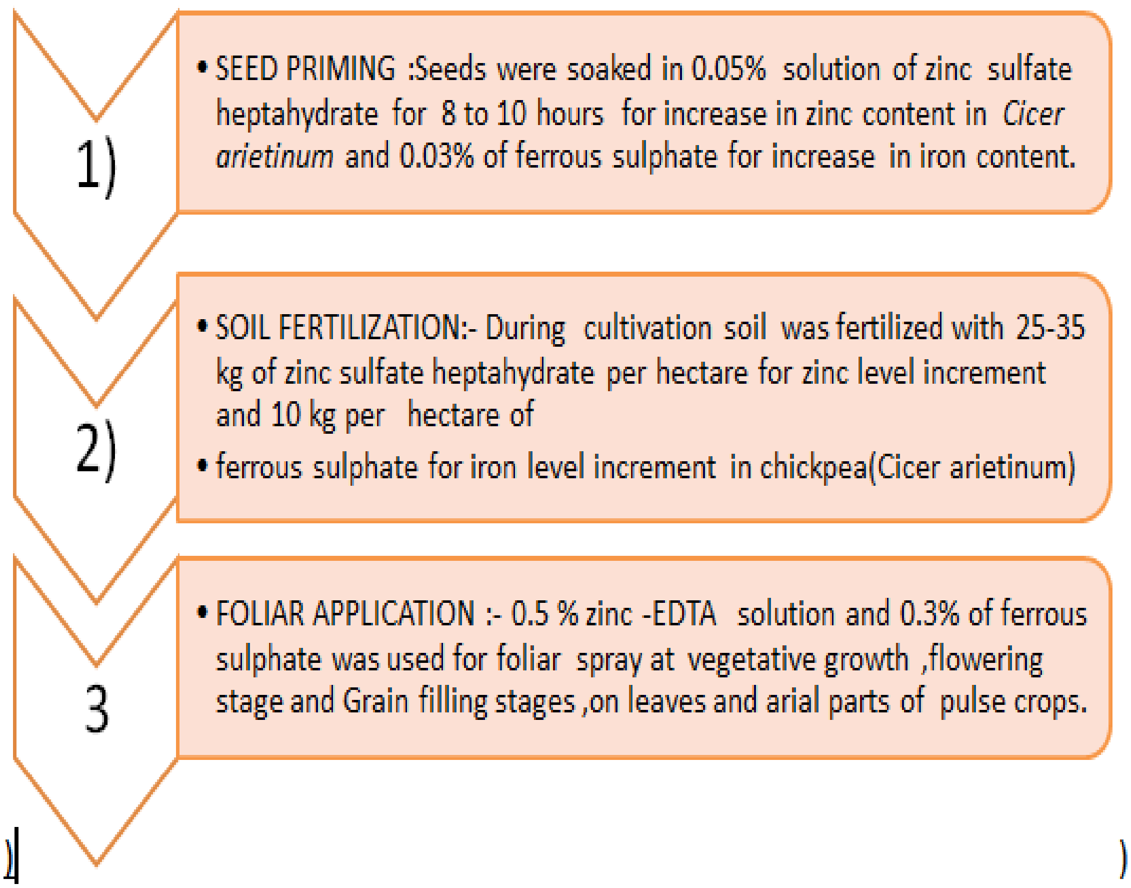


Fig:4 Methodology for transgenic approach for Biofortification in Pulses

WORKPLAN AND METHODOLOGIES TO BE FOLLOWED :-

AGRONOMIC INTERVENTION FOR BIOFORTIFICATION IN PULSE(*Cicer arietinum*



BIO-FORTIFICATION IN PULSES: - CURRENT SCENARIO

1. NUTRIENT CRAFTING OF PULSES BY NEXT GENERATION SEQUENCING

Advanced molecular techniques like genome wide association study **(GWAS)** and genome selection **(GS)** ,have been utilized for identification of beneficial traits in greater crop varieties. Substantial phenotypic variations expressed by QTL (quantitative trait loci) can be used to determine elemental or micronutrient concentration and their genetic basis in grain legumes eg. for Zn by Zhou et al 2010, for iron by Lungaho et al 2011,protein by Blanco et al 2006, and so on. Gene mapping for selenium content was done by Bohra et al 2015 and Ates et al 2016. Further with the use of Genome wide association study(**GWAS**) trait of interest can be extensively studied for diverse population.(Diapari et al 2014). chick pea gene mapping studies in 94 diverse genotypes provided eight markers in zinc and iron content in Marker trait association analysis(MTA). 19 MTAs on five different chromosomes for protein content was analysed in chickpeas in association studies with 187 accessions(Jadhav et al 2015).

Almost sixteen genes involved in iron and zinc accumulation in chick peas have been found in chickpea by association mapping and expression profiling by Upadhyaya et al in 2016. For seed protein content six genes have been found encoding ATP- dependent RNA helicase, DEAD –BOX, cystathione beta synthase, CMP and dCMP deaminases, G10 and zinc finger proteins , in chick pea**. Ionomics** ,another high advanced micronutrients profiling technique have made it possible for identification of genetic lines with high micronutrient content.(Swamy et al 2016) . Process of nutrient level measurement can be speed up by Atomic Absorption Spectrometry(AAS), inductively coupled plasma spectrometry, synchrotron X-ray fluorescence microscopy(XFM).

2. Bio-fortification of chick pea (Cicer arietinum) by arbuscular mycorrhizal fungi (Funneliformis mosseae and Rhizophagus irregularis) was done for iron and zinc grain content increment. Major reason attributed for the beneficial effects of AMF is due to increase in mobility of nutrients and their better uptake with the help of hyphae and increased rhizobial nitrogen fixation. Organic acids produced by fungi assists in enhanced nutrition uptake ,especially phosphorous. AMF inoculation increases NO3-N Content in soil when inoculated for chickpea . AMF application also improved iodine and selenium accumulation and protein and cellulose synthesis. This is due to increase in root surface area. Thus greater biomass at the end of crop cycle promotes high protein extraction and high energy production.

3. Genetic diversity of folate profile was studied in common beans ,lentil chickpea, and pea by ultra performance liquid chromatography coupled with mass spectrometry. Long beans contained 130 micrograms per 100 grams of total folate. Folates present in legumes and pulses are not readily available due to complex formation with the biomolecules(Neilson ,1994) . This analysis can used for its bio-fortification using conventional or transgenic approach. The 5-methyl tetrahydrofolate and 5- formyl terahydrofolate readily available in common beans lentil and chickpea.

4. Bio-fortification in Australian chickpea by Transgenic approach was done by *Grace Z.H , Sudipta Das , Hao long et al* in 2018 (centre for tropical crops and biocommodities , Brisbane , Australia). Two genes chickpea nicotianamine synthase 2(CaNAS2) and soybean ferritin(GmFER) responsible for iron transport and storage were used in this research. First baseline iron level in existing germplasm was established by elemental analysis using laser ablation inductively coupled mass spectroscopy. Novel gene CaNAS2 was isolated and its expression was studied . Then a binary vector was constructed to constitutively overexpress CaNAS2 gene and constitutively express GmFER gene ,with a neomycinphosphotransferase ll(NPTll) as selectable marker. Agrobacterium strain AGL-1 with expression vector was used to carry out transformation.

Fig 5 .BINARY VECTOR CONSTRUCT OF CaNAS2 and GmFER gene

Expression of CaNAS2 gene was studied in iron sufficient and Iron deficient conditions in different tissues and it was measured by quantitative real time Polymerase chain reaction. It was finally concluded that transgenic chickpea expressed both genes and greater seed iron bioavailability.

5. Iron common beans (*Phaseolus vulgaris*) ; During initial research phase from1999 to 2002 , more than 1000 beans germplasm have been screened for iron and zinc content. During HARVEST PLUS high iron containing genotypes were used for conventional breeding. Upto 50% increase in iron concentration have been obtained and nine varieties have released in Rwanda.

6. To overcome the constraints associated with soil of arid and semi arid regions , boron seed coating and inoculation with boron tolerant bacteria have been done by Mubshar et al in 2020 in chickpeas. Bacillus sp. MN54 was used for inoculation. Boric acid was used as source of boron and to increase the adherence of boron on seed surface ,arabic gum was used. Boron seed coating were beneficial at low concentration in all traits of chickpea ,while it is toxic at higher concentration. It was observed that boron coating of 1.5grams of boron per kilogram enhances nodulation, number of seeds per pods and plant height.

7. Utilization of pulse crop genetics

Molecular breeding techniques have demonstrated the role of legumes signaling in nodulation. In common beans (*Phaseoalus vulgaris*) nuclear factor y(NF-Y) and its transcription factors PvNF-YA1 and PvNF –YB7 have been supposed to enhance nodulation in legumes. Also Nod factor receptors (NFRs) in L. japonicus and G.max are main determinants of symbiosis specificity.(Ripodas et al2019),(Mus et al , 2016). This research study can be further adapted in many bio-fortification prospects with the help of genetic engineering. Also genome analysis has made it possible to enhance nutritional and other related traits of underutilized pulse crops like winged bean and lablab and to facilitate the breeding of improved varieties through marker assisted selection.(Varshney et al 2013).

Genome assemblies drafting of *pigeon pea (Cajanus cajun)* was done by Varshney et al in 2012 and of *common beans (Phaseolus vulgaris)* was completed by *Schmutz et al* in 2014.

Effective transcriptome assemblies ,gene predictions and sequence enabled databases of pulses crops have been generated. Nowadays focus is on generating pan genomes supporting multiple references genome for a single legume crop. This has been made possible by interspecific comparative analysis and study of their evolutionary trends. Further germplasm conservation and its stable maintenance is important for their maximum utilization for securing the global need of biofortified pulses.

8. Icarda pulses breeding program

Vast germplasm collection and its utilizaton have made ICARDA(International center for Agriculture Research in Dry Areas) developed almost 38000 accessions of chickpea, faba beans ,lentils ,field pea and grass pea. An extensive breeding program called as FIGS(focused identification of germplasm strategy) have been adopted at ICARDA .Selection –hybridization -selection cycle is the main mechanism to develop new breeding lines , which are promoted by NARS (National research and extension system) in the form of international nurseries .More than 350 varieties lentil(137) ,chickpea(162) ,faba beans (75)and grass pea (7) have been released by NARS for commercial cultivation between year 2005 to 2015. Important traits like abiotic and biotic resistance , greater iron and zinc content to be acquired is main focus of the program.

9. Silicon content increment in green beans

Soilless cultivation offers the possibility of modification of mineral nutrition to be added to the plants. Green beans cultivar *Saporro* was used for bio-fortification. The target silicon concentration was achieved by addition of potassium metasilicate to the nutrient solution. Silicon content in biofortified and unfortified samples was measured by spectroscopy and its bioavailability was analysed. Ariel parts were treated with silicon solution. Cumulative effect showed an increase of silicon content by 360% in stem ,240% in leaves and 310% in pods.(Francesco Fabiano Montesano et al 2016,)

CONCLUSION

Hidden hunger and malnutrition are two preliminary reasons leading to all major health issues ,not only in low income group countries but also in well developed nations. Solving this problem commenced after beginning of Green revolution, when Dr .Howarth Bouis , pioneer of HARVEST PLUS program , focused on the importance of nutritional quality and quantity in recommended daily diet. This led to the requirement of Bio-fortification techniques for all major crops of the world . A technique well accomplished by conventional breeding, agronomic practices and Transgenic methods.

Pulses being cheaper and being in extensive cultivation from many centuries have been in focus for nutrient bio-fortification. Three main stages been employed in the process are;-discovery, development and dissemination of developed pulses varieties in local markets for growth and consumption

Transgenic approach has been well exploited in all pulses bio-fortification by gene editing tools like CRISPR/CAS9, ZFN and TALENs .Advanced Next generation sequencing approaches like quantitative trait loci and GWAS(Genome wide association studies) have been advantageous in *Phaseolus vulgaris* ,*Cicer arietinum* and lentils bio-fortification with iron and zinc.

Conventional breeding for pulses bio-fortification has even greater success rate due to accessibility of wider gene pool and recombinant inbred lines. A few examples of biofortified Lentil varieties are *Pusa Vaibhav, Barimasur and Khajurah L4704.*

Agronomic approach for bio-fortification involves

- Seed Priming,
- Seed Coating ,
- Soil Fertilization,
- Foliar Spray &
- Microbial Intervention

Bio-fortification of pulse with selenium using sodium selenate, with zinc using zinc sulphate heptahydrate or zinc –EDTA chelate have been ushered with this approach. Further role of microbial interventions using PGP(Plant growth promoting) bacteria and Mycorrhizal arbuscular fungi cannot be overlooked in common beans, chickpea ,lentils bio-fortification.

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