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Biofortification of pea (*Pisum sativum* L.): A Review

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ABSTRACT

Biofortification refers to an approach to increase micronutrient concentrations in the edible parts of plants with increased bioavailability to the human population. Conventional, agronomic and transgenic breeding methods can be used to develop these biofortified crops, offering sustainable and cost-effective strategies. Pea has long been recognized as a valuable, nutritious food for the human diet, but there is a limited amount of information about it, which prevents the full micronutrient enrichment potential of this pulse crop to be reached. Considerations must include not only micronutrient concentrations but also the amount of the nutrient that can be absorbed by the consumer, after processing and cooking. Development of biofortified pea that retains nutrients during cooking and processing is not only essential for fighting micronutrient malnutrition, but also necessary to improve agricultural productivity.

KEYWORDS: Plant breeding, micronutrients, genetic variability, *Pisum sativum.*

INTRODUCTION

Biofortification is the improvement of the nutritional quality of the edible part of the plant. It offers a sustainable and long-term solution to providing micronutrients-rich crops for people.[1] So far, our agricultural system has not been designed to promote human health; instead, it only focuses on increasing grain yield and crop productivity. While the nutritional profile of cereals and tubers is not optimal for human or animal nutrition, their success in production has also led to displacement in some countries of legumes crops, which are nutritionally superior to cereals and have the capacity to incorporate nitrogen into the soil, favoring a more sustainable agriculture.[2,3]

This approach has resulted in a rapid rise in micronutrient deficiency due to a diet composed of food grains, thereby increasing micronutrient malnutrition among consumers.[1] It has been estimated that more than two thirds of the world population experiences an inadequate intake of one or more mineral nutrients, with more than 50% of the population with deficiencies in iron (Fe) and more than 30% in zinc (Zn).[4]

Biofortification is the development of micronutrient-enriched staple food crops through traditional plant breeding methods in conjunction with modern molecular biological techniques, allowing a sustainable intervention to combat global micronutrient deficiencies. Past attempts to address micronutrient malnutrition have included dietary supplements, food fortification, diet diversification, and supplementation. Biofortification offers the opportunity to change crop nutritional value within the production system adding little or no costs to consumer.[5] Evidence to date from randomized trials suggest that biofortified crops are an efficacious intervention to improve Fe status.[6] Furthermore, biofortified seeds are also likely to have an indirect impact in agriculture, as a higher trace mineral content in

seeds confers better protection against pests, diseases, and environmental stresses, thereby increasing yield.[7] Pea has long been recognized as a valuable, nutritious food for the human diet and it constitutes a good target for biofortification.

NUTRITIONAL QUALITY OF PEA

Peas, more specifically the yellow or green cotyledon varieties, are known as dry peas or field peas and are grown around the world for human and animal consumption. They have long been recognized as an inexpensive, readily available source of protein, complex carbohydrates, vitamins and minerals. Their high nutrient density makes them a valuable food commodity, capable of meeting the dietary needs of undernourished individuals worldwide.[8] Some researchers have reported that *Pisum sativum* L. is an excellent source of nutrients for all species of animal with high digestibility and palatability.[9]

The increasing demand for protein due to increasing population has shifted focus from animal protein towards plant proteins.[10] In some parts of the world peas are the main source of proteins for humans, with protein content ranging from 190 to 300 g Kg^{-1} in commercial varieties, equal to the protein content of meat (180-250 g Kg^{-1}).[11] Like other grain legumes, peas are deficient in the sulfur-containing amino acids methionine and cysteine but they are relatively high in lysine, hence its essential amino acid profile is complementary to that of cereal grains.[12]

Studies have been done on differences in protein content between various pea cultivars using different methods, obtaining values from 110.38 to 320.60 g Kg^{-1} (Table 1).[13,14,23–29,15–22] Coyne et al.[17], Valverde et al.[28] and Guindon et al.[18] reported that seed type,

wrinkled or smooth, affected the composition of pea seeds, with higher average protein values in wrinkled seeds.

Pea has multiple qualities, such as good functional properties in food applications, high nutritional value, availability, and relatively low cost, but they remain underutilized in the food industry. Proteins as concentrates or isolates are used as a functional ingredient primarily to increase nutritional quality and to provide desirable sensory characteristics such as structure, texture, flavour, and colour to formulated food products.[30] The protein concentrates and isolates used by the food industry today are mostly derived from soybean, whey and wheat. However, food manufacturers and consumers are looking for alternative protein sources. Pea proteins should be useful in a variety of formulations, such as bakery products, soups, dairy products, gluten-free foods, mayonnaise, and salad dressing, as well as new food products.[31] Different studies have been perform to evaluate techno-functional properties, including ratio of vicilin:legumin concentration, water and oil absorption capacity, solubility, emulsifying, foaming and gelling properties of pea protein isolates. [10,15,32–36] Some targets for legume seed protein improvement include the lack of cysteine and methionine, the removal of antinutritional factors and components that generate undesirable flavors, removal of potential allergens, improved digestibility and improved functional behavior for processing.[37]

Peas are recognized as a good sources of dietary carbohydrates, like many food legumes. A significant amount of starch, called resistant starch, defined as the sum of starch and products of starch degradation not absorbed in the small intestine of healthy individuals, can escape digestion. This starch passes through the digestive tract to the large intestine, where it serves as a substrate for microorganisms, so it behaves as dietary fiber, with

benefits in reducing the risk of colon cancer, reducing the glycemic index, acting as a prebiotic, showing a hypocholesterolemic effect, inhibiting fat accumulation and, when compared to digestible starch, allowing greater apparent absorption of calcium (Ca) and Fe.[38]

Although starch is the major component of pea, there are few studies in the literature concerning its composition, physicochemical and functional properties**.** Recognition of variation in starch properties among cultivars is very important for plant breeders to develop or select potentially useful cultivars with particular functional properties of their starches suited to specific applications. Values detected for total starch ranged between 270.6 and 560.3 g Kg^{-1} (Table 1).[19,20,24,27,29]

As a natural resource and food component with health benefits, pea starch is attracting increasing attention for its high amylose content, resistance to shear thinning, rapid retrogradation and high resistant starch content.[39] According to Ratnayake et al.[40], the amylose content can vary from 30-40% in the starch from smooth peas to 60-76% in the starch from wrinkled peas.

Starch is the principal component of many food matrices, it contributes to the important functional properties and nutritional characteristics of processed food products and can be utilized in many industrial applications. Although from a nutritional point of view, pea starch is interesting due to its considerable resistant starch and total dietary fiber contents[38], Ratyanake et al.[40] reported that it has been mainly used in industrial applications, but not much in food applications due to its poor functional properties. Pea starch is a very useful film-forming material due to its high amylose content which can improve mechanical strengths, including tensile strength and gas barrier properties.[41]

There have been some studies on the structure and functionality of starches from peas. Liu et al.[39] evaluated the physicochemical and in vitro digestibility properties of starches isolated from four varieties of field peas grown in China. They displayed variability in swelling power, pasting characteristics, thermal and textural properties, and in susceptibility to in vitro attack by α-amylase. Mehyar and Han[42] established that pea starch films were strong and elastic and possessed good barrier properties and physical integrity. They were either comparable or superior to other edible films such as whey protein, soy protein, pea protein, and wheat gluten films. Matta et al.[43] determined that the addition of xanthan gum and glycerol affected the mechanical properties of these films, reducing the maximum tensile strength, strain at break and puncture force and increasing the elongation and deformation. Mehyar et al.[44] proved that edible coatings made of whey protein isolate and pea starch were effective in preventing oxidative and hydrolytic rancidity of walnuts and pine nuts during storage. Finally, Saberi[45] results revealed that pea starch/guar gum edible films had appropriate physical and optical characteristics and can be effectively produced and successfully applied in the food packaging industry.

Thus, information on starch characteristics is important to improve the texture of food products such as frozen foods, extruded snacks, cookies, crackers, sauces, and soups. This is significant not only for food processing but also for consumer acceptance [46].

Phenolics are an important group of natural compounds that contributed significantly to the marked pharmacological properties of a number of plants including legumes. They have wide therapeutic and pharmacological activities against human and animal diseases.[47] It is widely accepted that significant antioxidant activity of food is related to high total phenolic content.[48] There is not much work done on the estimation of individual phenolic acids in

pea (Table 1). Amarowics and Troszynska[49] found vanillic, caffeic, *p*-coumaric, ferulic and sinapic acids, quercetin and kaempherol, procyanidin B2 and procyanidin B3 as active phenolic compounds in pea material using HPLC (High-performance liquid chromatography). Wang et al.[50] estimated values of total phenolics that ranged from 868 mg Kg^{-1} to 2059 mg Kg^{-1}, while Xu and Chang[48] estimated values that varied from 1040 to 1670 mg Kg^{-1} and Zia Ul Haq et al.[51] values in the range of 840–990 mg Kg^{-1}.

Pea seeds contain some phenolic compound that are considered antinutritional factors, such as condensed tannins which can reduce protein intake by precipitating proteins. Wang et al.[50] reported mean values of condensed tannins ranging from 890 mg Kg^{-1} to 5180 mg Kg^{-1} and Zia Ul Haq et al.[51] obtained values between 570 to 680 mg Kg^{-1}. Xu et al.[48] evaluated condensed tannins using different extraction solvents obtaining a value of 1710 mg Kg^{-1} using acidic 70% acetone (Table 1). Troszinska et al.[52] determined that condensed tannin extract from the seed coats of coloured varieties of pea exhibited a pronounced antioxidant activity and they could be effectively employed in food systems.

Carotenoids are natural pigments synthesized from plants. Humans and animals are incapable of carotenoid biosynthesis and therefore depend on dietary carotenoid sources. Ashokkumar et al.[53] determined that green cotyledon pea accessions were richer in β-carotene and total carotenoids compared to yellow cotyledon accessions (Table 1). Nemeskéri[23] results showed that the total carotene content of yellow seeds in pea was rather low (0.32 μg g^{-1}), but the total xanthophyll content was relatively high (10.20 μg g^{-1}). Edelenbos et al.[54] measured the concentration of carotenoid and chlorophyll pigments in six cultivars of processed (blanched, frozen, and thawed) green peas. On average over two years, the chlorophyll type a concentration varied from 48 to 73 μg g^{-1}, the chlorophyll type

b concentration from 21 to 28 μg g^{-1}, the lutein concentration from 12 to 19 μg g^{-1}, and the β-carotene concentration from 3 to 4.90 μg g^{-1}. According to Marles et al.[55] and Jin et al.[56] chlorophylls and carotenoids accumulated in the hulls split from the green and yellow field pea had potential as a value-added prospect in food supplements.

Micronutrients are crucial for plant growth and human health. Cheng et al.[57] evaluated 330 accessions from a core collection of the USDA pea collection obtaining the following means expressed in μg g^{-1}: boron (B): 7.8; Ca: 802.1; copper (Cu): 4.4; Fe: 50.4; magnesium (Mg): 1,685.8; manganese (Mn): 16.0; molybdenum (Mo): 23.2; nickel (Ni): 2.5; phosphorus (P): 5047.5; potassium (K): 12474.3, and Zn: 41.8 (Table 1). The concentrations of certain minerals, especially Fe and Zn, are low relative to animal food products [58] and these mineral deficiencies can be quite prevalent.

Fe is an essential component of many enzymes catalyzing redox reactions due to its ability to readily accept and donate electrons under physiological conditions. Zn is a cofactor with diverse structural and catalytic functions in about 10% of all human proteins. In addition, evidence has been accumulating for important regulatory roles of Zn ions in inter and intracellular signaling [59]. In the USA, Amarakoon et al.[9] reported peas naturally enriched in Fe (46-54 mg kg^{-1}) and Zn (39-63 mg kg^{-1}), and Ma et al.[60] found values from 37.3 to 71.2 mg kg^{-1} of Fe and 30.7 to 64.9 mg kg^{-1} of Zn using recombinant inbred lines. In Australia, Poblaciones and Rengel[25] reported a mean value of Zn of 34.1 mg kg^{-1}. In Turkey, Demirbas[61] found high diversity for Fe, from 38.6 to 320.9 mg kg^{-1} and for Zn, from 11.3 to 82.9 mg kg^{-1} evaluating 152 landraces and 5 commercial cultivars. In Canada, Wang and Daun[29] reported 43 to 79 mg kg^{-1} of Fe and 25 to 52 mg kg^{-1} of Zn, while Ray et al.[62] reported values from 47.7 to 58.1 mg kg^{-1} of Fe and 27.5 to 34.0 of Zn, and Liu et al.[63]

found values from 38 to 44 mg kg^{-1} of Fe in low phytate varieties (Table 1). Phytic acid is the main storage form of P and is a chelator of cations such as Ca, Mg, K, Fe and Zn, preventing its absorption in the human intestine.[64] Monogastric animals including poultry and humans are unable to metabolize phytic acid due to the lack of sufficient levels of phytate degrading enzyme activity in their digestive tract.[65] Poblaciones and Rengel[25] found values of grain concentration of phytate in the range of 6.3–7.0 g kg^{-1}, in accordance with the data reported by Amarakoon et al.[9] (Table 1). Warkentin et al.[66] using mutagenesis developed low phytate lines with high concentration of bioavailable Fe.[67–70]

Peas have high potential for nutritional quality improvement of food. Furthermore, a variety of technological processes are available to facilitate the inclusion of legumes into more innovative food products.[71] Biofortification offers a new approach to develop materials with better composition, offering a sustainable and cost-effective strategies. However, increasing micronutrients content of peas, without improving micronutrients bioavailability will not improve the micronutrients status of consumers.

BIOFORTIFICACIÓN

There are marked differences between traditional plant breeding and crop biofortification. Traditional breeding focuses on improving traits of known economic value and developing product concepts for existing markets. Biofortification breeding, on the other hand, seeks to make an impact on human micronutrient status, so it has to link directly to the human health and nutrition sectors.[72]

Biofortification of essential micronutrients into crop plants can be achieved through three main approaches, transgenic, conventional, and agronomic, involving the use of biotechnology, crop breeding, and fertilization strategies, respectively.[1]

The design of conventional plant breeding programs requires the screening to identify available genetic variability that can be exploited as a donor for transferring useful genes in into the background of cultivated genotypes and also for use directly as a biofortified variety, if the identified variant is already a high yielding variety. Wild species are a rich reservoir of useful alien genes, which are often no longer available within the cultivated gene pool.[73] However, they possess significant undesirable agronomic traits and transferring desirable alleles from such species often take long time, so it is important to determine the nutritional traits in cultivated materials.

Lines with high mineral concentrations in seeds can be crossed with lines with desirable agronomic traits over several generations to produce plants with desired nutritional and agronomic characteristics. This approach is a sustainable and a long-term solution to increasing the amount of micronutrients in pea genotypes, however, development of new varieties is long and expensive (8 to 10 years) and it would be very useful to reduce the time of this process. Molecular markers could be used for assistance in the selection of superior genotypes and for identification of regions associated with the traits of interest. Application of rapid generation technology can accelerate the production of recombinant inbred lines from crossing of selected materials.[74] Different protocols have been developed to accelerate generation time, maintaining population size and genetic diversity. Mobini and Warkentin[75] and Cazzola et al.[76] developed *in vivo* methods that allow the acceleration

up to six generations per year of pea. It consisted in a hydroponic system, with a 22-hour photoperiod, a temperature of 20 °C, flurprimidol antigiberelin and early grain harvest.

In agronomic biofortification, the concentration of essential mineral elements is increased by the application of inorganic fertilizers or by the inoculation of soil with beneficial microorganisms. This can be complemented by breeding crops with an increased ability to acquire and accumulate these minerals in their edible portions.[4]

In cases where agronomic and breeding approaches cannot achieve significant improvement in mineral concentration, transgenic techniques offer a useful alternative. Fe and Zn are relevant cases because their concentrations are particularly low in the starchy endosperm which is the part of grain commonly consumed by people, and because their bioavailability to humans is low due to the presence of the anti-nutrient phytate. These hindrances may be overcome by mutagenesis to reduce the synthesis of phytate, by transgenesis to express genes encoding phytase or by expression of genes that lead to accumulation of Fe in the endosperm.[3]

Peas are rich sources of nutrients especially when used as whole grains. However, they could be processed further after cleaning and grading to yield end products useful for industry. Processes applied to legumes can be classified into three groups: the preparation of raw materials involving washing, cutting, or chopping; preservation operations, such as sterilization, drying, freezing, or freeze-drying; and transformation processes all of which aim to increase the shelf life of the foodstuff. These operations alter the nutritional composition of resultant product to varying degrees. These could also modify the matrices, the surrounding in which nutrients are embedded in a grain, which in turn influences the nutrient availability *in vivo*. Processes like soaking and germination reduce the antinutrient

content and also increase the availability of nutrients, in particular of minerals.[77] Breeding to increase bioavailability can be done by manipulating plant structures or increasing promoters and/or decreasing inhibitors such as antinutrients. [78]

CONVENTIONAL BIOFORTIFICATION: GENETIC BASIS OF TRAITS TO IMPROVE

The first step to develop a biofortification breeding program is to search in existing seeds banks for genetic variation for nutritional traits. These data are useful for obtaining basic information about genetic relationships between accessions, for understanding the inheritance patterns of these traits, and for selecting parental lines for crosses. The availability of genetic variation is essential for achieving meaningful increments through conventional breeding and high rates of genetic progress under selection. The mineral variation within pea germplasm, described in the previous section, provides the potential to create new pea cultivars with greater mineral density.

All plant-breeding components (such as crossing strategies, breeding methodologies, years required for testing) are based on genetic parameters. Breeders can exploit additive gene effects, transgressive segregation and heterosis to improve micronutrient density. Genetics of yield and yield components in pea has been studied based on morphological[79,80] and molecular data[81,82,91,83–90] but there is less information about quality traits.

Knowledge of heritability as it relates to genetic progress and associated genetics is crucial for effective breeding. Guindon et al.[18] calculated broad sense heritability for quality traits obtaining high values (between 0.41 and 0.98), but this parameter is related to additive genetic variance and non-additive or non-fixable variance, so it is also necessary to

calculate narrow sense heritability to predict the amount of genetic progress that can be made in selection of a trait or traits of interest which can be fixed in the final inbred cultivars. Nemeskéri et al.[23] established values of broad sense heritability of 0.78 for xanthophyll content and 0.32 for carotene, while Ma et al.[60] found values of broad sense heritability from 0.84 to 0.98 for different minerals (B, Ca, Fe, K, Mg, Mn, Mo, P and Zn). These traits showed transgressive segregation except for Fe, Mo and S concentrations.

Micronutrient concentrations are affected by genotype by environment (G x E) interaction. Wang and Daun[29] reported that environmental conditions exhibited a significant effect on Fe, K, Mg, Mn, P and Zn. Amarakoon et al.[9] reported that Fe, Zn, Ca, P and Mg concentrations of six field pea genotypes are influenced to different extents by location, genotype and G x E, although G x E was not significant for phytic acid. Liu et al.[63] showed that variety, environment and their interaction affected P and phytic acid concentration, but for Fe concentration G x E was not significant. For carotenoids and polyphenolics Marles et al.[55] demonstrated that only chlorophyll a showed G x E interaction. For all other compounds assayed, the relative ranking of the genotypes was reasonably stable across environments, suggesting that breeding for increased levels should be possible. There were differences among locations and years, however, indicating that growing environment will have an effect on the total amount of these compounds, so testing at multiple locations would be advised before making selections. More studies are required to understand G x E interactions.

Correlation estimates are important in plant breeding because they quantify the degree of genetic and non-genetic association between two or more traits, allowing indirect selection. Significant negative correlation was observed between total starch and crude protein

concentration (r values from -0.57 to -0.88).[19,20,22,24,29] Wang and Daun[29] found correlations between minerals and macronutrients. Fe, Mg and Zn correlated positively with protein (r = 0.60; r = 0.54; r = 0.59) and negatively with starch (r = -0.73; r = -0.43; r = -0.47), while K, Mg and Mn correlated negatively with fat content (r = -0.54; r = -0.47; r = -0.57). They also found correlations between minerals, K and Fe (r = 0.54); K and Cu (r = -0.54); Mg and Fe (r = 0.65); Mg and K (r = 0.71); Mn and Fe (r = 0.41); Mn and Ca (r = -0.42), P and K (r = 0.51); P and Mg (r = 0.64); Zn and Fe (r = 0.52); Zn and Mg (r = 0.59). Ma et al.[60] observed negative correlations between seed weight and all the mineral nutrient concentrations (B, Ca, Fe, K, Mg, Mn, Mo, P, Zn). The highest positive correlations between different mineral concentrations were observed between Mg and Mn (r = 0.69) and between Ca and Mn (r = 0.69), Wang and Daun[29] observed the last correlation but with opposite sign. Demirbas[61] also found positive and highly significant correlation among various mineral elements: Fe with Zn (r = 0.42), Mn with Fe (r = 61), already observed by Wang and Daun[29], Mn with Zn (r = 0.48), Cu with Fe (r = 0.55), and Cu with Zn (r = 0.48). Kosev and Ilieva[22] observed a positive correlation between P and crude protein (r = 0.65) and a negative correlation between P and crude fiber (r = –0.59). Nikolopoulou et al.[24] determined that phytic acid content of pea seeds was positively correlated with fat (r = 0.47) but negatively correlated with starch (r = -0.52). Wang et al.[50] established that a highly significant negative correlation existed between condensed tannins and lightness of seed coat color, so pea lines with darker seed coats contained higher levels of condensed tannins. On the other hand, total phenolic and condensed tannins were positively correlated (r = 0.89). Understanding the association among traits allows the prediction of the positive or negative influence that

selection of a character of interest has in another related character, which is useful for the design of selection index.

Similar correlations were found in other legumes, particularly negative correlation between total starch and crude protein concentration were observed in lentil, dry beans and chickpea.[92–94] Negative correlation between seed weight and P y K were observed in chickpea[95] and negative correlation between phytic acid content and starch in dry beans[93]. These results may reflect similarities between crops in physiological processes.

Correlations between minerals were observed in legumes but there is more variation in magnitude and sign, which can be explained by the influence of genetic background of the materials, and environmental adaptability. The positive correlations between Fe and Zn in different legumes species[73,96] suggest the possibility of breeding for increased concentrations of these elements simultaneously.

Developing analytical methods and high throughput screening methods to assay micronutrients and establish germplasm screening are prerequisites for effectively assessing genetic variation. Inexpensive rapid screening methods boost breeding effectiveness and are crucial for assessing the large number of genotypes in plant population development. For metal determination coupled plasma optical emission spectroscopy and atomic absorption spectrometry have been used in pea studies. On the other hand, HPLC and spectrophotometric methods have been used for determination of carotenoids and phenolic compounds (Table 1). When interpreting these data, one must consider differences that can produce error, such as sampling, milling, analytical protocols, and the type of experimental screening design used.

Molecular markers and marker-assisted protocols to select for micronutrient density can greatly increase breeding efficiency. Recently, Jha et al.[97] have reviewed different studies performed in pea that found associations between micronutrients and markers. This information is summarized in table 2. Detected QTLs (quantitative trait loci) provide useful information to allow an understanding of genomic loci underlying quantitative traits allowing further identification of potential candidate genes, targeted marker design for fine mapping, or genetic/genomic experiments.

AGRONOMIC BIOFORTIFICATION

This method is simple and inexpensive, but it requires information about the source of nutrients, the method of application and the effects on the environment. Fertilizers must be applied in each growing season, reducing the cost effectiveness of the method. Sankaras et al.[98] established the importance of providing nutrients to the soil, because although they observed net remobilization of some minerals from different tissues into seeds, continued uptake and translocation of minerals to source tissues during seed fill is as important, if not more important, than remobilization of previously stored minerals. Peas have been enriched with foliar applications of Zn alone or in combination with soil applications.[25] Although doses could vary according to deficiencies of the soil, the combined application of Zn in the soil and foliarly (8 mg $ZnSO_4.7H_2O$ kg^{-1} soil + 0.25% w v^{-1} $ZnSO_4.7H_2O$ foliarly) increased grain Zn concentration by more than 40 mg Kg^{-1}, with good bioavailability. Thavarajah et al.[99] reported that reduction of phytic acid in low phytate line seeds may reduce seed Fe and total P concentrations, but these low phytate field pea lines may have high P fertilizer use efficiency, affecting their mineral content. Field peas, like other pulses,

are critical to soil P deficiencies, so more investigation of the genetic diversity of field pea in terms of P use efficiency is necessary.[100]

Taking into account that legumes are generally cooked before being consumed to improve their taste and palatability, Poblaciones and Rengel[25] evaluated the bioavailability of Zn in fertilized peas after their freezing and cooking, determining that processing caused a 30% decrease in Zn concentration, although it preserved a good amount of bioavailable Zn.

Utilization of soil microbes in improving Fe uptake processes by plants may be used in biofortification. The main microorganisms involved in improved Fe uptake are plant growth-promoting bacteria and Mycorrhizal fungi.[101] Plants form symbiotic relationships with Mycorrhizal fungi for greater P acquisition. For legumes specifically, high concentrations of P exist in the nodules to maintain the nitrogen-fixing function. Due to stress or senescence, P is remobilized from younger tissues and moves into upper leaves and seeds for storage as phytic acid.[100] Sugar, protein and nutrient uptake of *Pisum sativum* increases with the use of domestic sewage mixed soil, which also enhanced the maximum growth of rhizospheric microorganisms in the soil. [102]

TRANSGENESIS

Transgenesis can be used to incorporate genes involved in the improvement of concentration and bioavailability of micronutrients, the reduction of concentration of anti-nutrients and the redistribution of micronutrients between tissues. [103] Fe and Zn levels have been modified using different genes. Overexpression of the ferritin gene from soybean or common bean in the endosperm of rice doubled or tripled its Fe concentration.[104,105] The expression of a pea seed ferritin cDNA in transgenic mustard leads to a significantly

increased leaf Fe content in mature transgenics and an increased accumulation of Fe in transgenic seedlings.[106] The expression of the enzyme phytase of *Medicago truncatula* also increased the bioavailability of the minerals through a decrease in phytic acid.[107] Robinson et al.[108] have reviewed some challenges and solutions for the use of genetic engineering to improve pea nutritional content, which also include the inactivation of genes encoding less desirable proteins, the manipulation of starch biosynthetic genes and their control and the development of raffinose synthase mutants to knockout production of these compounds.

Transgenesis has been used in the improvement of many legumes.[109] Although various pea transformation protocols have been established, their transformation efficiency is still low and there is no regeneration method used routinely.[110,111] It is necessary to developed efficient protocols in order to achieve their massive use in improvement programs, with emphasis in the optimal conditions for the efficient regeneration of a wide variety of genotypes.

Transgenesis has the limitation that farmers and community are not receptive to this technology. Different countries have adopted regulatory processes for the acceptance and commercialization of transgenic crops that are very expensive and time consuming.[1] New gene editing tools like CRISPR/Cas9, with better acceptance, have been poorly studied in legumes.[112]

EFFECTS OF PROCESSING TECHNIQUES ON RETENTION OF NUTRIENTS AND BIOAVAILABILITY

To set target levels and determine the likely contribution to nutritional status, critical information is needed on the bioconversion and bioavailability of ingested nutrients;

retention of micronutrient after storage, processing, and cooking; human micronutrient requirements; and potential levels of consumption by target population.[72] Genotypic differences in retention, post-harvest micronutrient deterioration, and concentrations of anti-nutrients and promoters that inhibit or enhance micronutrient bioavailability have been established. Liu et al.[63] calculated the molar ratio of phytate:Fe as it can influence Fe bioavalaibility (molar ratios of phytate:Fe above 10 lead to reduced human Fe bioavailability). Fe bioavalaibility was significantly affected by variety, environment and their interaction. The estimated phytate:Fe molar ratios of two low phytate lines ranged from 10:1 to 14:1, while the ratios of normal phytate varieties ranged from 18:1 to 25:1 over four environments. Poblaciones and Rengel[25] also determined phytate:Fe ratios, that ranged between 6.0 and 8.2 in raw grains and were slightly lower in cooked grains, suggesting good bioavailability to humans. These ranges were lower than those found by Amarakoon et al.[9] (9–11 in field pea grains). According to Liu et al.[63] dehulling seeds removed most of the polyphenols and increased Fe bioavalaibility. Baking, soaking and cooking can also reduce the phytate concentration.[8] Poblaciones and Rengel[25] established that cooking of field pea grains resulted in decreased of protein (by about 7%), Zn (by about 32%) and phytate (by about 6%). However, cooking improved Zn bioavailability to humans. The molar ratio of phytate:Zn changed from 19.9 for uncooked grains to 2.0 for cooked grains. Values smaller than the critical target level of 15 ensures adequate bioavailability. Then, the authors evaluated bioavailability of Zn on peas biofortified with Se and Zn, reaching the same conclusions.[113] Trozsinka et al.[52] reported that antioxidant activity of tannin extract were slightly changed after the seed coat was cooked in water for 30, 60, and 90 min. Almeida Costas et al.[13] shown that thermal treatment, together with

freeze-drying, resulted in a small increase of available nutrient amounts, with exception of raw fiber possibly due to its softening.

The ultimate goal should be that improvement of nutritional quality of peas enables the prediction of impact on human nutrition. But currently there are not enough information about the effect of biofortification on bioavailability of micronutrients and about the level of retention after typical processing, storage, and cooking practices for legumes. A review of the literature on other legumes reported similar effects of different methods of preparation on nutrient content. According to Fabbri et al.[114] cooking fava beans, lentils and chickpeas produce a marked reduction in the content of vitamins. Soaking and cooking beans are effective in removing or reducing anti-nutrients such as tannins, trypsin inhibitor activity and phytic acid. In white beans, traditional cooking has a positive effect on the bioavailability of Fe. Hummel et al.[115] was the first retention study on beans that compares low phytic acid lines with biofortified and conventional beans after treatments included soaking, boiling and refrying. They concluded that developing beans with an increased mineral content combined with a low phytic acid trait, low concentrations of specific polyphenolic compounds, and shorter cooking times could be the research target for the next generation of biofortified beans attractive for consumers and lead to a higher nutritional intake. There are not similar studies performed in pea, however, Warkentin et al.[116] demonstrate the potential efficacy of low-phytate biofortified pea varieties on dietary Fe bioavailability, as well as on intestinal microbiome, energetic status, and brush border membrane functionality in vivo (*Gallus gallus*) comparing with control pea diet as well as a no-pea diet.

Producing nutritious and safe foods, sufficiently and sustainably, is the ultimate goal of biofortification. After the initial investment in developing fortified peas, biofortification has no extra costs, especially as germplasm may be shared internationally. Therefore, this strategy is accessible to those who produce peas for their own consumption, these are usually are rural-based populations more vulnerable to micronutrient malnutrition.

However, as we established in this work, there are limited number of studies related to pea biofortification, which prevents the full micronutrient enrichment potential of this pulse crop being reached. Breeding studies require the determination of genetic variation for micronutrients, their heritability and stability through genotype by environment studies, so it is necessary to apply screening methods on germplasm. On the other side, nutrition research and food science needs to investigate bioavailability and nutritional impact of biofortified cultivars.

Development of biofortified peas is not only essential for developing whole food-based solutions to micronutrient malnutrition but also necessary for improving agricultural productivity and sustainable development, because of pea´s capacity of incorporate nitrogen to the soil.

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TABLES

Table 1. Variation for nutrients in pea

Nutrient	Range	Method	Evaluated material	Reference
		Carotenoids (µg g^{-1})		
Lutein, β-carotene, zeaxanthin, violaxanthin	11.2 , 0.5 , 0.3 , 0.3 (means)	HPLC	94 pea accessions grown in Saskatchewan	53
Chlorophyll *a*, chlorophyll *b*, lutein, β-carotene	48 -73, 21-28 , 12-19 , 3-4.90	HPLC	Four cultivars produced at the Research Center in Aarslev	54
Total carotene, total xanthophyll	0.32, 10.20 (means)	Spectroscopy	Three yellow pea grown in Hungary	23
Carotenoids and phenols	Not provided exact values	HPLC	Five genotypes grown in Saskatchewan	55
		Condensed tannins (mg Kg^{-1})		
	682-1560 (coloured seed coat)	Vanillin method (spectroscopy)	Kwestor variety with white seed coat and Fidelia variety with coloured seed coat grown at the Wiatrowo National Agriculture Experiment Station	52
	890 -5180	Vanillin method (spectroscopy)	17 pea varieties grown in Manitoba	50
	1710 (mean)	Vanillin method (spectroscopy)	Capri yellow pea and Cruiser green pea	48
	71000 (mean) [A500/g]	Vanillin method (spectroscopy)	Pea (Pisum sativum) seeds were obtained from the Plant Breading Station in Olsztyn	49
	570-680	Vanillin method (spectroscopy)	Four cultivars commonly consumed in Pakistan.	51
Total Tannins	4500-9200	Prussian blue photometric method (Grahsm, 1992)	Three different white-flowered cultivars provided from the Fodder Crops and Pasture Institute	24
		Folates (µg Kg^{-1})		
	120.74–610.93	Liquid chromatography	85 accessions from University of Saskatchewan	68
	230-300	Ultra-performance liquid chromatography coupled with mass spectrometry	Four cultivars developed at University of Saskatchewan	69
		Metals (mg Kg^{-1})		
B, Ca, Cu, Fe, Mg, Mn, Mo, Ni, P, K, Zn	7.8, 802.1, 4.4, 50.4, 1685.8, 16.0, 23.2, 2.5, 5047.5, 12474.3, 41.8 (means)	Inductively coupled plasma optical emission spectrometry	384 accessions from a core collection of the USDA pea collection	57
N, P, K, Fe, Zn, Cu, Mn	22300–66700, 1408–8470, 6700–18700, 38.6–320.9, 11.3–82.9, 10.5–50.8, 10.2–37.9	Inductively coupled plasma optical emission spectrometer	152 landraces and 5 commercial cultivars collected from Turkey	61
Fe, Zn, Se	25.71-93.68, 14.39-92.51, 0.08-5.53	Atomic absorption spectrophotometry	94 accessions	67
B, Ca, Cu, Fe, K, Mg, Mn, Mo, Ni, P, Zn	4.19-14.06, 311-2566, 1.37-13.8, 23.16-105.2, 7126-20065, 1058-2473, 8.04-54.26, 5.87-56.47, 0.29-11.89, 2505-7655, 16.1-106.63	-	285 accessions from a core collection from USDA	21
Ca, P	7270-10280, 2790-3890	According to Sandev 1979	Seven varieties from the Institute of Forage Crops–Pleven pea collection	22
Fe	38.1-44.7	Atomic absorption spectrophotometry	Five low phytate varieties from University of Saskatchewan	63
B, Ca, Fe, K, Mg, Mn, Mo,	6.8–13.5, 521.7-2257.2, 37.3 -71.2, 7533.2-12954.2,955.2–1796.7, 8.6–	Inductively coupled plasma optical	158 RIL	60

P, S, Zn	21.1, 0.2–3.8, 2470.8–6013.9, 1381.6–2869.8, 30.7-64.9	emission spectroscopy		
Zn, Ca, Fe and Mg	34 (mean of Zn)	Inductively coupled plasma optical emission spectroscopy	Twilight cultivar	25
K Mg Fe Zn Mn Cu Ni Se	9265-11874, 1098-1279, 47.7-58.1, 27.5-34.0, 9.0-15.6, 5.2-6.3, 2.3-3.4, 0.405-0.554	Atomic absorption spectrometry	17 cultivars grown in Saskatchewan	62
Ca, Cu, Fe, K, Mg, Mn, P, Zn	59.6–106.9, 0.4–0.9, 4.3–7.9, 876.1–1463.9, 130.4–172.3, 0.8–2.4, 270.3–950.5, 2.5–5.2	Atomic absorption spectrophotometer	Four commercial varieties	29
Fe, Zn, Mg ,Ca, P	46–54, 39–63, 1350–1427,622–1219, 3500–5000	Inductively couple plasma-emission spectrometry	Six commercial field pea genotypes grown at seven locations in North Dakota, USA.	9
		Phytic acid (mg Kg^{-1})		
	2100-12600	According to AOAC 1998	Three different white-flowered cultivars provided from the Fodder Crops and Pasture Institute	24
	6300-7000	Spectrophotometry (According to Haug & Lantzsch, 1983)	Twilight cultivar	25
	650-1860	HPLC	One normal and two low phytate varieties from University of Saskatchewan	70
	4900–7100	Anion exchange chromatograph with a conductivity detector	Six commercial field pea genotypes grown at seven locations in North Dakota, USA.	9
	2937-3674	HPLC	Three cultivars	56
	1285-2963	Wade method (spectroscopy)	Five low phytate varieties from University of Saskatchewan	63
		Protein (g Kg^{-1})		
	180.5-210.9	According to AOAC 1975	Grains from the collection of National Center of Vegetable Research of the Brazilian Company of Farming Research and from The Campinas Agronomic Institute	13
	210.8-280.6	NIR	Four commonly grown cultivars and 32 breeding lines grown in Saskatchewan	14
	240.1 and 310.7	Kjeldahl method	Three varieties commonly grown in Serbia and three experimental lines	15
	120.38 -300.93	LECO FP-528 Nitrogen/Proteinein Determinator	504 accessions from the USDA *Pisum* core collection	17
	180.80-290.28	Qubit kit	16 varieties grown in Zavalla, Argentina	18
	180.6–270.3	NIR	169 accessions	19
	140.3-290.5	NIR	50 accessions	20
	130.2-300.93	-	285 accessions from a core collection from USDA	21
	160.54 to 200.23	Kjeldahl method	Seven varieties from the Institute of Forage Crops–Pleven pea collection	22
	190.80-220.41	Kjel-Foss method	Three yellow pea grown in Hungary	23
	240.3-320.5	According to AOAC 1998	Three different white-flowered cultivars provided from the Fodder Crops and Pasture Institute	24
	190.9-220.3	Dumas combustion method	Twilight cultivar	25
	110.38–300.25	NIR	18 genotypes	26
	130.7 -300.7	Dumas combustion method	54 lines from Cebeco Zaden B.V. and 5 wild relatives from the Center for Genetic Resources	27
	260-320	Kjeldahl method	18 lines from the germplasm collection of Valladolid	28

	200.16-260.66	Dumas combustion method	Four commercial varieties	29
		Starch (g Kg^{-1})		
	370.5–560.1	NIR	169 accesions	19
	270.5-510.0	NIR	50 accesions	20
	330.4-470.5	Enzimatically (with a kit)	Three different white-flowered cultivars provided from the Fodder Crops and Pasture Institute	24
	270.6 -560.3	Enzimatically (with a kit)	54 lines from Cebeco Zaden B.V. and 5 wild relatives from the Center for Genetic Resources	27
	410.6-470.5	Colorimetrically by AACC method 76-13.18	Four commercial varieties	29
Resistant starch	20.45 (mean)	Enzimatically (According to Faisant et al., 1995)	Grains from the collection of National Center of Vegetable Research of the Brazilian Company of Farming Research and from The Campinas Agronomic Institute	13
		Total phenolics (mg Kg^{-1})		
	1040-1670	Folin and Ciocalteau's method (spectroscopy)	Capri yellow pea and Cruiser green pea	48
	868-2059	Prussian blue assay (According to Price & Butler, 1977)	17 pea varieties grown in Manitoba	50
	22600 (mean)	Folin and Ciocalteau's method (spectroscopy)	Seeds from the Plant Breading Station in Olsztyn	49
	840–990	Folin and Ciocalteau's method (spectroscopy)	Four cultivars commonly consumed in Pakistan.	51
Free phenolics acids	46.36 (mean coloured seed coat), 7.75 (mean white seed coat)	HPLC	Kwestor variety with white seed coat and Fidelia variety with coloured seed coat grown at the Wiatrowo National Agriculture Experiment Station	52

AACC: American Association for Clinical Chemistry; AOAC: Association of Official Analytical Chemists; HPLC: High Performance Liquid Chromatography; NIR: Near-infrared spectroscopy; RIL: Recombinant inbred line; USDA: United States Department of Agriculture.

B: boron, Ca: calcium, Cu: copper, Fe: iron, Mg: magnesium, Mn: manganese, Mo: molybdenum, Ni: nickel, N: nitrogen, P: phosphorus, K: potassium, Se: selenium, S: sulfur, Zn: zinc.

Table 2. Identified quantitative trait loci (QTLs) for micronutrient concentration in pea.

Trait	*Population*	*Marker*	*Detection method*	*Environments*	*LG*	*R^2*	*QTLs*	*Reference*
B	158 RIL from Aragorn x Kiflica	1684 markers (SSR and SNP), 7 LG, 1310.1 cM	CIM	Whitlow and Spillman, 2014	1, 5, 6 ,7	7.0-42.0	5	60
Ca	384 accessions from a core collection of the USDA pea collection	SNP	Association mapping	Multiple location and years	2, 3, 4, 5	0.05-0.07	4	57
Ca	158 RIL from Aragorn x Kiflica	1684 markers (SSR and SNP), 7 LG, 1310.1 cM	CIM	Whitlow and Spillman, 2014	4, 5, 7	2.4-31.0	5	60
Ca	285 accessions from a core collection from USDA	15 SSR, 36 RAPD, 1 SCAR	Association mapping	-	Not assigned	0.03-1	1	21
Cu	285 accessions from a core collection from USDA	15 SSR, 36 RAPD, 1 SCAR	Association mapping	-	Not assigned	0.02-6	1	21
Fe	94 accessions	SNP	Association mapping	Saskatoon and Rosthern, 2011 and 2012	1,3,4,5,7, unnasigned LG	0	15	67
Fe	158 RIL from Aragorn x Kiflica	1684 markers (SSR and SNP), 7 LG, 1310.1 cM	CIM	Whitlow and Spillman, 2014	2, 5, 7	6.6-19.4	5	60
Fe	285 accessions from a core collection from USDA	15 SSR, 36 RAPD, 1 SCAR	Association mapping	-	Not assigned	0.02-3	1	21
Fe	94 RIL derived from Orb x CDC Striker,	1866 SNP, 14 LG, 951.9 cM	CIM	Two locations over three years	2b, 3b, 4b, 6	10.33-26.67	5	87
Fe	94 RIL derived from Carrera x CDC Striker	3355 SNP, 15 LG, 1008.8 cM	CIM	Two locations over three years	3a, 3b, 4, 5b, 5c, 7a	10.85-35.52	14	87
Mg	384 accessions from a core collection of the USDA pea collection	SNP	Association mapping	Multiple location and years	3	0.05	1	57
Mg	158 RIL from Aragorn x Kiflica	1684 markers (SSR and SNP), 7 LG, 1310.1 cM	CIM	Whitlow and Spillman, 2014	3, 4, 5	4.7-43.3	4	60
Mn	158 RIL from Aragorn x Kiflica	1684 markers (SSR and SNP), 7 LG, 1310.1 cM	CIM	Whitlow and Spillman, 2014	1, 2, 4, 5, 7	3.6-29.9	5	60
Mo	158 RIL from Aragorn x Kiflica	1684 markers (SSR and SNP), 7 LG, 1310.1 cM	CIM	Whitlow and Spillman, 2014	5	33.0-34.2	1	60
Mo	285 accessions from a core collection from USDA	15 SSR, 36 RAPD, 1 SCAR	Association mapping	-	Not assigned	0.026-0.036	2	21
Ni	285 accessions from a core collection from USDA	15 SSR, 36 RAPD, 1 SCAR	Association mapping	-	Not assigned	0.016-0.022	3	21
P	285 accessions from a core collection from USDA	15 SSR, 36 RAPD, 1 SCAR	Association mapping	-	Not assigned	0.028-0.034	2	21
P	158 RIL from Aragorn x Kiflica	1684 markers (SSR and SNP), 7 LG, 1310.1 cM	CIM	Whitlow and Spillman, 2014	3, 5, 7	5.9-15	5	60
K	285 accessions from a core collection from USDA	15 SSR, 36 RAPD, 1 SCAR	Association mapping	-	Not assigned	0.025	1	21
K	158 RIL from Aragorn x Kiflica	1684 markers (SSR and SNP), 7 LG, 1310.1 cM	CIM	Whitlow and Spillman, 2014	3, 4, 5, 7	3.8-43.0	6	60
S	158 RIL from Aragorn x	1684 markers (SSR	CIM	Whitlow and	3, 5, 6, 7	5.6-	5	60

	Kiflica	and SNP), 7 LG, 1310.1 cM		Spillman, 2014		16.3		
Se	94 RIL derived from Orb x CDC Striker	1866 SNP, 14 LG, 951.9 cM	CIM	Two locations over three years	4a, 5a, 7	7.14-18.76	4	87
Se	94 RIL derived from Carrera x CDC Striker	3355 SNP, 15 LG, 1008.8 cM	CIM	Two locations over three years	2b, 4, 5a, 5b, 7a, 7b	-	9	87
Zn	94 accessions	SNP	Association mapping	Saskatoon and Rosthern, 2011 and 2012	3	-	6	67
Zn	158 RIL from Aragorn x Kiflica	1684 markers (SSR and SNP), 7 LG, 1310.1 cM	CIM	Whitlow and Spillman, 2014	2, 3, 5, 7	9.1-14.7	5	60
Zn	94 RIL derived from Orb x CDC Striker	1866 SNP, 14 LG, 951.9 cM	CIM	Two locations over three years	1a, 3b, 6	12.72-25.83	4	87
Zn	94 RIL derived from Carrera x CDC Striker	3355 SNP, 15 LG, 1008.8 cM	CIM	Two locations over three years	1a,1b, 2b, 3b, 4, 7a	6.34-50.13	15	87
Phityc acid	94 RIL derived from 1–2347-144 x CDC Meadow	3408 SNP, 12 LG, 914.2 cM	CIM	Two locations over four years	3a, 5, 6a	16.09-33.19	9	87

CIM: composite interval mapping, LG: linkage groups; RAPD: random amplified polymorphic DNA, RIL: Recombinant inbred line; SCARS: sequence characterized amplified regions, SNP: single nucleotide polymorphisms, SSR: Simple sequence repeats, USDA: United States Department of Agriculture.

B: boron, Ca: calcium, Cu: copper, Fe: iron, Mg: magnesium, Mn: manganese, Mo: molybdenum, Ni: nickel, P: phosphorus, K: potassium, Se: selenium, S: sulfur, Zn: zinc.