

Reaching for the pulse of the planet and its population

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Seeds provide humans and farm animals with a rich supply of dietary proteins, fibre, carbohydrates and micronutrients. Natural variation, mutagenesis and advances in genetics and genomics support the development of plant variants which provide better nutrient bioavailability, promote health in consumers and provide advantages to industry. As we consider the adoption of more healthy and sustainable plant-based diets, there is a renewed focus on legume crops which also provide benefits as an essential component of a sustainable agriculture. This article discusses significant compounds that are deposited in the seeds of a major legume crop, pea (*Pisum sativum*), and the possibilities for changing these using genetic variation.

Introduction

There is an urgent need to re-design the food systems which are currently used to feed and nurture the human population, as highlighted by recent research papers and reports, including those of the Intergovernmental Panel on Climate Change and the EAT–Lancet Commission. The latter report provides a comprehensive overview of the potential of our food systems to nurture and support both human health and the environment, while emphasizing that current trajectories threaten the sustainability of both. The need for the global population to have an adequate and healthy diet while causing minimal damage to our planet is clear. However, the global food system has been described as a principal driver of accelerating biodiversity loss, largely through degrading natural habitats. Furthermore, it is accepted that agriculture is a major contributor to greenhouse gas emissions and environmental pollution. Added to this is the fact that a significant proportion of the food we consume currently is linked to human ill-health, with negative consequences for health care and society.

A successful re-design of food systems to relieve pressures on land, while providing healthy and sustainable food, is needed. Legume foods rank highly among plant foods which can sustain us, while promoting human and planetary health. As nitrogen-fixing crops legumes provide savings in fertilizer use, thus minimizing emissions of N_2O , the main greenhouse gas from arable agriculture. Pulse crops are legumes which are harvested as dry seeds for food or feed, in contrast to legumes which are harvested fresh as vegetables or grown primarily for oil extraction.

The seeds of many pulse crops provide a valuable source of protein, starch and micronutrients. Although deficiencies are cited in their concentration of the

essential amino acids methionine and tryptophan, their amino acid composition is complementary to that provided by other plant foods. For example, many cereal grains contain adequate sulphur-containing amino acids (methionine and cysteine) to support human and animal health but are deficient in lysine. Pulses such as pea, on the other hand, are a rich source of lysine and a pulse-cereal diet provides a balanced amino acid profile. Antinutrients exist in pulse seeds to varying degrees, and strategies exist to diminish their effects. Largely, heat treatment is used to inactivate lectins, inhibitors of digestive enzymes, and enzymes which produce compounds associated with negative flavours.

Here we consider how genetic improvement of the composition and bioavailability of seed components could increase the acceptability of pulse food products and enhance their ability to replace a higher proportion of animal food products. A major consideration in assessing the quality of foodstuffs is digestibility or bioavailability. Recent advances in protocols which mimic human digestion conditions now permit these parameters to be assessed.

This article focusses on pea (*Pisum sativum*) with excellent genetic resources (Figures 1 and 2) and seeds that are very amenable to biochemical study. Pea seeds are now in high demand by a food industry seeking alternatives to animal products in response to a growing demand for novel plant-derived foods and high-value ingredients for sports nutrition. This has resulted in a demand for increased diversity among the many constituents that are stored within seeds and determine the functionality of end-products.

Seed protein

The ability of legumes to fix atmospheric nitrogen gives these species an ecological advantage in the colonization

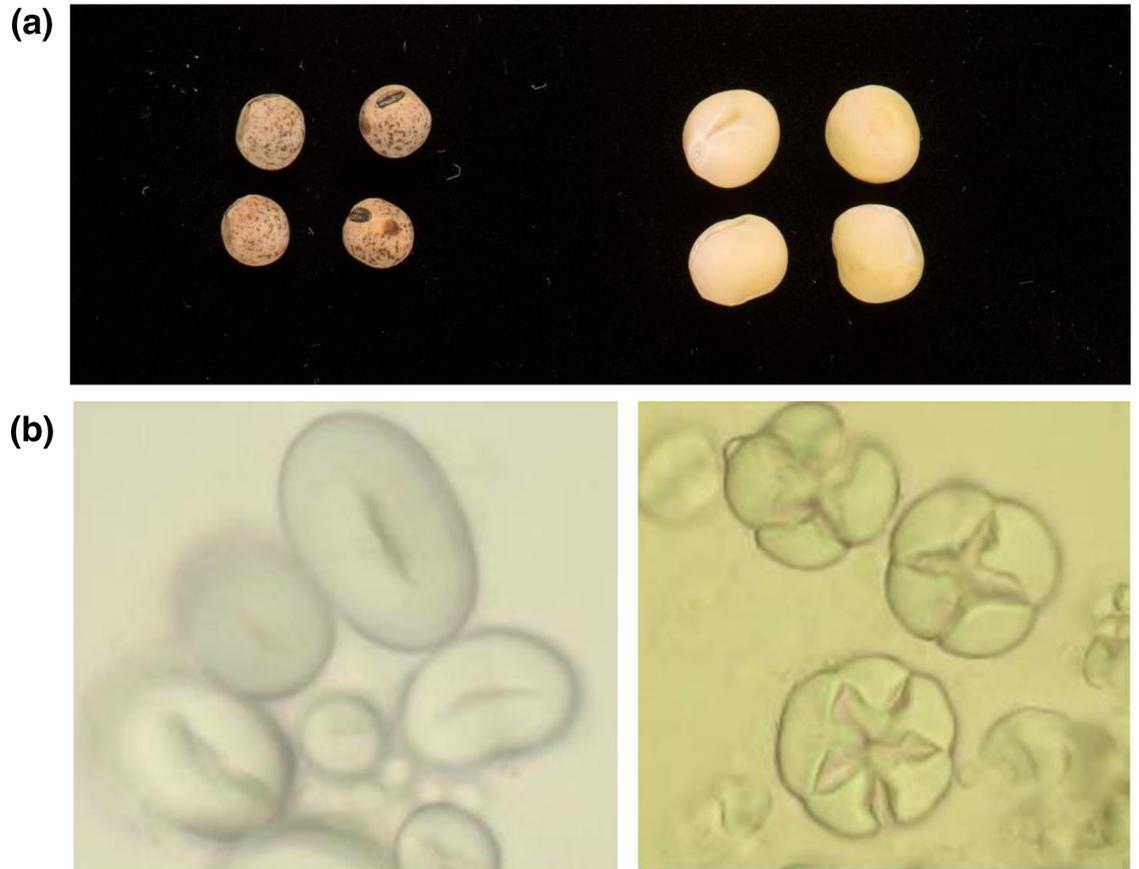


Figure 1. (a) Seeds from the wild accession collected in Turkey, which carry a mutation resulting in very low protease inhibitory activity (left), compared with seeds of a cultivar (right); (b) starch grains from control (left) and high-resistant starch (right) variants of pea.

of N-depleted soils. Their ability to sequester nitrogen and other resources is an evolutionary strategy which goes some way to account for their nutritional advantages. Most nitrogen and amino acids present in pulse seeds are stored in the form of protein. Sufficient intake of protein in the human diet is critical to well-being and is recognized as particularly important in the prevention of muscle protein loss and the onset of frailty, which can be initiated at a relatively early age. Although hydrolysis of protein and measurement of constituent amino acids provides some measure of protein quality and amino acid balance, a Protein Digestibility Corrected Amino Acid Score provides a more meaningful assessment of nutritional quality. Seed or grain storage proteins were classified historically according to solubility characteristics, whether water-soluble (albumins), salt-soluble (globulins) or alcohol-soluble (glutenins). This more than 100-year-old classification is well supported by proteomic, genetic and genomic studies across many crops, where families of proteins are now recognized in terms of both their sequence similarity and associated functional properties.

It seems likely that some abundant seed proteins have roles beyond storage. Besides a negative effect of some on predation by insects, others have been implicated in anti-microbial activity, some possess enzymatic activity, while one vicilin-related protein (p54) which is post-translationally processed, gives rise to a subunit which is located to the nucleus. Mutations which systematically remove proteins from seeds allow us to address fundamental questions on function and provide material which can be tested in an industrial setting for desirable properties for the consumer, such as emulsification, foaming and whipping. These functions, determined by protein structure, identify seed proteins which can act as alternatives to animal products, e.g., as egg-substitutes.

In pea seeds, stored proteins include the major globulin proteins, vicilin and legumin. Whereas legumins have a relatively low concentration of methionine and cysteine, most vicilins contain neither of these amino acids. In contrast, the albumin fraction includes proteins with a more balanced amino acid profile but some of these are comparatively poorly digested. One type of

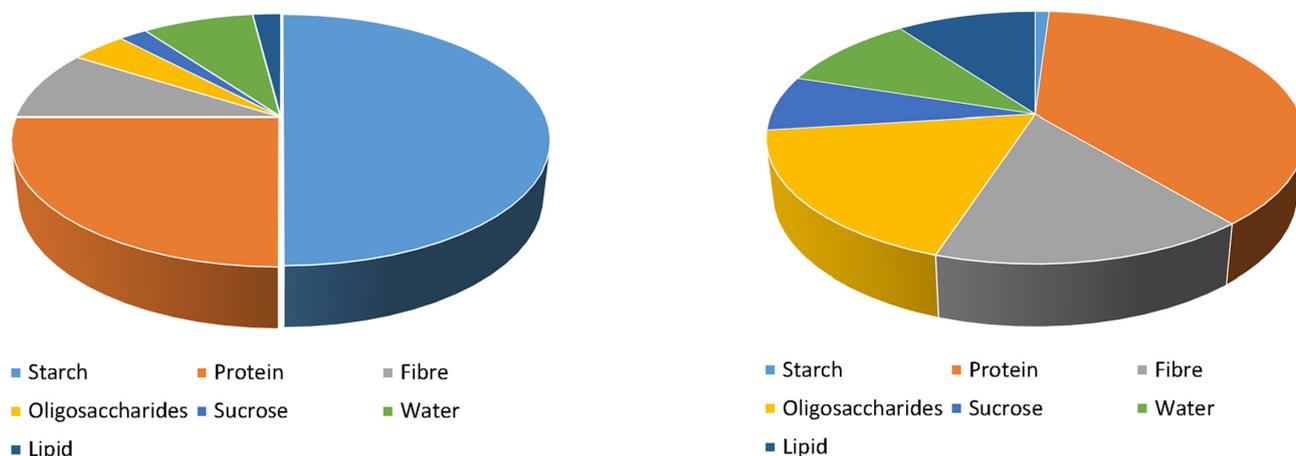


Figure 2. Average composition of wild-type pea seeds (left) compared with that of seeds derived from a phosphoglucosyltransferase mutant line (right), where the synthesis of starch has been drastically reduced.

albumin which contains 10% cysteine is classified as an anti-nutritional protein, based on its inhibition of digestive proteases. The cysteine residues contribute to extensive disulphide linkage, rendering the protein highly heat-stable and resistant to digestion. Two loops, stabilized by disulphide bonds, can simultaneously bind and inhibit the action of a trypsin and chymotrypsin enzyme molecule at each distinct inhibitory site.

Several genetic approaches have been taken to identify dispensable proteins in seeds, where their elimination might improve quality without negatively impacting yield. A first example in pea used an ethylmethanesulphonate (EMS)-mutagenized population to identify mutations affecting genes encoding the trypsin–chymotrypsin inhibitors. One mutation resulted in a cysteine being altered to a tyrosine residue, destabilizing the inhibitory loop and the protein. The specific inhibitory activity of the mutant seeds was less than half of the parental line, reflecting the fact that two genes encode the major inhibitors. In a second approach, a screen of almost 3000 *Pisum* germplasm accessions revealed a variant which carried a deletion of part of this gene and, interestingly, the same mutation was present in both genes encoding the major seed inhibitors, which are genetically closely linked. This natural variant is a *Pisum elatius* accession from Turkey. It has small seeds with a thick seed coat (Figure 1a) but could be inter-crossed readily with *Pisum sativum*, allowing the effects of the mutations to be determined more easily in progeny mutant lines. Seeds carrying the two mutant genes have extremely low inhibitory activity.

Screening germplasm also identified an accession lacking pea albumin 2, an abundant albumin which is not stored in protein bodies along with the globulin proteins. This protein has been implicated in binding polyamines within seeds of pea and *Lathyrus sativus* (grass pea)

but is associated with poor digestibility in pea and with allergenic responses to *Cicer arietinum* (chickpea). Combining the pea albumin 2 null mutant with additional nulls for proteins with poor digestibility is providing novel germplasm where the impact of major changes in protein composition on digestibility, functionality and overall amino acid composition can be investigated.

In a third approach, an alternative mutagenized population of pea has been deployed to identify null variants for genes encoding several seed proteins, targeting particularly the proteins which are encoded by several genes at any one genetic locus. Here EMS-induced mutations of single encoding genes have limited impact due to gene redundancy. In contrast, fast neutron mutagenesis can result in large genomic deletions, eliminating several genes which are in close genomic proximity. Genetic screens of such a mutant population in pea identified several deletions, which result in the loss of specific classes of vicilin-related proteins. Mutations at distinct genetic loci can be readily combined, resulting in vicilin-less mutants.

Lipoxygenase is an enzyme which is present in many plant organs. Different isoforms of this enzyme exist and some have a defence role. In seeds, however, the activity of one isoform (LOX-2) leads to the generation of an array of compounds associated with flavour. While early studies attempted to remove negative flavours, but retain positive ones, the current demand is for new bland food ingredients associated with high nutritional value. These needs are at least partially met by the discovery of a natural mutation which eliminates LOX-2 from pea.

Seed carbohydrate

Starch is the principal storage form of complex carbohydrate in plants and is stored in many seeds and

grains as amylose and amylopectin, which differ in the extent to which they are degraded during digestion. Recent studies indicate that health benefits may be derived from consumption of starch which is resistant to digestion, including slow release of glucose into the bloodstream, which can impact positively on the release of insulin. The so-called 'resistant' starch can be fermented by microbial communities in the large intestine, leading to the formation of short-chain fatty acids, which in turn benefit several aspects of human physiology.

The use of pea seeds in human intervention trials has been enabled by the availability of near-isogenic genetic stocks, differing by the presence or absence of a mutation in the starch-branching enzyme I gene (*SbeI*). The mutation reduces the concentration of amylopectin in the seeds and alters the shape of the starch grains (Figure 1b). Thicker cell walls further reduce accessibility of starch-degrading enzymes, amylases, to their substrate. The results suggest that food plants, which carry an *sbeI* or equivalent mutation, may have a role in preventing the onset of type 2 diabetes. Mutations which impair starch synthesis have secondary consequences for other polymers, including fibre (cell walls) and protein; an extreme example is a mutation affecting phosphoglucomutase activity in pea, where starch can be reduced to 1% (Figure 2).

Additional oligosaccharides which are stored in many seeds include the raffinose family of oligosaccharides. As dietary components, these cannot be hydrolysed in the human digestive tract but require the microbial or plant enzyme, α -galactosidase, to hydrolyse to D-galactose and sucrose. Overconsumption of legume products by some individuals is associated with vigorous intestinal fermentation and discomfort. Mutations which impair the early steps of this pathway may offer a strategy for the provision of nutritional benefit with greater comfort.

Micronutrients

The seeds of many pulse crops are rich in a range of micronutrients including iron, zinc and selenium, of which iron has been studied most intensively. A large proportion of the iron in pea seeds is stored as an iron-containing protein, ferritin, within plastids. Ferritin-iron extracted from pea seeds has been shown to have good bioavailability. Mutants which hyperaccumulate iron have been described in pea: *brz* (*bronze*) and *dgl* (*degenerate leaves*).

However, phytic acid accumulates during late seed maturation and reduces the bioavailability and absorption of iron, zinc, calcium and other minerals. This compound presents itself as a target for reduction through genetics.

Conclusion

Genetic variation in the macromolecules stored within seeds provides fundamental information regarding their synthesis, modification and dispensability. The same is true of micronutrients, where seeds can make an important dietary contribution, and antinutrients which can reduce the bioavailability of nutritional components. ■

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