Genetic Control of Anti Nutritional Factors and Their Dietary Implications

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ABSTRACT

Anti nutritional factors (ANFs) represent a very heterogeneous group of plant metabolites which diminish nutrient bioavailability, induce digestive retardation or cause toxicity in humans and livestock. Major ANFs are phytic acid, raffinose family oligosaccharides (RFOs), saponins, tannins, protease inhibitors, lectins, gossypol, glucosinolates, cyanogenic glycosides and oxalates, erucic acid and 2 N oxalyl L 2 -diaminopropionic acid (2 ODAP). Traditional breeding of crops has lowered the ANFs in certain crops, whereas genetic relationships and pleiotropy have slowed down. Recent developments in genomics, multi omics, marker assisted selection, RNA interference (RNAi) and CRISPR/Cas based genome editing make it possible to do ANF biosynthesis genes very specific and precise. The review is a synthesis of the information on ANF biosynthesis, dietary effects, and genetic control of them using the viewpoints of a plant breeder.

Keywords: Metabolomics, Metabolite profiling, Analytical techniques, Crop improvement, Stress tolerance, Multi-omics integration

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Introduction

Plants have a great diversity of secondary metabolites which protect against herbivores and pathogens or store nutrients or control physiology. Most of these compounds are anti nutritional in their consumption as they chelate the minerals, block digestive enzymes or release toxins and are consumed by the animal or human body [1]. ANFs are found in high amounts in legumes, oilseeds and certain cereals. Conventional food processing techniques like soaking, fermentation, heat and germination can lower ANF levels, however, all these techniques are labour intensive and have the disadvantage of destroying desirable nutrients [2]. Plant breeders are thus in high priority to develop cultivars with low concentrations of ANFs intrinsically and at the same time maintain crop performance. Reduction of ANFs genetically should be done in a way that does not affect the nutritional value of the plants; they should also be aware of the physiological functions of these compounds in plants [3]. An example is a primary phosphorus storage in seeds, phytic acid, which is involved in the myo inositol signalling pathway; high levels of phytic acid can inhibit seed germination and stress tolerance. Breeders have to determine and regulate genes that regulate ANF biosynthesis or transport and reduce adverse pleiotropic effects [4].

Figure 1: The dual role of ANFs: vital plant defenses that concurrently lock away nutrients and inhibit digestion in consumers.

Significant Anti Nutritional Factors and Dietary Impact.

Phytic acid (PA) contains phosphorus in the seed of plants but binds cations like iron, zinc and calcium, and makes them less available to humans and other animals. Genetic options are to select low phytate mutants (e.g. lpa1, lpa2 and lpa3 in maize), and marker assisted backcrossing.

Raffinose Family Oligosaccharides.

Raffinose family oligosaccharides (RFOs) raffinose, stachyose and verbascose are carbon reserves and prevent desiccation. But human beings do not have the α galactosidase enzyme to break down the sugars causing intestinal fermentation and flatulence [5]. Low levels of RFO generated by breeding or gene editing enhance the digestibility of legumes.

Saponins

Saponins are amphipathic glycosides which give them the property of being bitter and foaming. Selective breeding is based on low saponin genotypes or silencing triterpenoid biosynthetic genes; as an example, RNAi silencing of β amyrin synthase decreases the level of saponins in soybean [6].

Tannins

Breeding has taken advantage of less alleles that are recessive tannin (e.g. in lentil and faba bean), and full elimination may make it easier to pest attack, necessitating integrated pest management [7].

Protease Inhibitors

Other inhibitors of trypsin, like Kunitz trypsin inhibitors (KTIs) and Bowman-Birk inhibitors (BBIs) are used in inhibiting digestive enzymes, leading to hypertrophy of the pancreas, and decreasing animal growth [8].

Lectins and Proteins that are Allergenic.

Lectins are carbohydrate binding proteins, which agglutes red blood cells and may result in gastrointestinal distress. IgE mediated reactions are elicited by soybean lectin and seed allergens including Gly m Bd 30K (GmP34) [9].

Gossypol

Gossypol is a cotton (Gossypium spp.) phenolic aldehyde, which contains insect- and pathogen-protective properties. Knockout of GhCAD genes decreased gossypol levels by at least 64% in seeds and leaves; editing GhCAD1 A alone resulted in a reduction of gossypol in seeds by nearly 46% and no reduction of gossypol in leaves, leading to pest resistance [10].

Goitrogens and Glucosinolates.

Glucosinolates Brassica crops have sulfur containing compounds that hydrolyse to isothiocyanates, nitriles and goitrin. Moderation will decrease the risk of cancer, whereas excessive intake will affect the state of the thyroid negatively by blocking the absorption of iodine [11]

Oxalates

Oxalates form insoluble complex with calcium and magnesium which leads to the formation of kidney stones. Oxalates are accumulated in crops like spinach, beet and taro [12].

Erucic Acid

Mustard and rapeseed oils contain a very long chain monounsaturated fatty acid, erucic acid. Cardiac lipidosis is related to high levels (> 2% of total oil) [13]. Introgression of recessive alleles of FAE1 gene (fatty acid elongase) by breeding of so called double low canola varieties substituted high erucic cultivars.

Genetic Control and Strategies of breeding.

Traditional breeding and mutagenesis.

Selection has been traditionally used by breeders to reduce ANFs, induced mutagenesis and backcrossing. Maize (lpa1/lpa2/lpa3) and common bean have been found to have mutants with low PA (lpa), but there are mutations that decrease seed weight and germination [14]. Non-pigment glandless varieties of cotton are resistant to gossypol, but vulnerable to insects; breeding species of g2 and g3 mutated alleles backcrossed onto cultivar elite lines is a more moderate solution [15]. Introgression of low glucosinolate alleles into rapeseed and low tannin alleles into lentill has been speeded up by Marker assisted selection [16]. However, genetic association and pleiotropic penetration tend to frustrate developments.

Modern Biotechnologies

RNA Interference

RNAi gene silences by destroying the mRNA of the target gene to provide tissue-specific silencing of ANFs [17]. Transgenic soybean with the expression of dsRNA against Kunitz trypsin inhibitors genes gives rise to seeds with KTI free without any change in leaf expression. Specific RNAi of the gene 2 amyrin synthase decreases saponins in soybean and RNAi of MIPS (myo inositol 3 phosphate synthase) decreases phytic acid in rice.

Genome Editing

The ANF genes can be modified in a very specific way through CRISPR/Cas systems, which have revolutionized breeding of plants [18]. ANFs reduction examples of genome editing include:

- CRISPR/Cas9 knockouts of GhCAD and PGF (cotton) show that disruption of GhCAD gene decreases seed gossypol content by 64 and disruption of GhCAD1 A A decreases seed gossypol by 46, and does not affect leaf gossypol. The modification of PGF or GhDIR5 leads to seed selective low gossypol cotton plants [19].
- The knockout of the MeCYP79D1 gene (cassava) reduced the levels of linamarin and evolved cyanide to a maximum of seven folds, but did not prevent cyanogenic glycosides [20].
- The GMp34 and homologs - Multiplex CRISPR manipulation of GmP34 allele of the allergen gene and homologs generate soybean without the protein that causes allergy [21].
- FAE1, FAD2 and ECR genes - Genes editing fatty acid elongase(FAE1) and desaturase(FAD2) genes of oilseed rape can reduce the level of erucic acid and alter fatty acid compositions; editing of BnFAE1 can abolish the accumulation of erucic acid [22].

Nevertheless, off target effects, low homology directed repair efficacy and regulatory control are issues.

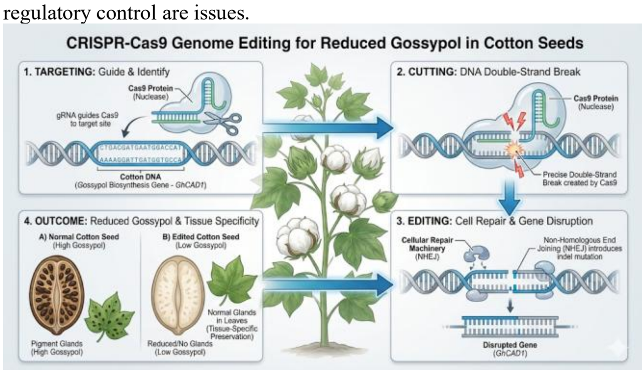

Figure 2: Precision genome editing (e.g., CRISPR-Cas9) disrupts specific ANF genes, reducing toxins while preserving nutritional and agronomic value.

Food Implications of ANFs Reduction.

The decrease in ANFs can have a great impact on the nutrition of humans and the productivity of livestock. This would reduce allergenic proteins such as GmP34 to decrease the allergy to soy induced and reduce β ODAP to decrease neurolathyrism. Breeders however need to think about trade-offs ANFs can be sources of plant defence, seed longevity or resistance to stress. The complete eradication may lead to making it more vulnerable to pests or decreasing the seed viability. Therefore, there is a need to have a middle way solution between partial reduction and agronomic solutions [23].

Difficulties and Future Projections.

Despite significant improvement, there are still some issues. Of significant concern are pleiotropic effects; low phytate mutations can lead to reduced seed weight or stress tolerance, and gossypol reduction may lead to a loss of pest resistance [24]. It might be necessary to edit several genes to realize important ANF decreases without affecting the performance of the plants. Gene edited crop regulatory frameworks in the world are different and affect commercialisation. Future studies must employ systems biology to determine the master regulators of ANF pathways, use base and prime editing to make specific alterations and combine biofortification with ANF depletion [25]. Other methods that can be utilized by breeders to control ANFs without altering DNA permanently are gene drive systems and epigenetic editing. Significantly, field analyses in a variety of settings are required to know the interactions between genotypes and the environment and to be able to maintain the trait constancy.

Conclusion

Anti nutritional factors are inherent constituents of plant metabolism that may be of a challenge to nutrition and food safety. Traditional breeding has been successful to some extent in the reduction of ANFs, however with newer developments in genomics, RNAi and above all, CRISPR/Cas genome editing, it would be possible to precisely manipulate ANF biosynthesis genes. The examples of cotton, cassava, soybean and grass pea case studies show how genome editing can be used to develop low ANF cultivars without compromising agronomic performance. Plant breeders are central in the process of integrating such technologies, they help to find the right alleles and make certain that nutritional enhancement does not interfere with plant resilience. The application of biofortification with specific gene editing into breeding programs in the future is likely to provide nutritious yet robust crops.

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