Cereals breeding challenges to reduce anti-nutritional factors from feed

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Cereal grains, such as wheat, maize, barley, sorghum and oats are foundational components of animal feed globally, providing essential nutrients such as carbohydrates, proteins, lipids, vitamins, and minerals. In addition, cereal grains have high amounts of dietary fiber and antioxidants which have beneficial effects on human health linked with the utilization of whole grain products. The **germ** has high content of vitamins B and E, unsaturated fats, high quality protein, carbohydrates, and minerals while the **bran** mostly consist insoluble carbohydrates such as cellulose, antioxidants, protein, traces of vitamin B and minerals, and also **anti-nutritional components like phytic acid**. In cereal grain endosperm is the major component consisting mainly of starch and protein, and small amount of minerals and vitamins (Ram et al., 2020)

Despite their nutritional benefits, cereals often contain various **anti-nutritional factors (ANFs)** that can impair animal growth, nutrient absorption, and overall health. These ANFs include phytic acid, tannins, non-starch polysaccharides (NSPs), protease inhibitors, and lectins, among others. Overcoming the challenge of ANFs in animal feed is increasingly relevant as the demand for efficient and sustainable animal production rises. Cereal breeding, particularly through advanced biotechnological methods, has emerged as a promising strategy to reduce ANFs while maintaining or enhancing the nutritional quality of feed. However, numerous challenges hinder progress in breeding cereals with reduced ANF levels. **This paper** explores these challenges and the future directions for cereal breeding to improve animal feed quality.

The Impact of Anti-Nutritional Factors in Cereal Feed

Anti-nutritional factors have negatively affect on nutrient bioavailability and the digestibility of animal feed.

Phytic acid (identified as inositol hexaphosphate (IP6), or phytate the primary storage form of phosphorus in several plant tissues), for instance, is a primary ANF in many cereals, where it binds essential minerals such as calcium, magnesium, and zinc, reducing their bioavailability to monogastric animals like pigs and poultry and humans, which lack the enzyme **phytase** to break down phytate-bound phosphorus (Kumar et al., 2010, Ram et al., 2020). Other ANFs, such as **tannins**, interfere with protein digestion and reduce amino acid availability, impacting growth rates and feed efficiency (Reddy, 2002). **Protease inhibitors** and **lectins** inhibit enzymes involved in protein and starch digestion, potentially causing gastrointestinal distress in animals (Gilani et al., 2005).

Tannins - because they precipitate proteins, block digestive enzymes, and interfere with the absorption of vitamins and minerals, tannins are regarded as nutritionally undesirable. Excessive tannin consumption can have negative health effects (Ram et al., 2020). The seed coat of grains contains the majority of the tannins.

Protease inhibitors (PIs) - The regulation of proteolytic activity depends on protease inhibitors (PIs), which are tiny molecules that prevent proteases from doing their jobs (Rawlings et al., 2004). PIs interact with their substrate in two most typical ways that are found in nature (Rawlings et al., 2004). Irreversible trapping reactions are the first type, where the inhibitor's internal peptide bond is cleaved by PI contact, changing its conformation. Since inhibitors are irreversible and never restore their former structure, they are sometimes known as suicide inhibitors. Second, inhibitor-substrate interaction is comparable to that of enzyme-substrate interaction in reversible tight-binding interactions. The intact version of the inhibitor and its changed variants coexist in balance within the protease inhibitor complex.

The protease inhibitor complex co-exists in equilibrium among the intact form of the inhibitor and the modified forms of the inhibitor enabling inhibitor to be dissociated to its intact or its modified form (Ram et al., 2020).

Lectins are proteins that bind to a specific carbohydrate reversibly without altering their structure. Wheat germ agglutinin (WGA) is a specific lectin that has been designated extensively for their adverse effects on health.

Thus, reducing these ANFs in cereal feed is essential for optimizing animal productivity and minimizing the need for supplementary feed additives.

Challenges in Cereal Breeding for Reduced Anti-Nutritional Factors

1. ***Genetic Complexity of Anti-Nutritional Factors***

Many ANFs are complex traits controlled by multiple genes, and environmental factors also influence their expression. For instance, phytic acid accumulation involves several metabolic pathways, making it challenging to identify and target specific genes for modification. Tannins and protease inhibitors are secondary metabolites, with biosynthesis regulated by a network of genes responding to various stresses (Venkatesh et al., 2018). Thus, selecting traits related to ANF reduction often requires sophisticated genomic tools and significant research into the genetic basis of these factors.

2. ***Trade-Offs Between Nutritional Quality and Plant Defense Mechanisms***

Many ANFs play a protective role in plants, defending against pests, pathogens, and environmental stressors. Tannins, for example, contribute to the plant's defense against herbivory and microbial invasion. Reducing these compounds may render cereal crops more susceptible to diseases or pests, requiring additional inputs in the form of pesticides or herbicides (Simmonds, 2003). Achieving an optimal balance between reduced ANF levels and maintaining plant resilience is a central challenge for breeders.

3. ***Limited Genetic Variability in Commercial Cereal Varieties***

Most modern cereal varieties have undergone extensive selective breeding, often for high yields and resistance to specific diseases. This has resulted in reduced genetic diversity in commercial cultivars, limiting the available genetic pool for ANF-related traits. Wild relatives of cereals and landraces, which may harbor desirable traits such as lower levels of specific ANFs, have been identified as potential sources of genetic material. However, introgression these traits into high-yielding commercial lines through conventional breeding is time-consuming and challenging due to genetic incompatibilities and undesired traits linked to lower yield or adaptability (Dwivedi et al., 2008).

4. ***Difficulty in Measuring and Screening ANF Levels***

Accurately measuring ANFs in cereal grains is essential for breeding programs, but it remains labor-intensive and costly. Analytical methods such as high-performance liquid chromatography (HPLC) and spectrophotometry, although precise, are not feasible for large-scale screening of breeding populations. As a result, breeders face limitations in efficiently evaluating large numbers of samples for ANF content, slowing down the breeding process (Liu et al., 2015).

5. ***Public Acceptance and Regulatory Hurdles***

Modern techniques like genetic engineering and genome editing hold significant promise for reducing ANF levels in cereals. For instance, CRISPR-Cas9 has been used to modify genes involved in phytic acid biosynthesis, reducing phytic acid content while maintaining yield (Liang et al., 2017). However, public skepticism and stringent regulatory requirements for genetically modified organisms (GMOs) complicate the development and commercialization of such crops. Consumer concerns about the safety and environmental impact of GM crops may deter breeding efforts, limiting the application of these technologies in reducing ANFs.

6. ***Adaptation to Diverse Environmental Conditions***

Climate variability adds another layer of complexity, as ANF expression can fluctuate based on environmental conditions. For instance, environmental stressors such as drought or nutrient deficiency can enhance ANF production as a protective response in plants. Consequently, cereals bred to have reduced ANFs under controlled conditions may exhibit increased ANF levels in response to field stress, impacting feed quality unpredictably (Savary et al., 2017). Breeders must therefore develop varieties that can maintain low ANF levels across diverse environmental conditions, which can require extensive multi-location field trials.

Current Strategies and Future Directions

1. ***Marker-Assisted Selection (MAS) and Genomic Selection (GS)***

MAS and GS have improved breeding efficiency by enabling the selection of ANF-related traits based on genetic markers. These techniques help breeders focus on favorable alleles, especially for traits with low heritability or complex inheritance. However, despite the potential, these methods require extensive genetic information and are dependent on well-developed reference genomes, which are not always available for all cereal crops (Varshney et al., 2014).

2. ***Biotechnological Approaches***

Genetic engineering, such as CRISPR-Cas9, is promising for reducing ANFs by directly targeting genes involved in ANF biosynthesis pathways. Additionally, RNA interference (RNAi) can silence genes responsible for ANF accumulation, effectively reducing their levels in the plant (Zhang et al., 2020). Despite the promise, adoption depends heavily on overcoming public resistance and regulatory hurdles.

3. ***Exploiting Genetic Diversity from Wild Relatives***

Pre-breeding programs using wild relatives of cereals are exploring the introgression of naturally low-ANF traits into commercial lines. For example, some wild barley varieties exhibit reduced levels of protease inhibitors and NSPs, presenting opportunities to transfer these traits into cultivated varieties. This approach can increase genetic diversity and resilience to environmental stressors (Dempewolf et al., 2017).

4. ***Breeding for Enhanced Digestibility and Nutritional Enhancements***

Apart from reducing ANFs, breeders are also exploring approaches to enhance the digestibility of nutrients in cereals, either by increasing the production of endogenous phytase or by optimizing the amino acid profile of cereal proteins. Such enhancements can partially offset the impact of ANFs, making feed more efficient (Shewry & Hey, 2015).

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