

Enhancing wheat (*Triticum Aestivum* L.) Yield Through Genetic Modification

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Abstract

Triticum aestivum L. or bread wheat, is an important crop species grown as a primary food source. With the increasing population, finding new ways to grow wheat genotypes with better qualities is important. The current task is to create varieties that produce more, are highly technologically advanced, and can handle a wide range of biotic and abiotic stresses. It is important to make healthier wheat types, like those with higher amounts of protein, zinc, iron, and other nutrients, because people's nutritional needs are worsening.

Keywords: bread wheat; biotic and abiotic stress; nutrients; genetically modified

Introduction

Triticum spp. is a type of grass that is in the tribe Triticeae and the family Poaceae. For each group, the genus has between 5 and 27 species (Yen & Yang, 2020). Depending upon the uses, wheat is usually grown in two main kinds: bread wheat (*Triticum aestivum* L.) and durum wheat (*Triticum durum* L.). There are still uses for wild types of wheat because they have traits that can be used to make cultivated wheats even better. Much work has gone into changing wheat hybrids' chromosomes and improving wheat's genes by crossing them with wild type wheat plants. Changes in pairing control mechanisms and forced translocations are some of the ways scientists have moved disease- and pest-resistant genes from annual (like rye) or permanent Triticeae plants to other plants in the same family. It is now easy to find traits people want and add genes to wheat. It is now easier to add foreign genes to different kinds of wheat through gene transfer techniques. This is what led to the development of modified bread and durum wheat. Studies by Munaweera et al. (2022) and Gomez et al. (2021) say that transgenic technology has not only made wheat resistant to pesticides but has also changed the protein and starch profiles of the grains to make them better. Biofortification of food crops is another fascinating topic right now. This means adding more micronutrients (like vitamins and minerals) by breeding plants or using transgenic methods. Gene transfer is a technique that improves quickly and is likely to be very important in plant breeding. Biotechnology advances will not completely replace traditional ways of growing plants, but will instead work with them (Tylecote, 2019). Evolution of wheat has been a complicated and interesting process. For example, it comes from

the Fertile Crescent in Asia and some parts of Africa, mainly from Syria to Kashmir and further south to Ethiopia. It is thought that taming likely happened near Diyarbakir in Turkey, as shown by the genetic links between einkorn and emmer. While farming was spreading during the Neolithic period, wheat became common in places like India, Great Britain, Ireland, and Spain. China didn't get it until a thousand years later (Bittman, 2021; Headrick, 2020). All types of wheat belong to the genus *Triticum*. They can be broken down into three groups based on how many chromosomes they have in their cells. These types have between 14 and 42 chromosomes, called chromosomes. The different types of *T. aestivum*, or bread wheat, are grouped by how they grow and the structure of their kernels. For example, they can grow in the winter or spring, have white or red berries, or be hard or soft. According to Garvin and Dykes (2021), the D set of chromosomes is what gives these cultivars their special milling and baking qualities. It is amazing how well wheat can grow in a variety of climates. It may be grown in various settings, from the Arctic Circle to higher heights near the equator and at different altitudes, even though it is commonly grown in temperate climates with an ideal temperature of 25°C. Because it can be grown in short seasons and produces a lot of grain, wheat is very valuable in foreign trade (Ali et al., 2016; Asif et al., 2020; Metwally et al., 2020). Wheat's history is long and interesting, and it's been through many changes that J. Phys. Biomed. Biol. Sci. Volume, 2: 11 Abbas et al., (2023) 2 have made it so important in world trade and agricultural production. Around the middle of the 20th century, wheat breeders had to deal with a big problem. The output growth

rate was not keep up with the fast population growth. As the Mexican government asked and with support from the US, a program began in 1944 to create wheat varieties that were immune to disease and produced a lot of grain. Norman Borlaug and his team first made disease-resistant varieties, and then they crossed them with the Japanese dwarf variety Norin 10 to make semi-dwarf, high-yielding, and disease-resistant wheat varieties (Azzopardi; Farooq et al., 2021; Ghafoor et al., 2020; Kashif et al., 2021). The "Green Revolution" began with these new types, and many countries, like India, Pakistan, and China, could produce enough food to feed their growing populations (Ameen & Raza, 2017; Conway, 2019). Slovakian farmers grew tall traditional wheat types (landraces) with low seed yield before the Green Revolution. They finally began using wheat varieties from the Czech Republic and the Soviet Union. But since 1967, the country hasn't grown any Slovakian wines. There was a lot of success in breeding wheat in Slovakia between 1945 and 1970, when sixteen breeding stations were opening. Eventually, modern semi-dwarf varieties with higher yields and better disease protection replaced the traditional tall varieties. This is what other countries around the world have also seen happen. The National List of Released Varieties had 47 wheat varieties from Slovakia from 1976 to 2008 (Adhikari et al., 2022; Bilgin et al., 2016). Wheat production must double to 1200 m MT by 2025 to meet future and growing world needs. This growth must come from making already-cultivated land more productive, not cutting down forests or grasslands. Because wheat and its close relatives don't have a lot of genetic diversity, standard breeding methods, and even marker-assisted breeding methods aren't likely to lead to such big yield increases (Edge et al., 2018; Iqra et al., 2020; Masood et al., 2015; Ngcamphalala, 2018). Because of this, we need to find new ways to include helpful genes from any living thing, like microorganisms, plants, or animals, to get the enormous amounts needed. This means cutting down on or getting rid of the production losses caused by pests, pathogens, weeds, drought, salinity, and food going bad after harvest during storage. Improving the plant's basic physiology, like making photosynthetic processes more efficient and improving how it uses nutrients, is also important for higher outputs (Nowicka et al., 2018). Plant breeders also need to improve the nutritional value of wheat as a staple food because more and more people are not getting enough micronutrients (Das et al., 2019; Naseem et al., 2020; Nawaz et al., 2020; Shelenga et al., 2021). Getting these goals done requires genetic engineering to add new, sometimes unknown, genes to different types of wheat. This creates transgenic kinds with the desired traits (Borisjuk et al., 2019; Yali, 2022). But, this process is currently not widely used in breeding efforts because it is costly. Even though genomics research could make crop improvement much more effective and

accurate, it is not meant to replace current breeding and testing methods fully (Gao, 2021; Mir et al., 2019; Wang et al., 2018). Genetic trait enhancement strategies Different types of landrace cereal crops have different genes that are important for farming. These landraces are in the same main gene pool as the crop species they belong to, which makes it easy for them to breed with each other. There is also a high amount of chromosome pairing between the two plants, which makes it easier for foreign genes to move from the landrace to the crop plant (Mir et al., 2019). Gene transfer from non-native donors into cereal crops requires chromosome pairing between the donor and recipient organisms. Although this is a straightforward procedure in diploid cereals, hexaploid wheat offers a problem due to three genomes and genetic control impacting matching (Parray et al., 2019). Here, the homologous pairing suppressor gene Ph1 is essential for maintaining stable inheritance through diploid-like pairing. But Ph1 also prevents chromosomes from wheat and foreign species from pairing, which makes it hard to transfer alien genes. Successful gene transfer between wheat and alien species is now possible because of the discovery of techniques to decrease Ph1 (Johansson, Henriksson, et al., 2020; Singh, 2018). Wheat has had many disease-resistant genes introduced through selective hybridization and chromosomal modification. There are still barriers to the widespread application of transformation technologies based on protoplasts, such as *Agrobacterium*'s inability to infect monocots (Mohammed et al., 2019; Su et al., 2023). However, in the last quarter century, the use of *Agrobacterium* for genetic transformation has progressed considerably, allowing for the successful transfer of genes in some cereal crops (Guo et al., 2019; Wang et al., 2020). In the last 25 years, scientists have made great use of the unique ability of *Agrobacterium tumefaciens*, a bacterium that lives in soil and causes crown gall disease, to move DNA into the genomes of damaged plant cells (Kuzmanović et al., 2018). Many different types of higher plants have had their genes changed using this method Figure. 2. *Agrobacterium* is known to infect many different types of plants, but it only does so on monocots (one-celled plants) very rarely (Zaman, 2019). Cereal foods, which are very important to the economy, are one example. People used to think that the fact that *Agrobacterium* couldn't infect monocots in its natural setting was a big reason why it couldn't get into cereal crops and change them (Riseh et al., 2022). There was a delay in the transformation of cereal by *Agrobacterium* because damaged cereal cells could not respond well. However, this problem was solved by growing embryogenic cells actively dividing along with highly virulent strains of *Agrobacterium tumefaciens* (Gheysen et al., 2022). This was done with acetosyringone, a potent inducer of virulence genes (Gheysen et al., 2022; Jain et al., 2022; Pratiwi & Surya, 2020).

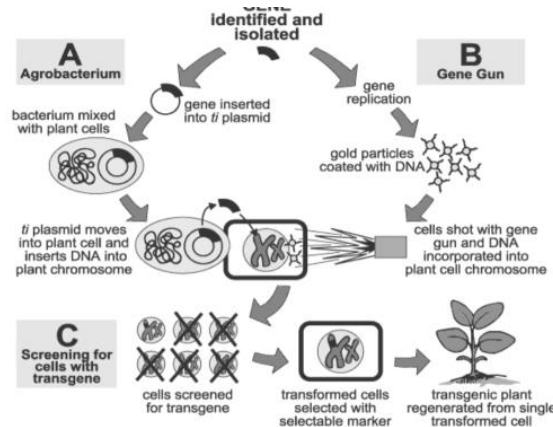


Figure 1 Possible ways to improve the genetics of wheat. A: transformation by *Agrobacterium*, B: biological way

bombardment, which uses tiny projectiles covered with DNA (see Figure 1), is the most common way to get DNA into plant tissues and callus. When SANFORD and his colleagues developed this method in 2000, it solved many problems using protoplasts. It quickly became the best way to change cereals, especially wheat (Derman, 2020; Purwantoro et al., 2022). To get the best effects, there must be a balance between how deeply the particles go into the tissue and how much damage they do. Early on, scientists were able to grow transgenic wheat plants. They used bombardment to insert the pBARGUS plasmid, which had the maize Adh1 promoter and intron 1 driving the GUS reporter gene, along with the CaMV35S promoter driving the bar gene that made the plants resistant to Basta pesticide (Dosad & Chawla, 2018; Shrawat & Armstrong, 2018). Later, different plasmids and regulators were used to improve the production of transgenic wheat plants. Randomly adding transgenes to the genome didn't cause any changes to the chromosomes, and the expression of the genes depended more on the promoters used than the integration site (Lowe et al., 2018). Biolistic methods and systems built on *Agrobacterium* have some things in common. Their change rates are about the same (Gheysen et al., 2022), and they both use the same target explant tissues. Both methods are also affected by the changing plant genetics (Bednarek & Orłowska, 2020). Recent research, on the other hand, has shown that the supposed problems with particle bombardment, like the fact that it can make transgene arrays that are too big, messed up, or broken (Ozyigit & Yucebilgili Kurtoglu, 2020), don't happen when *Agrobacterium* assists transformation. In-depth structural studies of transgene loci made by each method have not shown any major differences. Also, there isn't strong proof that these two methods of transformation make a big difference in the amounts of transgene instability and silencing in important cereal crops (Clark & Maselko, 2020; Tonosaki et al., 2022). Resistance to herbicides, pathogens, and pests Weeds compete with crop plants for nutrients and light, reducing crop yields. Weeds reduce wheat productivity by 12.3% worldwide and 23.9% without crop protection (Acharya et al., 2022; Zahan et al., 2021). Due to wheat's ability to naturally break down only one herbicide, several weed species in wheat fields have evolved herbicide resistance (Hussain et al., 2022). The production of herbicide-resistant crops has involved inserting one or two genes that can deactivate the herbicide in one of three ways: a) overproduction of an herbicide-sensitive biochemical target, b) structural changes in the biochemical J. Phys. Biomed. Biol. Sci. Volume, 2: 11 Abbas et al., (2023) 4 target's binding to the herbicide, c) detoxification or degradation before the herbicide reaches its target site in the plant cell. Roundup uses glyphosate. Glyphosate-tolerant wheat plants have grown and reproduced without harming yield (Meftaul et al., 2020; Sarwar et al., 2022). Recent advances have created a highly effective detoxifying enzyme that protects wheat plants against glyphosate and weeds (Nakka et al., 2019; Padgett et al., 2018). Many fungal diseases hurt wheat production around the world. These include rust caused by *Puccinia* spp., smut and bunt caused by *Tilletia* and *Ustilago* spp., blotch caused by *Septoria* spp., *Fusarium* blight/scab, *Helminthosporium* leaf blight, powdery mildew caused by *Blumeria graminis*, and bacterial leaf streak caused by *Xanthomonas translucens* (Ngwangum et al., 2022). Estimates show that these pathogens cause a 12.4% drop in wheat production, which can go up to 16.7% without good crop protection (Jägermeyr et al., 2020). However, chemical treatments and cultural practices can only guard against these diseases to a certain extent. It is hard and takes a long time to make resistant cultivars because many genes control resistance. Even when resistant cultivars are made, they might not be enough in the long run because pathogens change (Nelson et al., 2018). There haven't been many successes in making wheat resistant to the main

fungus and viral pathogens that affect it. One study put the coat protein gene from the barley yellow mosaic virus into wheat, but it wasn't said whether it worked against pathogens (Pretorius, 2018; Sarwar et al., 2021; Shafique et al., 2020). The *rnc70* gene, which codes for a changed form of bacterial ribonuclease III, made transgenic wheat plants resistant to the barley stripe mosaic virus (Shrawat & Armstrong, 2018). The wheat plants that had the viral replicate gene *Nib* or the coat protein gene of the wheat streak mosaic virus were also resistant to the virus in the lab, but they weren't resistant in the field and produced less than their parent cultivars (Ljubojić, 2021). Overall, there hasn't been much progress in making wheat resistant to these bugs. Genetically altering the *PINA* gene could control several plant diseases. Morris (2019) created altered durum wheat cultivars that express the antibacterial and antifungal *PINA* protein. These transgenic plants were more leaf rust-resistant in greenhouses and fields. The inclusion of barley seed class II chitinase (*pr3*) and apoplastic ribosome inactivation protein (*RIP*) genes enhances powdery mildew resistance (Zribi et al., 2021). In other research, Zribi et al. (2021) showed that expressing genes for an *Aspergillus giganteum* antifungal protein and a barley class II chitinase reduced powdery mildew and leaf rust. Developing wheat lines resistant to diseases that reduce production requires additional research. The mapping of the *Lr10* leaf rust resistance gene, the *Fhb1* gene for *Fusarium* head blight resistance, and the *Yr34* stripe rust resistance gene will help isolate and sequence relevant genes for marker-assisted breeding and genetic transformation of resistant varieties (Börner, 2021; Ghimire et al., 2020). Bugs reduce wheat output by 9.3% worldwide, which can rise to 11.3% without crop protection, but the timeconsuming breeding process and pesticide-resistant bugs have limited wheat resistance gene introduction (Liu et al., 2019; Singh et al., 2023). However, future research should include resistance genes from related and unrelated species, such as the recently studied *Hfr-3* gene, which encodes a putative chitin-binding lectin that resists the Hessian fly, a major pest. This method may boost crop yields, reduce pesticide use, and reduce post-harvest losses (Collins et al., 2018; Nemacheck et al., 2019). Tolerance to abiotic stresses Abiotic stresses, like drought, saltness, flooding, high mineral levels, and extreme temperatures, greatly affect plant growth and can cut wheat crop yields by 80% (Hossain et al., 2021). Using natural differences found in the crop or related wild plants has been the most effective way to deal with these stresses. For example, *Lophopyrum elongate* can handle salt better than other plants in the same family, and *Aegilops uniarisfata* can handle aluminum better (Zhang et al., 2018). More recent progress in knowing how genes affect abiotic stress tolerance, such as finding and cloning related genes, has led to the creating of plants that can handle these stresses without losing their yield (Xiong et al., 2021). But breeding for stress tolerance takes a long time and is hard to do because stress tolerance is multigenic and you have to pick the good genes while getting rid of the bad ones simultaneously (Gupta et al., 2020). Scientists have found that stress-responsive genes are important for how plants react to living and nonliving pressures. Shailani et al. (2021) found that changing the production of these genes is an excellent way to make rice more resistant to stress, like drought and salt. A study found that about 19% of wheat genes respond to salt stress, but more research is needed to fully understand how they help plants handle salt (Amirbakhtiar et al., 2019; Zubair et al., 2016). After adding the abasensitive barley gene *hva1* to wheat, some transgenic lines showed better plant growth in dry soil, with some showing higher biomass and grain yield than controls (Srivastava et al., 2018). In the same way, wheat plants that produce a vacuolar *na⁺/h⁺* antiporter gene have also shown better salt tolerance (Fu et al., 2022; Sen, 2021). modern wheat breeding projects aim to improve wheat handling frost (also called winter hardiness)

(Kumar et al., 2022). finding c-repeat-binding factors (CBF) in the frost tolerance locus *fr-a''2* in *Triticum monococcum* by Sheehan and Bentley (2021) has led to new ways of making wheat types more resistant to frost. This is very helpful in places where harsh winters can cause big losses in crop yields. Farmers also have to deal with the effects of cutting down too many trees, which often causes devastating floods that J. Phys. Biomed. Biol. Sci. Volume, 2: 11 Abbas et al., (2023) 5 can damage crops when they are buried for long periods. To solve this problem, the *sub1a* gene was found in rice, and studies have shown that increasing its expression in a type of rice that can't handle being submerged makes the plant more likely to survive a flood (Sarkar et al., 2021; Yousuf & Alim, 2020). It would be helpful to look into the pros and cons of genetically engineering this gene into wheat to see if it could similarly help wheat varieties grown in places prone to flooding. Gaining yield Increase seed number or weight to boost wheat output. Increase the amount of starch, which makes up over 70% of the seed weight and is the main component of wheat endosperm (Tian et al., 2018; Twizerimana et al., 2020). Controlling endosperm development can increase seed weight. Seed sink strength depends on ADP-glucose pyro-phosphorylase (AGP), which regulates cereal starch synthesis (Ahmad et al., 2022; Sehgal et al., 2018). A mutant maize *Sh2* gene (*Sh2r6hs*) that encodes an altered AGP large subunit increased seed weight/plant by 38% and overall biomass by 31% (Chachar et al., 2023). The number and size of tillers on wheat and rice plants determine panicle and seed production. The gene *MONOCULM1* (*MOC1*) regulates rice tillering (Shao et al., 2019). Modifying plant architecture by causing more evenly distributed tillers and leaves (Huang et al., 2021) increases leaf surface area for better sunlight absorption and photosynthesis, increasing productivity. Gibberellins, auxins, cytokinins, and brassinosteroids can cause dwarfing and boost grain output (Castro-Camba et al., 2022). These discoveries can help design high-yield crops by improving our understanding of genetic control of plant architecture and phytohormones (Huang et al., 2021). Other ways to boost crop productivity include changing C3 wheat to C4, which loses less carbon through photorespiration. Heterosis has increased maize and rice yields over 75 years (Raza et al., 2021). Wheat productivity also depends on blooming management and modification. RNA interference can change the flowering time or convert winter to spring wheat by manipulating verbalization response genes such as *VRN1*, *VRN2*, and *VRN3* (Gao et al., 2020). Improved grain quality Traditional wheat quality ratings are based on grain hardness and protein content. Grain hardness is inherited, although severe rainfall during harvesting can affect it (Johansson, Branlard, et al., 2020; Ratajczak et al., 2020). Although protein content is less inheritable, environmental factors like soil nitrogen availability and growth season wetness can considerably impact it. Protein quality also depends on end-use. With its most challenging grain and high protein content, Durum wheat cultivars are suitable for pasta production due to their high milling yield and unique storage protein combination (Ali et al., 2013; Ratajczak et al., 2020; Zingale et al., 2023). The cooked product's attractive look depends on its strong yellow pigment content. Traditional breeding can considerably increase these three traits. New research suggests that γ -gliadin 45 is a dependable indication of cooking quality, making it a popular screening tool for durum wheat breeding efforts (Yıldırım et al., 2019). Bread wheat (common or hexaploid) has different grain hardness and protein content. These toughest wheat, with the most protein, are suitable for pan bread. Other bread and noodles prefer medium-hard, low-protein wheat (Guzmán et al., 2022). The softest wheat with little protein is best for cakes and cookies. Starch quality is also vital in Chinese-style noodles, so choose wheat accordingly. Wheels' intended use should be considered while checking their quality with specialized

screening methods (Hasan et al., 2018). One important thing that can lower grain quality is sprouting, which happens when grains start to grow. Alpha amylase, an enzyme that breaks down starch, levels rise because of this process (Takahama & Hirota, 2018). Sprouting is bad for foods made from wheat because it weakens the ability to mix, makes dough sticky, and changes the size and shelf life of loaves (Espinosa-Ramírez et al., 2021; Olaerts et al., 2018). When sprouts are used to make pasta, the pasta may not last as long, lose more water during cooking, and be softer when it's done. Alpha-amylase damages flour, which means it can't hold as much water when mixed and can't receive as much water when baked. Finding the "falling number" can help you figure out how badly damaged the sprouts are in a sample of wheat (Williams et al., 2019). The falling number goes down as the amount of enzyme activity goes up. If the reading of the falling number is 350 seconds or more, it usually means that there aren't many enzymes working and the wheat is of high quality. Because preharvest sprouting (PHS) makes wheat much less valuable and of higher quality, PHS tolerance is a vital feature to breed for in wheat. Hexaploid wheat (*Triticum aestivum*), the only type of cereal that can be used to make leavened bread, is the only one that can make dough with the right rheological qualities (de Sousa et al., 2021). The presence of gliadins and glutamines that make up gluten together is to blame. Omedi et al. (2019) have found that the high molecular weight glutenin subunit (HMW-GS) is crucial for figuring out the viscoelastic qualities needed to make good yeast-raised bread. There are two more HMW-GS subunits: high Mr, x-type, and low Mr, y-type. There are two types of HMW-GS alleles at the *Glu-1* locus on the long arms of homologous chromosomes 1A, 1B, and 1D in hexaploid wheat cultivars (Bo et al., 2022). These alleles code for an x-type and a y-type subunit. They all have six HMW-GS genes, but only three, four, or five subunits because some genes are not working. It is usual for all types to have the 1Ay gene turned off. Every subunit of HMW-GS makes up about 2% of the extractable protein, meaning the whole seed protein can make up to 10% (Wieser et al., 2023). J. Phys. Biomed. Biol. Sci. Volume, 2: 11 Abbas et al., (2023) 6 Wheat landraces are valuable biological resources due to their genetic variety. Some landraces have genes not found in current cultivars that may improve their quality (Azeez et al., 2018). Due to this, many breeding efforts screen landraces for distinctive HMW-GS alleles. TOSI et al. created transgenic wheat cultivars with extra LMW subunits from *GluA3* and *GluB3* loci 2005. These variants had weaker dough due to co-suppression of the key endogenous HMW subunits. Novel LMW-GS detection and characterization are improving wheat quality. Genetic transformation and marker-assisted selection may soon allow wheat processors to introduce genes associated with bread-making properties into agriculturally desirable cultivars, eliminating the need to blend flour from different cultivars during milling (Pandurangan et al., 2021).

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