

Green Revolution – genes in modern crop varieties

Introduction

Global crop grain production grew from 1 billion tons in 1960 to 2 billion tons by 2000 (Khush, 2001). This growth was a result of a series of plant breeding and agronomy research initiatives, known as the Green Revolution, led by Norman Borlaug, who received the Nobel Peace Prize in 1970 for being the "Father of the Green Revolution". A part of these activities was an innovative wheat research program, and the foundation of the International Maize and Wheat Improvement Centre (CIMMYT) based on Borlaug's pioneering work in Mexico. The motivation for this program was the increasing pressure of food-population imbalance which was expected to lead to worldwide famines.

During the 1940s years, tall wheat varieties (e.g. Mentana) (Fisher and Stapper, 1987) were grown and the average wheat yield in Mexico was 2 t/ha. From 1900 to 1949, height of the plants ranged from 120.9 cm to 113.7 cm (Würschum et al., 2017). These were susceptible to lodging which led to loss of grains and therefore, yield. The harvest index, calculated from the yield of a crop species versus the total amount of biomass, was 0.3 (Kush, 2001). This meant that wheat plants produced 30% grain and 70% straw and their yield potential was 4 t/ha. The addition of fertiliser caused the plants to grow even taller, were more prone to lodging and tillering. The crops could not fully exploit the additional nutrients and the maximum yield was predicted not being able to cope with the growing population.

Wheat stem rust (*Puccinia graminis*) infection was spreading in Mexico. Borlaug noticed that new wheat varieties could be grown twice in a year in between the Ciudad Obregon and Toluca field sites (Figure 1) (Ortiz et al., 2007). This meant the varieties were grown from spring to October at Toluca (2,500m in altitude), and 'shuttled' to Ciudad Obregon from November. This 'shuttle breeding' led to more resistant wheat varieties as they were exposed to different environments and diseases and sped up the breeding process. CIMMYT defined 12 mega-environments and the breeding would take place at two different sites and the varieties' yield would be compared in the different environments. This was the beginning of the Green Revolution.

The major breakthrough was the introduction of a Japanese cross between Daruma with an American variety which led to Norin 10 (Hedden, 2003). It was Norin10-Brevor 14, produced by Norman Borlaug, which had the Norin 10 dwarfing gene and was suitable for temperate climate. The introduction of the dwarfing gene increased the harvest index to 0.5 by shifting the biomass production to more grains, rather than straw production (Khush, 1993). Shorter plant height led also enabled the optimisation of fertiliser usage by the crops without lodging or tillering. Their maximum yield potential



Figure 1. Shuttle breeding started in Mexico (left) to enhance wheat rust resistance and international shuttle breeding has led to even more resistant modern varieties (Ortiz et al., 2007). The introduction of Norin 10 dwarfing genes has reduced the height of wheat plants (right).

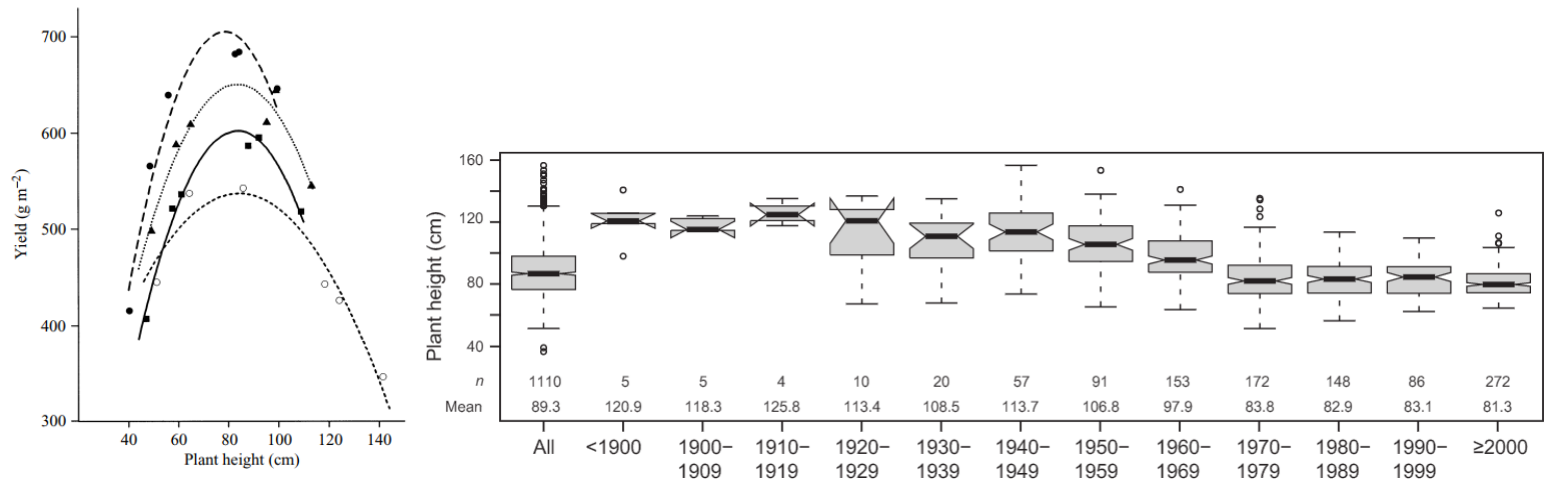


Figure 3. Different *Rht* genotype means (back circles - Maris Huntsman, black squares - Maris Widgeon, black triangles - Bersee, white circle - April Bearded varieties) show a decline in yield after 85 cm plant height (Flintham et al., 1997). Overall height reduction of 1,110 winter wheat from 1900 to 2000 (Würschum et al., 2017)

Wheat *Rht* genes

The introduction of Norin 10 genes in traditional wheat breeding, formerly called *Gai/Rht₁*, *Gai/Rht₂* genes, started the modern wheat (*Triticum aestivum*) varieties selection to shift towards semi-dwarf phenotypes (Brooking and Kirby, 1981). Twenty-one genes have been described on the wheat's chromosome B and D. *Rht1* to *Rht21* have been described to be responsible for reduced plant height in wheat (McIntosh, 2013). Not all genes were used for crop improvement as some can result in adverse effects of biomass production, decreasing yield.

The four major genes are *Rht-B1b* and *Rht-B1c* on chromosome 4BS, *Rht-D1b* on chromosome and *Rht-D1c* on chromosome 4DS. *Rht-B1b* and *Rht-D1b* encode DELLA proteins which are negative regulators of gibberellins (GA) (Peng et al., 1997; Li et al., 2012). This was identified by Peng et al. (1999; 2002) (Figure 4).

Rht-B1a	GEEVDELLAALGYKVRASDMADVAQKLEQLEMAMGMGGVGAGAAPDDSFATHLATDTVHYNPDLSSWVESMLS
Rht-B1b	GEEVDELLAALGYKVRASDMADVAQKLE* MAMGMGGVGAGAAPDDSFATHLATDTVHYNPDLSSWVESMLS
Rht-D1b	GEEVDELLAALGYKVRASDMADVAQKLL* MAMGMGGVGAGAAPDDSFATHLATDTVHYNPDLSSWVESMLS
Rht-D1a	GEEVDELLAALGYKVRASDMADVAQKLEQLEMAMGMGGVGAGAAPDDSFATHLATDTVHYNPDLSSWVESMLS

I

II

Figure 4. Mutations in the N-terminal regions I (DELLA region) and II (VHYNP region) of wheat *Rht* genes cause GA-insensitivity (Peng et al., 1999).

Rht-B1 was found to contribute 2.7% of the genotypic variance which led to on average 7.6 cm decrease in plant height, and has been identified resistance to *Fusarium* pathogen. *Rht-D1* was found in 49% of the cultivars with the allele substitution effect of -9.4 cm. *Rht8* on chromosome 2D, close to *Ppd-D1* (photoperiod controller) was identified for future uses as it explained 4.8% genotype variance and led to 9.4 cm height reduction. *Rht24* on chromosome 6A, is found in cultivars worldwide and its frequency has been increasing. It was responsible for 15% of genotypic variance on average but the Polish lines showed up to 37.5% while German lines 8.5% genotypic variance.

By gaining an insight to how much variation can be produced by these genes, it enables crop breeding to fine tune these genes and breed selectively to optimise crop reduction to its maximum capacity (Figure 5). These molecular methods have to be tested under field conditions once a new variety is

produced. In Europe now, Community Plant Variety Office (CPVO) and International Union for the Protection of New Varieties of Plants (UPOV), test new varieties by growing them in the field for three years, recording plant characters, combined with molecular work before a new crop variety becomes commercially available.

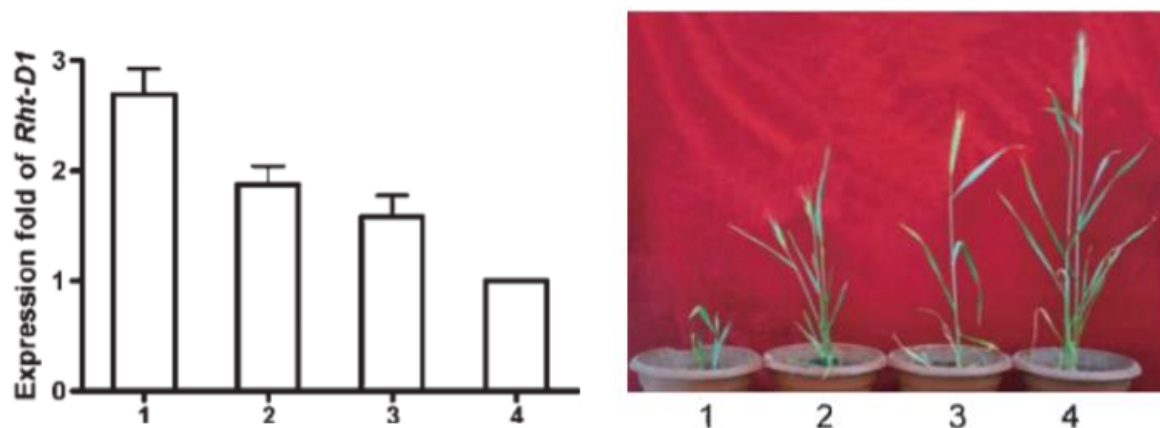


Figure 5. The expression of Rht-D1 expression in transgenic wheat was showed to have the strongest effect on plant height reduction by Li et al. (2012).

Rice modern varieties

At the same time as the modern wheat varieties were developed in 1950s, breeders at the International Rice Research Institute (IRRI) also introduced dwarfing genes into rice (*Oryza sativa*) plants (Peng et al., 2010). There are two main rice cultivars; *indica* cultivars require submersion in water and are grown on lowland around tropical Asia and *japonica* is grown mostly in drier field in temperate East Asia, or at higher altitudes in South Asia. The latter can be *temperate* or *tropical japonica* and there are also *aromatic*, and *aus* rice cultivars (Wang et al., 2014). The rice dwarfing gene originated from the Chinese *indica* cultivar, Dee-geo-woo-gen, and was first used in Taiwanese crosses. In 1950, Taichung Native 1 (TN-1) was produced and in 1966 (Hedden, 2003), IR8 was the new, fertiliser-responsive variety that exceeded the normal yield (6 t/ha^{-1}) to 10 t/ha^{-1} on irrigated lands (Peng et al., 2010). This was called the 'miracle rice'. The shortened height had the same benefits as in wheat. This TN-1 and IR8 was widely used for semi-dwarf rice *indica* and *japonica* cultivars. This was a single recessive allele called *sd1* chromosome 1, which was responsible for the semi-dwarf trait. However, addition of GA could counter effect *sd1* (Sasaki et al., 2002). This gene has also been shown to control culm length in *japonica* landraces (Asano et al., 2011). The two genes involved in GA20ox enzyme production, which is involved in GA biosynthesis, were *OsGA20ox1* and *OsGA20ox2* in rice. The *OsGA20ox2* gene originated from the Dee-geo-woo-gen cultivar with a 383-bp deletion in the GA20ox gene (Ashikari et al., 2002). Mutations in these two genes can be used for rice breeding while it was shown that *SLR1* gene acts a negative regulator in the GA signalling and therefore leads to a variety of plant heights (Ikeda et al., 2001).

While *sd1* is a gene leading to semi-dwarf phenotype, *d2* is a dwarf gene from *japonica* cultivars (Houghton et al., 2001). A recent study by Yan et al. (2012) gamma radiated KDM105, elite aromatic cultivar from Thailand and produced a *ser* mutant line by a single recessive mutation. This reduced plant height, leaf length, panicle and leaf angle which led to a better performing crop in the field.

Not only the height of the rice plants was selectively bred, but seed shattering was also important; this pattern is controlled by the *sh4* gene (Lin et al., 2007). Tillering capacity is controlled by *moc1* controls in *japonica* cultivar (H89025) while *SCM2* increases spikelet number per panicle in *japonica* (Ookawa et al., 2010).

These improvements led to 132% increase in global rice production from 1966 to 1999 and currently the price of rice is 40% lower than in the 1960s (Khush, 1993).

Maize modern varieties

Maize (*Zea mays*) dwarf mutants were first described in 1912 by Emerson. Both recessive and dominant alleles are known to cause semi-dwarfism and dwarfism (Harberd and Freeling, 1988). Recessive mutants have been identified on chromosome 3 (*d1*, *d2*), *d3* on 9S, *d5* on 2S and *an1* on 1L. Dominant alleles are *D8* and *Mpl1*. The dwarf varieties are reduced in height, and have shorter but broader leaves. The most dominant dwarfing allele was *D8-1* described by four amino acid deletion in the DELLA domain, two codon change causing amino acid substitutions.

The mutant alleles of wheat (*Rht*), maize (*d8*) and rice (*sd1*) using the *gai* locations from Peng et al.'s (1999) study, found the N-terminal regions of the *GAI* to be closely related in two regions and was able to locate these regions across the different species (Peng et al., 1999c).

Conclusion

The Green Revolution enabled the human population growth followed by enormous growth of food production systems. However, it has also led to varieties that can take up more fertilisers the use of fertilisers has increased by sevenfold since 1960s (Tilman, 1998). Crop fields are intensive and mostly monocultures which makes them vulnerable to plant pathogens which can lead to total harvest losses (Cleaver, 1972).

Human population is expected to reach 9 billion by 2050 (FAO, 2003) and working towards sustainable food production system is of upmost importance. Climate change is predicted to a 5.8°C temperature rise by the end of the 21st century (Houghton et al., 2001). Over 40% of global food production depends on irrigation systems (Chaves and Oliveira, 2004) and by 2050, 6.5 billion people are predicted to be living in countries facing chronic water shortage (Rockström et al., 2009). Therefore, optimising drought resistance in crops is in the frontline of crop breeding.

Green Revolution could not take place in Africa and some developing countries (Rockström et al., 2007; Toenniessen et al., 2008) without the facilitated help of organisations. Uncovering the functions of genes and fine tuning them with novel molecular techniques to produce optimal harvest quantity and quality with disease resistance (Figure 8), is the future of global food production.

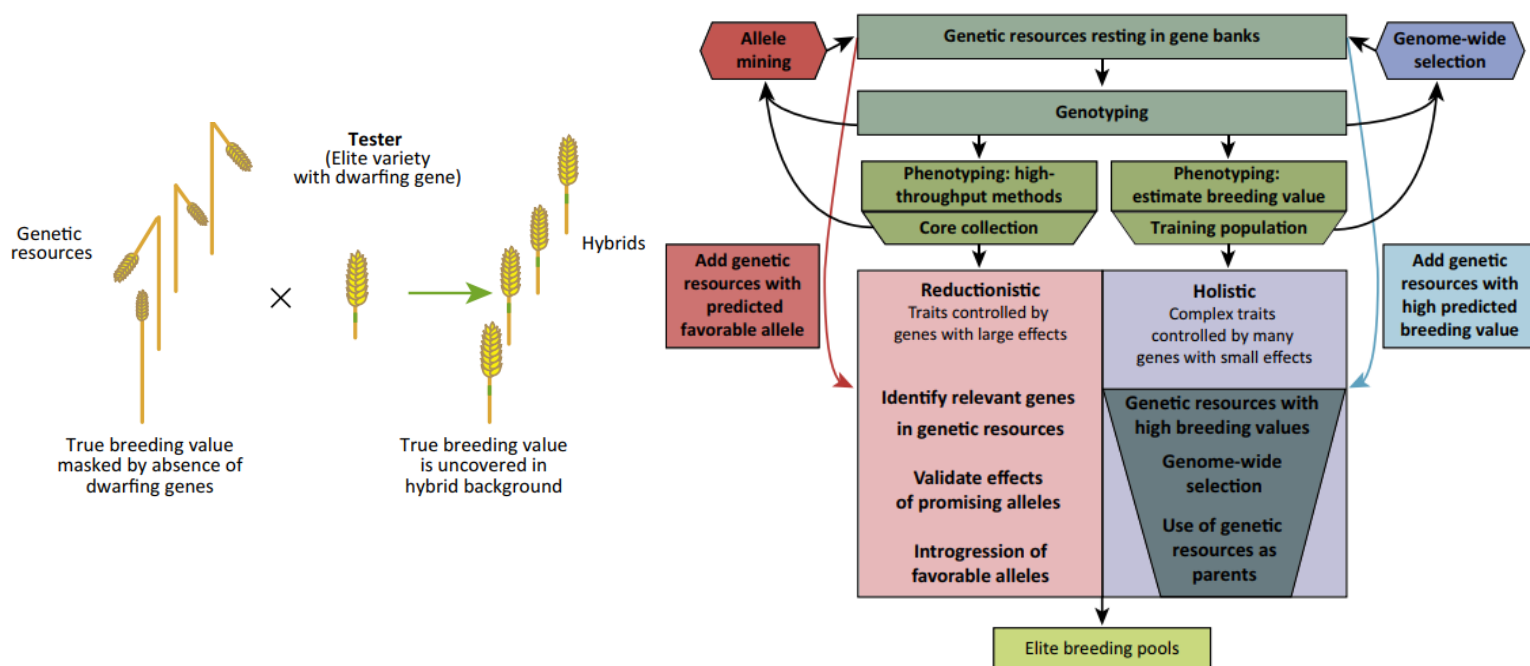


Figure 8. A novel method (left) of wheat variety breeding was proposed (Longin and Reif, 2014) to fully exploit wheat genetic resources by using elite varieties without dwarfing genes, re-introducing dwarfing genes and hybridising the new elite varieties. Selection process can be reductionist or holistic (right), depending if only few traits and genes are focused on or whole genome-wide selection is used.

Word count: 1882

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