

Cereal breeding takes a walk on the wild side

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Elite cultivated crop gene pools of the Triticeae tribe (wheat, barley and rye) exhibit limited genetic diversity, raising concerns about our ability to increase or simply sustain crop yield and quality in the face of dynamic environmental and biotic threats. Although exploiting their wild relatives as a source of novel alleles is challenging, it has provided notable successes in cereal improvement for >100 years. Increasingly facile gene discovery, improved enabling technologies for genetics and breeding and a better understanding of the factors limiting practical exploitation of exotic germplasm promise to transform existing, and accelerate the development of new, strategies for efficient and directed germplasm utilization.

The foundations of agriculture

Since the beginnings of agriculture ~10 000 years ago, cereals have provided the main source of calories for mankind. Recognized for their high yields, nutritional value and ease of transport and storage, a range of different cereals were domesticated by the world's original farmers (Figure 1). Of these, wheat and barley, two members of the tribe Triticeae (taxonomically belonging to the *Poaceae* family, commonly known as the grasses), have been particularly important because they served as the principal grain stock that enabled the founding of agriculture in the Middle East and led to its successful spread around the world [1]. The Triticeae are an unusual group of plants. Together they account for more than one third of global cereal production (Table 1). They include diploids (barley and rye), tetraploids (durum or pasta wheat), hexaploids (bread wheat, spelt and Triticale) and even some octaploid Triticales. As a group, they are so closely related that fertile hybrids can be produced between them. Most Triticeae are in-breeders, but rye is an obligate out-breeder. We know a great deal about Triticeae origins, and our understanding of key agronomic and quality traits has expanded dramatically as a result of the development and application of contemporary genetic technologies. Because of their agricultural importance, the Triticeae species have been bred intensively for the past hundred years, resulting in spectacular improvements in yield and quality. However, this success has been associated with a narrowing of the available genetic diversity within elite

germplasm, and there is concern that the prospects for continued genetic gain are becoming increasingly limited.

A high strategic priority for practical cereal improvement worldwide is to enrich the cultivated gene pools by incorporating favourable alleles, genes or gene complexes from wild relatives. Consequently, gene pool expansion is already under way. The development of extensive genomic resources for the Triticeae, if coupled with innovative breeding strategies and biologic understanding emerging from both crop and model plants, will provide new opportunities and innovative approaches to meet this challenge head on.

Glossary

Alien chromosome: Alien chromosomes originate from a different species. They are introduced by hybridization and subsequently eliminated almost completely either spontaneously or through crossing. They replace an existing chromosome, add to or replace part of an existing chromosome or add an extra pair.

Amphiploids: Diploid plants have one paired set of homologous chromosomes. Polyploids have more than one set. Amphiploids (or Allopolyploids) are artificial polyploids with homoeologous chromosomes derived from different species.

Colchicine: A poisonous alkaloid that inhibits microtubule polymerization and thus affects chromosome segregation during mitosis. Applying colchicine to haploid plants induces chromosomal doubling.

Embryo culture: A process in which immature embryos are manually dissected from developing grain and nurtured to form 'green plants' in special tissue culture media.

Gametocidal chromosomes: Some alien chromosomes from *Aegilops*, called gametocidal (Gc) chromosomes (see Ref. [28]), harbour unique genes that cause chromosome breakage by an unknown mechanism.

Genetic manipulation (GM): Insertion of a fragment of DNA (often a gene) into plant cells by transgenesis. The introduced sequences provide new functions (e.g. drought tolerance) or alter existing functions (e.g. changing its level of expression).

Homoeologous chromosomes: Chromosomes that exhibit different degrees of ancestral homology. In polyploid wheat, homoeologous chromosomes can pair and recombine only in the absence of *Ph1*.

Hybrids: Interspecific hybrids are the product of crosses between two different species within the same genus. Intraspecific hybrids are from crosses between different subspecies within a species and intergeneric hybrids from crosses between different genera.

Introgression: The transfer of DNA segment from one species into another. This can occur both through contemporary breeding processes and in the wild. In breeding, introgression is usually done into an elite cultivated background.

Linkage drag: The reduction in fitness in a cultivar because of the introduction of deleterious genes along with a beneficial gene during backcrossing.

***Ph1/ph1*:** Hexaploid wheat contains three sets of seven chromosomes from the A, B and D genomes. Although each of the seven chromosomes can potentially pair with either its strict homologue or its other homoeologues, in the presence of *pairing homoeologous (Ph)* genes, in particular *Ph1*, pairing is largely restricted to homologues. Thus, hexaploid wheat behaves like a diploid at meiosis. In *ph1* deletion mutants, association occurs between homoeologous chromosomes, thus allowing recombination between related species.

Synteny: A term describing the preserved order of genes along whole, or sections of, chromosomes that derive from a common ancestor.

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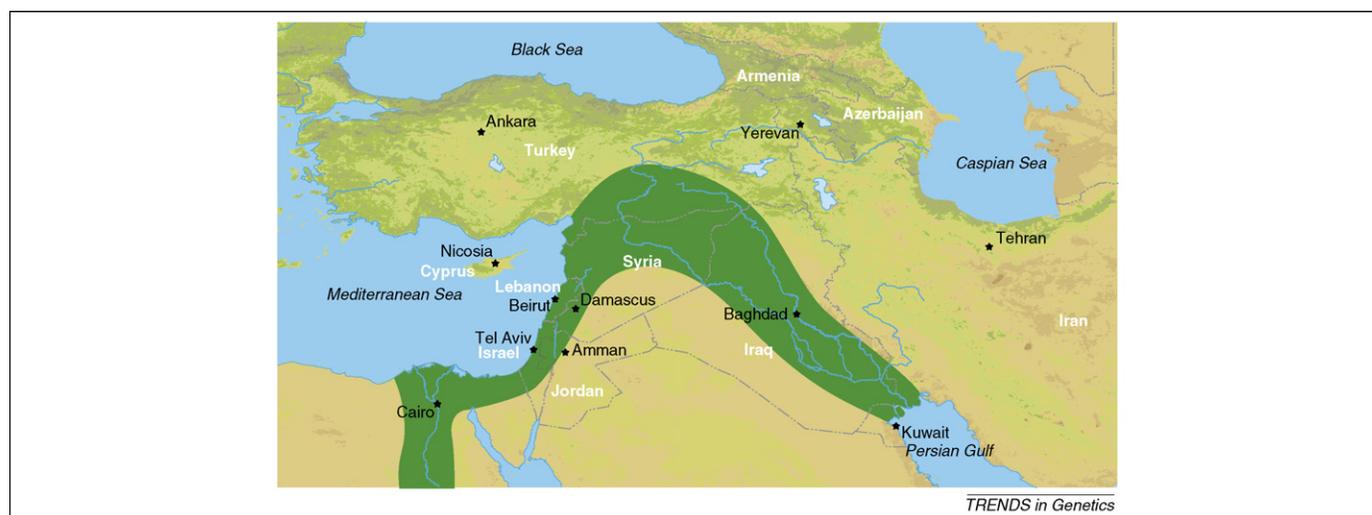


Figure 1. The domestication of cereals. The dark green shaded area on the map shows the region of the near east (Israel, Jordan, Turkey, Syria, Iran, Iraq) known as the Fertile Crescent from where the earliest archaeological remains of barley, einkorn and emmer wheat have been recovered. The natural diversity of their wild relatives in the region and molecular marker and DNA sequence information support different regions of Fertile Crescent and its northern fringes as the site of domestication of the Triticeae cereals, and the cradle of agriculture ~10 000 years ago. More recently, a second site of barley domestication has been identified ~1500–3000 km east in central Asia.

Origins of wheat, barley and rye

The word ‘wheat’ is used to describe several related grain crops. The most important wheat species grown today are bread wheat (*Triticum aestivum*) and pasta wheat (*T. turgidum*) (Figure 2). A third species, einkorn wheat (*T. monococcum*), has great historic but very little current agricultural significance. Einkorn was probably the first wheat species to be widely cultivated, ~10 000 years ago in southeastern Turkey. This primitive, hardy and low-yielding wheat constituted the main crop in the region for several thousand years. Einkorn survives to this day as a cultivated animal feed in mountainous regions of Turkey, Italy and Spain and as a wild species in the mountains surrounding the Fertile Crescent [2].

Besides einkorn, several additional wheats and wheat relatives evolved in and around the fertile crescent of the Middle East (Figure 2). These species all share the same basic set of seven chromosomes, mostly in diploid form, and are given a ‘genomic constitution’ classification according to their meiotic pairing characteristics in diploid hybrids (Box 1). The first evolutionary event leading to polyploid wheats was hybridization of a diploid wheat closely related to *T. urartu* (genomic constitution AA) with a yet unknown species from the *Sitopsis* section that provided the

B genome and was closely related to *Aegilops speltoides* (SS) (Figure 3). Eventually, this fertile tetraploid (AABB) became a species in its own right and, >10 000 years ago, was domesticated and became known as emmer wheat, or *T. turgidum* [3]. Having the combined genetic resources of both ancestral diploids, tetraploid wheat is, generally, more vigorous, higher yielding and more broadly adapted to different environmental conditions than its progenitors. *T. turgidum* has been of great historical significance because it provided a range of subspecies that were cultivated widely across the globe for thousands of years. Although most are of little economic significance now, one subspecies, *T. turgidum* ssp. *durum*, which gave rise to the pasta wheat cultivars of today [4–6], is still grown widely. A second evolutionary event that led to the bread wheat lineage occurred when tetraploid emmer wheat reached the region south of the Caspian Sea and crossed with *Ae. tauschii*, a wild diploid species with a DD genome (Figure 3). As with the hybridization event that generated *T. turgidum*, this cross would normally yield sterile hybrids; however, a doubling of the chromosomes in gametes or progeny gave rise to a fertile, hexaploid species with an AABBDD genome—a species now known as *T. aestivum* or bread wheat. This hexaploid species

Table 1. Global production and yield of cereals

	Production (million tonnes)	Area (million hectares)	Yield (t/ha)	Percent total cereal production
Triticeae				
Wheat	619	213	2.9	28
Barley	137	53	2.6	6.1
Rye	15	7	2.2	0.7
Triticale	13	3.7	3.5	0.6
Total Triticeae	784	273	2.8	35
Maize	703	143	4.9	31
Rice	626	150	4.2	28
Sorghum	58	42	1.4	2.6
Millet	29	32	0.9	1.3
Oats	23	11	2.1	1.0
Others	25	13	2.0	1.1
Total	2249	663	3.4	100

Data from Food and Agriculture Organization of the United Nations, FAO, July 2007, <http://faostat.fao.org/>.



Figure 2. The grain-bearing spikes of wild and domesticated forms of wheat and barley. (a) Diploid ancestral wild species related to the tetraploid and hexaploid wheat species: (i) *Triticum urartu* (A^uA^u), (ii) *Aegilops speltoides* (SS) and (iii) *Aegilops tauschii* (DD). (iv) Cultivated diploid wheat *T. monococcum* (A^mAm^m). Wild and domesticated forms of tetraploid wheat: (v) *Triticum turgidum* ssp. *dicoccoides* (AABB) and (vi) *Triticum turgidum* ssp. *durum* (AABB). *Triticum aestivum* (bread wheat, AABBDD) (vii) originated from hybridization between the domesticated tetraploid species *T. turgidum* ssp. *dicoccum* and the wild diploid *Aegilops tauschii* (Figure 3). (b) Different subspecies of barley: (i) the wild progenitor *H. spontaneum* (HH) showing the brittle rachis character (right) that causes the seed to shatter from the spike and (ii) the two main cultivated types of *Hordeum vulgare* (HH)—six row (left) and two row (right). Genome constitutions (see Box 1) are given in brackets beside the species names.

first emerged in cultivated wheat fields ~7000 years ago [1].

Because the D genome of bread wheat originated from *Ae. tauschii*, it carried genes and alleles adapted to the more continental climate of central Asia, thus enabling bread wheat to be cultivated both geographically and environmentally more widely than emmer wheat. Significantly, the D genome encoded proteins that restored the softness of the grain endosperm [7], thereby improving bread-making properties. It also contains proteins that trap CO₂ during yeast fermentation, making it suitable for leavened bread production. Combined, these factors led to the widespread cultivation of bread wheat around the world and to the development of several different subspecies. Hexaploid bread wheats account for ~90% of world wheat production today (<http://faostat.fao.org/>).

Barley (*Hordeum vulgare*) is also one of the world's ancient cereal crops, with archaeological remains revealing that it was first domesticated in the Fertile Crescent ~10 000 years ago, at much the same time as wheat (Figure 1). Although generally regarded as an inferior

staple to wheat and as 'poor man's bread', its continued cultivation has been ensured because it is the hardier of the two species [1]. More recently, barley has become valued particularly for its use in beer and whisky production. Compared with wheat, the taxonomy and evolution of barley is relatively straightforward, with the term 'barley' used only to describe one species, *H. vulgare*. However, several different subspecies have been identified growing wild in and around the Fertile Crescent and in secondary habitats from the Mediterranean to the Himalayas. Of these, the subspecies *H. vulgare* ssp. *spontaneum* is considered the progenitor of cultivated barley (Figure 1). This wild barley, along with a range of other *Hordeum* species, can be found today in its original habitat in the Middle East [8,9].

Rye (*Secale cereale*) is also thought to have originated in the Anatolian Plateau of Turkey and has its center of diversity in Southwest Asia (Turkey, Armenia, Iran). More than likely, rye first appeared as a tolerated weed and was picked up later as a crop [1]. The genus is made up of several weedy ryes (*S. montanum*) and wild types

Box 1. Genome constitution in the Triticeae

Genomic and phylogenetic relationships between species are determined from observing both the fertility of F1 hybrids developed from interspecific and intergeneric crosses and the meiotic pairing characteristics of their chromosomes. The latter cytogenetic analyses identify the extent and nature of chromosome pairing among species. In taxonomic groups of species with high levels of polyploidy, such as the Triticeae, this type of analysis establishes the relationships between the homoeologous chromosomes in diploid and polyploid species. The outcome of these studies provides a designation of the 'genome constitution' or 'haplome' of the species. Different genome constitutions are defined as having <50% complete meiotic pairing in F1 hybrids (see <http://www.herbarium.usu.edu/Triticeae/genmsymb.htm>). Genome constitutions are denoted by uppercase letters of the Roman alphabet. Unknown or unverified genome constitutions are denoted by a capital X with a lowercase letter indicative of the species name. Within the Triticeae tribe, extensive studies carried out over the past 50 or more years have clarified the 'genomic constitution' of most of the component species. Thus, the genomic constitution of durum wheat is AABB, bread wheat is AABBDD, barley is HH and rye is SS.

(*S. vavilovii* and *S. sylvestre*) that, usually, can form fertile hybrids with cultivated rye.

The key domestication event for both wheat and barley was the identification of plants expressing advantageous traits that control features of the grain-bearing spike. Through genetic research, we now know that these are caused by mutations at loci such as *brittle rachis* (*Br*), *Q* and *Tg* (*Tenacious glume*) [10–14]. These mutant types were more attractive to the first farmers than their wild relatives because they produced spikes that did not shatter and disperse their seeds before harvest or they had naked or hullless seeds that simplified flour milling. Recently, the isolation of the genes underlying these traits has been of great interest [10,14,15]. Functional and phylogenomic analyses of the cloned genes will advance our understanding of the domestication syndrome in cereals [the process of adaptation from the wild to the human (cultivated) environment] and provide an opportunity to transfer this knowledge for the domestication of new plants.

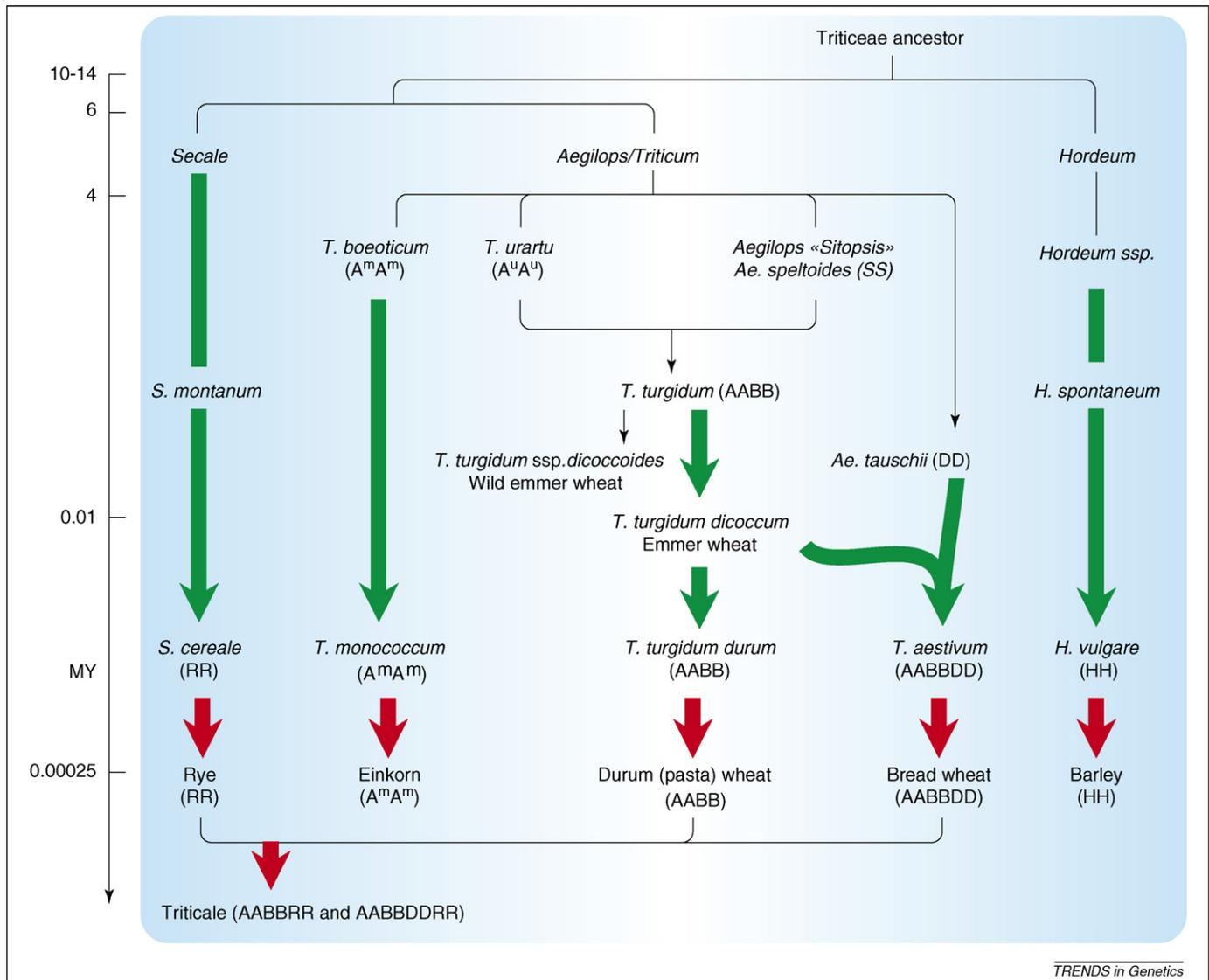


Figure 3. The evolution of the Triticeae species from a common ancestor. The different evolutionary hybridization (black arrows), domestication (green arrows) and selection (red arrows) steps that have resulted in modern wheat, barley and rye cultivars are described along the time scale in million years (MY). Genome constitutions (see Box 1) are given in parentheses beside the species names.

New synthetic amphiploid crops

Wheat, barley and rye are all closely related and share a basic chromosome number of seven, although bread wheat is hexaploid and durum or pasta wheat is tetraploid. The close relationship between the chromosomes of these three species enables the generation of fertile amphiploid hybrids between different cultivated members of this tribe and also between the cultivated species and their wild relatives. The first hybrid between wheat and rye, ‘Triticale’ (*Triticosecale*), was generated in Scotland in 1876, but the first fertile hybrid was not produced until 1938 (for review see Ref. [16]). Unfortunately, the processing and quality characteristics of bread wheat were not transferred to Triticale, and it is used mainly as an animal feed. Despite the fact that the first systematic breeding of Triticale only really began in the 1960s, production has grown from < 2 million tonnes in the late 1980s to 13 million tonnes in 2005 (<http://faostat.fao.org/>). Triticale is thus a new crop species that has found a strong niche because of its high yield, relative to its parental species, and ability to grow in environments not suited to wheat or barley production. Although fertile hybrids between wheat and barley have also been produced, the resultant ‘synthetic species’, *Tritordeum*, has failed to find commercial acceptance.

The value of wild relatives for crop improvement—opportunities for genetic gain

Cultivation, domestication and breeding have resulted in today’s elite, cultivated crop gene pools that contain only a fraction of the available diversity in the species. As an extreme example, only five hard red winter wheat varieties were cultivated in the United States in 1919, with one, cultivar Turkey, occupying almost the entire acreage [17]. This situation hardly changed until 1949, when Turkey and a second cultivar, Tenmarq, were replaced by four less closely interrelated cultivars (although all were derivatives of Turkey!). Since then, additional less related germplasm has been introduced, but the gene pool remains relatively narrow. The remarkable diversity of regional landraces, local cultivars and related species offers a reservoir of genetic variation that has the potential to impact positively on crop improvement and sustainable agricultural production (Box 2). Exploiting biodiversity for genetic gain is not a new concept. As noted above, it was both recognized and practiced over a century ago in the creation of the whole genome hybrid Triticale. Since then, several major genes have been introgressed (see Glossary) from wild relatives into the cultivated gene pools of many crops [18]. Despite some highly significant successes, including the incorporation of dwarfing genes (i.e. Reduced Height Loci Rht-B1 and Rht-D1) and genes conferring durable resistance against a wide spectrum of insects and diseases into wheat by Norman Borlaug that fueled the Green Revolution, introgression remains laborious and, for complex characters, largely unfulfilled.

Introgression has two key steps: sexual hybridization to bring the wild or ‘alien’ genome into a cultivated background and homologous and/or homoeologous recombination to eliminate or replace the deleterious alleles and/or genes that ‘come along for the ride’ with the selected locus,

Box 2. Triticeae gene pools

The tribe Triticeae contains >500 species in 26 genera (<http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi>). In relation to the cultivated species, each of these can be considered to be part of the primary, secondary or tertiary gene pool, depending on how closely related they are at the genomic level. Species are given a genome constitution determined by their meiotic pairing characteristics in diploid hybrids (Box 1)

Primary gene pool

Species in the primary gene pool include landraces, early domesticates and wild species that hybridize directly with the cultivated types. Their chromosomes are homologous to the cultivated types and will generally undergo homologous recombination, which can be exploited in breeding and selection schemes. The primary gene pool is that which has been best used for crop improvement. In the case of the polyploid wheats, the primary gene pool includes polyploid species (e.g. *Triticum turgidum*, AABB) and diploid donors of the A and D genomes [*T. urartu* (AA) and *T. tauschii* (DD)]. In barley (HH) and rye (RR), the primary gene pool includes the enormously diverse and geographically widespread, but sexually compatible, diploid progenitors *Hordeum spontaneum* and *Secale vavilovii*, *S. montanum*, respectively.

Secondary gene pool

The secondary gene pool of wheat contains polyploid species that share at least one homologous genome with the cultivated types. Gene transfer from these species is possible through homologous recombination when the target gene is located on the homologous genome. This includes polyploid *Triticum* and *Aegilops* species, such as *T. timopheevii* (AAGG) and the diploid S-genome (related to the B genome) species from *Aegilops* section *Sitopsis*. For barley, this includes diploid and tetraploid *Hordeum bulbosum* (ll) and *Elymus* (HHStSt), and for rye, *S. sylvestre*.

Tertiary gene pool

Species in the tertiary gene pool contain more distantly related diploids and polyploids. They possess none of the cultivated species genome constitutions. Homologous recombination cannot be exploited, and special strategies (e.g. irradiation, gametocidal chromosomes) are required for gene transfer. This group includes most members of the Triticeae that are not within the primary or secondary gene pools. A large proportion in this group are perennials and for wheat includes important species from *Secale* (RR), *Thinopyrum* (EE) and *H. marinum* (XX). For barley, the American wild barleys and *H. bogdanii* fall into this group, whereas for rye, the tertiary pool would include *Triticum* and *Aegilops* species.

a phenomenon known as ‘linkage drag’. There are many barriers affecting the success of these steps, with their scale broadly reflecting the evolutionary distance between the species involved. In polyploid wheats, understanding the origins of the genomes has provided one key to broadening the genetic base of cultivated elite material. In a process akin to the development of Triticale, bread wheat (AABBDD) can be recreated to produce ‘synthetic wheats’ by developing hybrids between durum wheat (AABB) and *Ae. tauschii* (DD) and doubling the chromosome number through the use of colchicine. Immature embryos that would otherwise perish are rescued using embryo culture [19]. Breeding programs, such as those at CIMMYT (http://www.cimmyt.org/english/wps/news/wild_wht.htm), have produced >1000 synthetic wheats since the early 1990s, and the new genetic diversity represented in this material is being incorporated increasingly into wheat breeding programs worldwide.

The diploid barley genome is much less buffered against major genomic perturbations than those of the polyploid cereals. The exploitation of diverse germplasm has focused generally on interfertile, regionally adapted landraces, particularly as donors of genes for resistance against pests and pathogens. For example, broad spectrum resistance against *Blumeria graminis* f.sp. *hordeii* (mildew) was found in barley landraces collected from Ethiopia in the 1930s and was described as the *mlo* locus in 1942. One particular allele, *mlo-11*, introgressed into the cultivated European spring barley gene pool, has provided robust and durable resistance for >30 years [20]. According to Hajjar and Hodgkin [18], the use of undomesticated wild barley species in breeding has not met with much success. However, lines from *H. vulgare* × *H. spontaneum* crosses that are suitable for general cultivation and use in stressful environments are emerging now from ICARDA (<http://www.icarda.org/>). In diploid tomato and rice, hidden but beneficial allelic variation can come from the most unlikely un-adapted germplasm sources [21]. Tanksley and Nelson [22] described a method termed ‘advanced backcross-quantitative trait locus (QTL)’ to discover and mobilize desirable alleles from wild into cultivated material, an approach that is being deployed currently in barley [23,24] and wheat [25].

Challenges of using wild species in Triticeae crop improvement

A major drawback to the use of wild genetic resources in breeding is that the introgressed alien fragments often come as large linkage blocks that carry genes with a potentially negative impact on the traits selected for in the elite varieties. This phenomenon, linkage drag, has limited the efficient exploitation of wild relatives in breeding programs because the process to eliminate the negative alleles is tedious and time consuming. The underlying reason is that recombination between an introgressed alien chromosome and its homoeologue is, generally, completely restricted or at best severely depressed. The challenge to overcome this ‘recombination barrier’ promoted the emergence of the field of ‘chromosome engineering’ (CE) that focuses on enhancing the fragmentation of donor chromosomes and promoting their recombination with recipient genomes [26–29]. In CE, ionizing radiation and gametocidal chromosomes have been exploited to induce chromosome breaks that, after rejoining, result in translocations and substitutions between the host and alien genome.

More important, however, for wheat was the discovery of the *Ph1/ph1* locus. This locus regulates pairing and recombination between homoeologous (as opposed to homologous) chromosomes [30,31]. Since its characterization, *Ph1* has been used widely and successfully in wheat to induce homoeologous recombination, and the molecular structure of this locus has been defined recently [32]. By allowing recombination between homoeologues, introgressed genome segments can be trimmed repeatedly to eliminate most of the linked undesirable alleles and/or genes. Cytogenetic [e.g. fluorescence in situ hybridization (FISH) and genomic in situ hybridization (GISH)] and molecular marker techniques greatly facilitate the process

of ‘tracking and trimming’ introgressed segments and help minimize linkage drag. Understanding how *Ph1* and several related loci work should afford new opportunities for manipulating recombination in a broad range of species.

Perhaps the best example of introgression of chromatin from a relative into wheat is the 1BL/1RS chromosomal translocation. The 1RS chromosome from rye carries several genes whose protein products increase grain yield by providing race-specific disease resistance to major rust diseases (including *Lr29/Yr26* leaf and yellow rust resistance genes), improved adaptation and stress tolerance, superior aerial biomass and higher kernel weight [33]. Between 1991 and 1995, 45% of 505 commercial cultivars of bread wheat from 17 countries carried this wheat–rye translocation [34]. Despite its success, the 1RS translocation negatively impacted bread wheat end-use quality and led to poor gluten strength. Recent dissection of the chromosome arm has succeeded in separating the yield advantage from the quality problem [35].

Other examples include the introgression of functional alleles of the *Pin* genes, whose protein products are responsible for endosperm texture in wheat [36] from the diploid wheat *T. monococcum* (A^mA^m). This was beneficial in that it led to softer grains, but it negatively influenced agronomic performance. Rounds of selection in backcross (BC) populations using molecular markers tightly linked to the *Pin* genes allowed Bonafede *et al.* [37] to select individuals with an introgression segment reduced from >40 to 6.3 cM. Extensive backcrossing and marker-assisted selection was also required to separate the *Sr22* rust resistance gene derived from *T. boeoticum* from a gene that had an undesirable effect on time to maturity [38]. CE has permitted the transfer of many disease and pest resistance genes that were not available previously within the domesticated wheat gene pool from sources including other members of the *Triticum* genus (such as *T. urartu*, *T. monococcum* and *T. turgidum*), rye (*Secale*), *Aegilops*, *Thinopyrum* and *Agropyron*. Primary hybrids have been developed with tertiary gene pool species, and there are groups working on transferring salinity tolerance from *Hordeum* spp. into bread wheat [39]. In barley, relatively little germplasm from outside the primary gene pool has been introduced into elite cultivated material, although recently some success has been achieved by introgressing resistance alleles (particularly against scald) from *H. bulbosum* [40].

Reducing the size of an introgressed chromosomal segment relies on recombination; however, recombination is not distributed homogeneously along the chromosomes of wheat and barley and depends strongly on the level of sequence similarity. Comparative sequencing between homoeologous sequences in wheat [7,41–43] and between cultivars in barley [44] has shown that sequence similarity is restricted mainly to the genic areas and that the intergenic regions are highly divergent. The more divergent the species used for wide crosses, the higher the probability that decreasing sequence similarity restricts recombination. Because there are long stretches of intergenic regions, the complete suppression of recombination at an introgressed locus (e.g. at R disease loci [45–47]) results in increased linkage drag. Thus, recombination between introgressed fragments from wild relatives and their allelic

sequence from elite breeding cultivars is reduced compared with that observed in crosses within the elite breeding pool.

How can these problems be overcome? The Triticeae research community through the International Triticeae Mapping Initiative (ITMI; <http://wheat.pw.usda.gov/ITMI/>) has focused on the development of genomics tools and resources to enable a thorough understanding of genome structure and behavior. The hope is that new methods for improving the use of wild germplasm will be developed. Numerous gene-based molecular markers are now available, and these are underpinning genetic diversity analyses and comparative studies between wheat, barley and model genomes (http://germinate.scri.ac.uk/barley_snpdb/; <http://wheat.pw.usda.gov/GG2/index.shtml>). Such genome comparisons have increased our understanding of synteny between the grass genomes [48] and cemented the position of rice as a 'genomic model'. Despite significant structural rearrangements, rice genomic data provide a template for the development of additional markers for wheat and barley at target genetic loci. Moreover, the large blocks of repetitive elements that comprise the bulk (i.e. >80%) of the genome can be exploited to generate a large number of specific polymerase chain reaction markers based on the amplification of junctions between repeats [49]. Positional cloning, often a laborious task, will be enhanced greatly by whole genome- and chromosome-specific physical maps of barley and wheat, respectively, that are currently being constructed under the auspices of international consortia (www.wheatgenome.org and www.barleygenome.org). These new resources will accelerate the isolation of genes underlying key agronomic traits and provide a new generation of gene-specific diagnostic markers for breeding and genes for genetic manipulation (GM). Importantly, they also generate a template for sequence-based diversity analyses, providing a precise and unchanging description of allelic variation. Although efficiently relating gene sequence variation with functional variation remains a challenge, a better knowledge of Triticeae genome structure, recombination distribution and regulation will accelerate the development of strategies such as 'allele replacement' through sequence specific homologous recombination to reducing linkage drag.

Concluding remarks and future directions

By exploiting the power of genetics, the Triticeae research community has already established the location of many genes that control both simple and complex traits. Through practical marker-assisted selection, this information is being widely used [50], and unpublished reports suggest that significant gains have been achieved in some breeding programs. Molecular evidence has affirmed that the genetic base available in cultivated wheat and barley, although expanding, remains very narrow. Whereas it is unclear whether continued reshuffling of alleles by breeding in the existing elite gene pool can achieve the same level of genetic gain achieved over the past 50 years, most agree that enrichment of the cultivated gene pool will be necessary to meet the challenges that lie ahead. However, despite extensive use of wild germplasm for wheat and barley improvement, the overall expansion of the germplasm base remains limited, indicating that this, alone, will likely be insufficient to maintain breeding progress.

Box 3. Advances needed to improve diversity in wheat and barley breeding programs

1. An increased understanding of the molecular basis of key traits. The recent cloning of the gene underlying a grain protein trait quantitative trait locus (QTL) from wild emmer wheat [51] has provided the first example of positional cloning of a QTL from wheat. Many more are needed to improve our understanding of key traits and realize the potential of predictive breeding.
2. Phenotypic and genotypic germplasm screening should be modernized and expanded to better quantify and partition genetic diversity [52,53]. New single nucleotide polymorphism (SNP)-based marker platforms and sequencing technologies should provide the impetus for germplasm characterization (e.g. in barley, [54,55], and in wheat, <http://wheat.pw.usda.gov/SNP/new/index.shtml>).
3. An improved molecular understanding of recombination. The manipulation of recombination is critical for the improvement of introgression techniques and for the fragmentation of alien chromosomes. The recent cloning of *Ph1* [32] has provided new insights into the regulation of pairing behaviour. Homologous recombination, the efficiency of which has been recently enhanced in rice [56], is key to allele replacement and coupling sequence to functional variation.
4. A better understanding of the Triticeae genomes structure and the relationship between coding and noncoding regions. Recent studies have helped clarify the nature of the relationship between genome evolution and recombination [57], but understanding the underlying mechanisms will depend on more detailed and extensive information. The new genomics initiatives (www.wheatgenome.org; www.barleygenome.org) should tackle this issue. To fully understand and exploit natural variation, they should be coupled tightly to genomic diversity analyses.
5. New breeding strategies are required to use the information gathered from genetic and genome analysis programs. How can breeders deal with complex traits more effectively? If new recombinational strategies are available, how will this impact breeding methodology? Can we devise approaches that introgress multiple loci or genome regions simultaneously?
6. We must re-engage the public in the genetic manipulation (GM) debate. GM offers an efficient alternative for increasing genetic diversity in the Triticeae crops without the inadvertent incorporation of deleterious alleles by linkage drag. Many valuable genes (e.g. for increased protein quality and drought tolerance [58,59]) have been introduced into these species, and the transgenic lines have been evaluated extensively. However, no commercial transgenic wheat or barley has been released, although field trials have been conducted in many countries [60]. Cisgenesis [61], where transgenes originate from the same or a related species, may promote wider acceptance of GM.

The very nature of current introgression strategies essentially eliminates the transfer of multiple or complex interacting loci into commercial varieties, although these loci might provide great advances in cereal improvement.

To fully capitalize on the extensive reservoir of favourable alleles within wild germplasm, many advances are still needed (Box 3). Thankfully, recent progress has shown that each of these challenges is tractable and within reach. Several areas of Triticeae research activity are underway, and new, international collaborative frameworks have been established through the International Triticeae Mapping Initiative and the Wheat and Barley Genome Sequencing Consortia. These international projects will ensure that available resources are used as efficiently as possible and that significant progress continues to be made in using

the extensive reservoir of wild species germplasm for cereal crop improvement.

Acknowledgements

The authors thank Tzion Fahima, Avi Levi and Kellye Eversole for critical and constructive reading of this manuscript.

References

- 1 Zohary, D. and Hopf, M. (2000) *Domestication of Plants in the Old World*, Oxford University Press
- 2 Heun, M. *et al.* (1997) Site of einkorn wheat domestication identified by DNA fingerprinting. *Science* 278, 1312–1314
- 3 Luo, M.C. *et al.* (2007) The structure of wild and domesticated emmer wheat populations, gene flow between them, and the site of emmer domestication. *Theor. Appl. Genet.* 114, 947–959
- 4 Feldman, M. (2001) Origin of cultivated wheat. In *The World Wheat Book: A History of Wheat Breeding* (Bonjean, A.P. and Angus, W.J., eds), pp. 3–56. Lavoisier Publishing
- 5 Gepts, P. (2002) Ten thousand years of crop evolution. In *Plants, Genes, and Crop Biotechnology* (Chrispeels, M.J. and Sadava, D.E., eds), pp. 328–359, Jones and Bartlett Publishers International
- 6 Morris, P.C. and Bryce, J.H. (2000) *Cereal Biotechnology*, Woodhead Publishing Limited
- 7 Chantret, N. *et al.* (2005) Molecular basis of evolutionary events that shaped the hardness locus in diploid and polyploid wheat species (*Triticum* and *Aegilops*). *Plant Cell* 17, 1033–1045
- 8 Badr, A. *et al.* (2000) On the origin and domestication history of Barley (*Hordeum vulgare*). *Mol. Biol. Evol.* 17, 499–510
- 9 Nilan, R.A. and Ullrich, S.E. (1993) Barley: taxonomy, origin, distribution, production, genetics, and breeding. In *Barley Chemistry and Technology* (MacGregor, A.W. and Bhatta, R.S., eds), pp. 1–5, American Association of Cereal Chemists
- 10 Azhacuvél, P. *et al.* (2006) High-resolution linkage mapping for the non-brittle rachis locus btr1 in cultivated x wild barley (*Hordeum vulgare*). *Plant Sci.* 170, 1087–1094
- 11 Dubcovsky, J. and Dvorak, J. (2007) Genome plasticity a key factor in the success of polyploid wheat under domestication. *Science* 316, 1862–1866
- 12 Faris, J.D. *et al.* (2003) A bacterial artificial chromosome contig spanning the major domestication locus Q in wheat and identification of a candidate gene. *Genetics* 164, 311–321
- 13 Jantasuriyarat, C. *et al.* (2004) Identification and mapping of genetic loci affecting the free-threshing habit and spike compactness in wheat (*Triticum aestivum* L.). *Theor. Appl. Genet.* 108, 261–273
- 14 Nalam, V. *et al.* (2006) Map-based analysis of genes affecting the brittle rachis character in tetraploid wheat (*Triticum turgidum* L.). *Theor. Appl. Genet.* 112, 373–381
- 15 Simons, K.J. *et al.* (2006) Molecular characterization of the major wheat domestication gene Q. *Genetics* 172, 547–555
- 16 Oettler, G. (2005) The fortune of a botanical curiosity - Triticale: past, present and future. *J. Agric. Sci.* 143, 329–346
- 17 Cox, T.S. *et al.* (1986) Changes in genetic diversity in the red winter wheat regions of the United States. *Proc. Natl. Acad. Sci. U. S. A.* 83, 5583–5586
- 18 Hajjar, R. and Hodgkin, T. (2007) The use of wild relatives in crop improvement: a survey of developments over the last 20 years. *Euphytica* 156, 1–13
- 19 Mujeeb-Kazi, A. *et al.* (1996) Conservation of the genetic variation of *Triticum tauschii* (Coss.) Schmalh. (*Aegilops squarrosa* auct. non L.) in synthetic hexaploid wheats (*T. turgidum* L. s.lat. x *T. tauschii*; 2n = 6x = 42, AABBDD) and its potential utilization for wheat improvement. *Genet. Resour. Crop Evol.* 43, 129–134
- 20 Jørgensen, J.H. (1992) Discovery, characterization and exploitation of Mlo powdery mildew resistance in barley. *Euphytica* 63, 141–152
- 21 Tanksley, S.D. and McCouch, S.R. (1997) Seed banks and molecular maps: unlocking genetic potential from the wild. *Science* 277, 1063–1066
- 22 Tanksley, S.D. and Nelson, J.C. (1996) Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. *Theor. Appl. Genet.* 92, 191–203
- 23 von Korff, M. *et al.* (2006) AB-QTL analysis in spring barley: II. Detection of favourable exotic alleles for agronomic traits introgressed from wild barley (*H. vulgare* ssp. spontaneum). *Theor. Appl. Genet.* 112, 1221–1231
- 24 von Korff, M. *et al.* (2005) AB-QTL analysis in spring barley. I. Detection of resistance genes against powdery mildew, leaf rust and scald introgressed from wild barley. *Theor. Appl. Genet.* 111, 583–590
- 25 Narasimhamoorthy, B. *et al.* (2006) Advanced backcross QTL analysis of a hard winter wheat x synthetic wheat population. *Theor. Appl. Genet.* 112, 787–796
- 26 Jiang, J. *et al.* (1993) Recent advances in alien gene transfer in wheat. *Euphytica* 73, 199–212
- 27 Qi, L. *et al.* (2007) Homoeologous recombination, chromosome engineering and crop improvement. *Chromosome Res.* 15, 3–19
- 28 Endo, T.R. (2007) The gametocidal chromosome as a tool for chromosome manipulation in wheat. *Chromosome Res.* 15, 67–75
- 29 Fedak, G. (1999) Molecular aids for integration of alien chromatin through wide crosses. *Genome* 42, 584–591
- 30 Riley, R. and Chapman, V. (1958) Genetic control of the cytologically diploid behaviour of hexaploid wheat. *Nature* 182, 713–715
- 31 Riley, R. *et al.* (1959) Genetic control of chromosome pairing in intergeneric hybrids with wheat. *Nature* 183, 1244–1246
- 32 Griffiths, S. *et al.* (2006) Molecular characterization of Ph1 as a major chromosome pairing locus in polyploid wheat. *Nature* 439, 749–752
- 33 Zarco-Hernandez, J.A. *et al.* (2005) Durum wheat (*Triticum turgidum*, L.) carrying the 1BL/1RS chromosomal translocation: agronomic performance and quality characteristics under Mediterranean conditions. *Eur. J. Agric.* 22, 33–43
- 34 Rabinovich, S.V. (1998) Importance of wheat-rye translocation for breeding modern cultivars of *Triticum aestivum* L. In *Wheat: Prospects for Global Improvement* (Braun, H.J. *et al.*, eds), pp. 401–418, Kluwer Academic Publishers
- 35 Lukaszewski, A.J. (2006) Cytogenetically engineered rye chromosomes 1R to improve bread-making quality of hexaploid triticale. *Crop Sci.* 46, 2183–2194
- 36 Giroux, M.J. and Morris, C.F. (1998) Wheat grain hardness results from highly conserved mutations in the friabilin components puroindoline a and b. *Proc. Natl. Acad. Sci. U. S. A.* 95, 6262–6266
- 37 Bonafede, M. *et al.* (2007) Reduction of a *Triticum monococcum* chromosome segment carrying the softness genes Pina and Pinb translocated to bread wheat. *Crop Sci.* 47, 821–828
- 38 Paull, J.G. *et al.* (1994) RFLP markers associated with Sr22 and recombination between chromosome 7A of bread wheat and the diploid species *Triticum boeoticum*. *Theor. Appl. Genet.* 89, 1039–1045
- 39 Islam, S. *et al.* (2007) Salt tolerance in a *Hordeum marinum*-*Triticum aestivum* amphiploid, and its parents. *J. Exp. Bot.* 58, 1219–1229
- 40 Pickering, R. *et al.* (2006) The transfer of a gene conferring resistance to scald (*Rhynchosporium secalis*) from *Hordeum bulbosum* into *H. vulgare* chromosome 4HS. *Plant Breed.* 125, 576–579
- 41 Gu, Y.Q. *et al.* (2004) Rapid genome evolution revealed by comparative sequence analysis of orthologous regions from four triticeae genomes. *Plant Physiol.* 135, 459–470
- 42 Gu, Y.Q. *et al.* (2006) Types and rates of sequence evolution at the high-molecular-weight glutenin locus in hexaploid wheat and its ancestral genomes. *Genetics* 174, 1493–1504
- 43 Wicker, T. *et al.* (2003) Rapid genome divergence at orthologous low molecular weight glutenin loci of the A and A^m genomes of wheat. *Plant Cell* 15, 1186–1197
- 44 Scherrer, B. *et al.* (2005) Large intraspecific haplotype variability at the Rph7 locus results from rapid and recent divergence in the barley genome. *Plant Cell* 17, 361–374
- 45 Alberto, C. *et al.* (2003) Genetic analysis of the *Aegilops longissima* 3S chromosome carrying the Pm13 resistance gene. *Euphytica* 130, 177–183
- 46 Stein, N. *et al.* (2000) Subgenome chromosome walking in wheat: a 450-kb physical contig in *Triticum monococcum* L. spans the *Lr10* resistance locus in hexaploid wheat (*Triticum aestivum* L.). *Proc. Natl. Acad. Sci. U. S. A.* 97, 13436–13441
- 47 Wei, F.S. *et al.* (1999) The Mla (powdery mildew) resistance cluster is associated with three NBS-LRR gene families and suppressed recombination within a 240-kb DNA interval on chromosome 5S (1HS) of barley. *Genetics* 153, 1929–1948

- 48 Salse, J. and Feuillet, C. (2007) Comparative genomics of cereals. In *Genomics-Assisted Crop Improvement: Volume 1: Genomics Approaches and Platforms* (Varshney, R.K. and Tuberosa, R., eds), pp. 177–205, Springer Verlag
- 49 Paux, E. *et al.* (2006) Characterizing the composition and evolution of homoeologous genomes in hexaploid wheat through BAC-end sequencing on chromosome 3B. *Plant J.* 48, 463–474
- 50 Reece, J.D. and Haribabu, E. (2007) Genes to feed the world: the weakest link? *Food Policy* 32, 459–479
- 51 Uauy, C. *et al.* (2006) A NAC Gene regulating senescence improves grain protein, zinc, and iron content in wheat. *Science* 314, 1298–1301
- 52 Balfourier, F. *et al.* (2007) A worldwide bread wheat core collection arrayed in a 384-well plate. *Theor. Appl. Genet.* 114, 1265–1275
- 53 Jana, S. (1999) Some recent issues on the conservation of crop genetic resources in developing countries. *Genome* 42, 562–569
- 54 Rostoks, N. *et al.* (2005) Genome-wide SNP discovery and linkage analysis in barley based on genes responsive to abiotic stress. *Mol. Genet. Genomics* 274, 515–527
- 55 Rostoks, N. *et al.* (2006) Recent history of artificial outcrossing facilitates whole-genome association mapping in elite inbred crop varieties. *Proc. Natl. Acad. Sci. U. S. A.* 103, 18656–18661
- 56 Terada, R. *et al.* (2007) Gene targeting by homologous recombination as a biotechnological tool for rice functional genomics. *Plant Physiol.* 144, 846–856
- 57 Akhunov, E.D. *et al.* (2003) The organization and rate of evolution of wheat genomes are correlated with recombination rates along chromosome arms. *Genome Res.* 13, 753–763
- 58 Barro, F. *et al.* (2003) Functional properties of flours from field grown transgenic wheat lines expressing the HMW glutenin subunit 1Ax1 and 1Dx5 genes. *Mol. Breed.* 12, 223–229
- 59 Bahieldin, A. *et al.* (2005) Field evaluation of transgenic wheat plants stably expressing the HVA1 gene for drought tolerance. *Physiol. Plant.* 123, 421–427
- 60 Bhalla, P.L. (2006) Genetic engineering of wheat—current challenges and opportunities. *Trends Biotechnol.* 24, 305–311
- 61 Jacobsen, E. and Schouten, H.J. (2007) Cisgenesis strongly improves introgression breeding and induced translocation breeding of plants. *Trends Biotechnol.* 25, 219–223

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