Review

Knowledge and technologies for sustainable intensification of food production

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Knowledge and technologies will always continue to be developed, as they have always, to bring new efficiencies to plant breeding and crop production, which suffer from many constraints and inefficiencies. These constraints need to be overcome throughout the world to help increase the rate of improvements in food production and intensify production on less land. The recent discoveries and technical innovations that are revealing the full complement of genes in crops, the ability to define genetic variation and use DNA markers to follow chromosome segments with known functions through breeding programmes are leading to new efficiencies in breeding. The ability to isolate and redesign genes and transfer them into different plants also offers the breeder solutions to several key limitations. These benefits are described together with some of the current issues associated with the use of transgenes. Generation after generation can look forward to new knowledge and technologies, many of which we cannot know at present, and thus there is no reason to be despondent about meeting future goals, if the right decisions and investments are made globally and locally. These decisions include putting optimal use of land at the top of the world agenda to sustain both the planet and an adequate quality of life for mankind. As always has been the case, more investments are urgently required into the dissemination of successful technologies in crop breeding and production, into teaching and training as well as into innovative research. Failure to invest adequately in innovative technologies will leave future decision-makers and citizens with fewer options and greatly enhance the risks for mankind and a healthy planet.

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Introduction

It is understandable that citizens who have not had the chance to gain knowledge and insight into the frontiers of genetic technologies should be wary of the innovative technologies being built to improve food production. But we must learn from history, the ancestral farmers and entrepreneurs who have brought us the food we enjoy. Without their genetic innovations we would not have corn, wheat or almost any of the foods we enjoy and that keep alive a global population. The world would be in a hopeless position. Mankind would still be in the dark ages. To bring about a better world where people have enough healthy food to eat and our planet is sustainable, we need wise decision-making and new technologies to make food production more efficient. We also need to transfer the best of existing technologies to food production worldwide.

Progress often depends on new technologies and it has always been so in the breeding of crops and in their production by farmers. It takes some 10 years to breed, test and commercialise a new variety starting with two parent plants. Ten years ago plant genetics and genomics were in their infancy compared with now. Also we had no Google, no Facebook, no You Tube, no blogs nor twitters. Farmers in poorest Africa were not in touch with the outside world. Today we are in a new enlightenment in plant genetics and breeding and have all these personalised communication systems that empower the individual, decentralise societies and bring people together with knowledge, systems and choices as never before. In consequence, the African farmer is in touch with the outside world via the cellphone and small villages in India have a computer and the World Wide Web. Where will societies be in 10, 20, 30, 40 and 60 years in the poorer parts of the world? What will the farmers and citizens be achieving and demanding, having become connected to global communication systems, knowledge and new markets. What will be the standards in plant breeding and farming? Not as today, that is certain. Many of today's technologies will be seen as old fashioned, hopelessly short of what is possible and required. Plant breeding and farming need to change radically in many parts of the world and will do so, driven by new knowledge and innovations in technology.

The idea that technology will stand still over the coming decades is obviously nonsense. Many thoughts today may seem fanciful, including some mentioned in this paper, but then so to people in Europe and USA in the early years of the 20th century would have been civil aviation, space exploration, organ transplants, nuclear power, computers, the Internet, nanotechnology and mobile phones. While plant breeding and solving the food and energy crop production issues may seem daunting to the average citizen, science will evolve beyond ways we understand today, to provide new options. Placing today's needs in the context of what has been overcome in the past, the opportunities of technology development and where we seek to be in the future can legitimately generate optimism, providing wise decision-making and appropriate investments are sustained. When wise decisions are not made, then technical developments have to be even more successful to meet the needs. Planners and opinion formers need to have all this at the heart of their decision-making. This paper seeks to draw attention to the new knowledge and technologies that are available and will become available to increase food production sustainably and more intensively so that all are fed and land is available for sustaining the planet and quality of life. It is recognised that hunger is most often a consequence of poverty, absence of markets, inadequate land reform and poor education systems. However, sound agriculture is a source of wealth for many rural people and societies in general. This justifies the focus of this paper on the knowledge and technologies associated with agriculture as one of the sources of relief from hunger. The paper is not designed to be in any sense a technical handbook for practitioners. Other treatises fulfil this need [1–3].

The issues we all face

There are many examples of successful increases in food production over the past 40 years, especially in Asia [4]. Figure 1 illustrates the large increases in total food production in different continents [5]. This means that breeders have produced suitable varieties, farmers have heard about them, invested in them and thereby increased food production. Much of Asia's food production increases have involved the use of modern varieties [6]. Even in Africa yields have increased. This would be a very satisfying position, except that food increases have not kept pace with population increases and so the increases *per capita* shown in Figure 1B are less positive. Africa is now only just beginning to restore the *per capita* food position that it had 40 years ago. Thus in spite of all these increases and successes there are still 1 billion people suffering from poverty and lack of food [4].

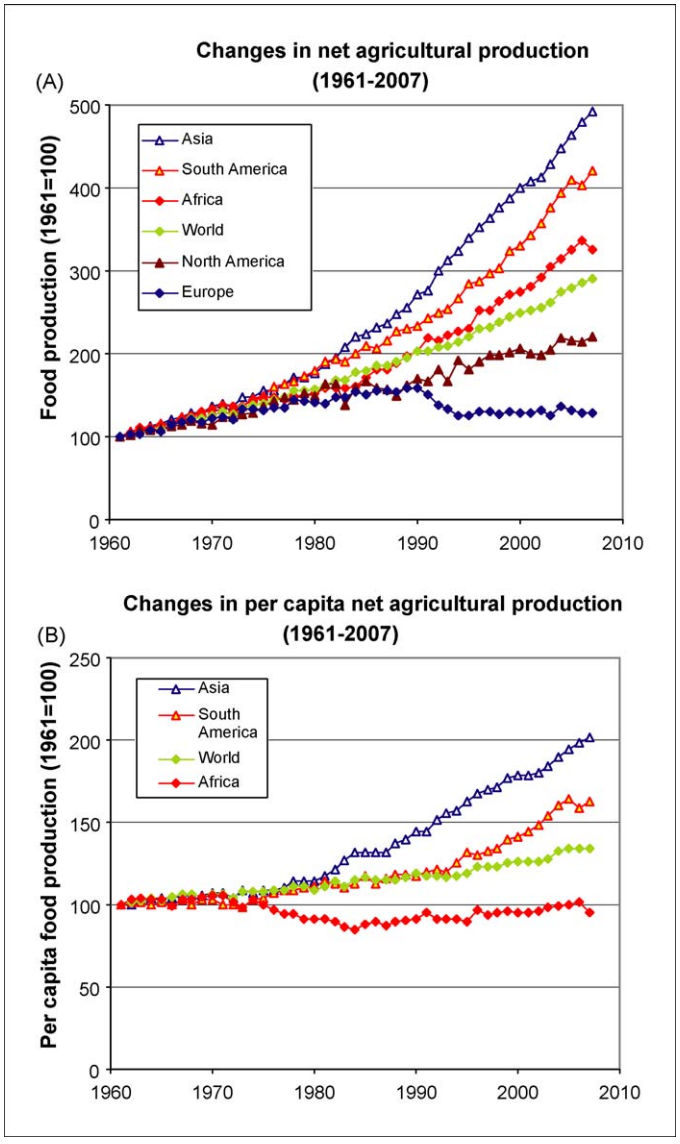

FIGURE 1

(A) Changes in net agricultural production in continents. **(B)** Changes in *per capita* net agricultural production. From Jules Pretty and Royal Society, 2009.

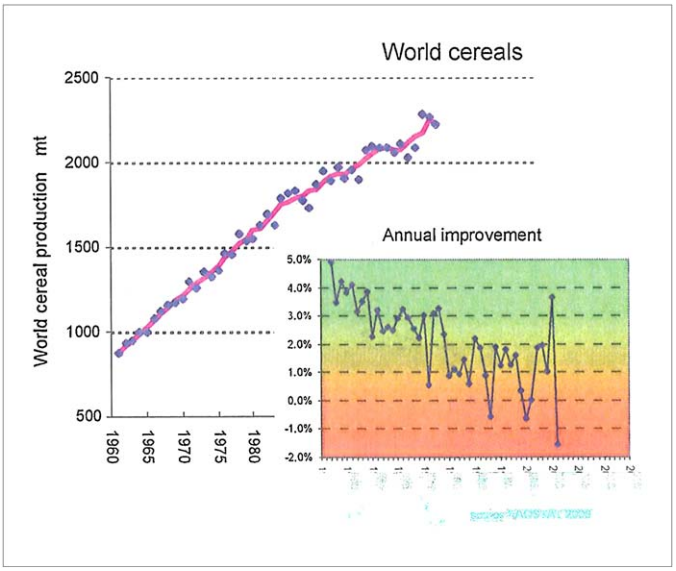

FIGURE 2

Changes in world cereal production and annual rate of improvement (FAOSTAT, 2008).

Can we sustain the good increases illustrated in Figure 1A throughout the world in all environments? If plant breeding were easy and we could simply make higher yielding crops more quickly by scaling up existing methods then the outlook would be more hopeful. However the results for world cereal production show that the *per capita* gains produced have fallen since 1985 and the rate of annual increase is declining (Figure 2). Thus plant breeders and farmers are not making gains. This is a serious position, given that we need to increase global food production by 40% in the next 20 years [4,7]. Figure 1 shows this to be an enormous challenge. Also these trends do not reveal the levels and the diversity of food needed to bring a healthy and satisfying life for all. Furthermore they do not draw attention to the amounts of land required to keep the planet and its populations sustainably healthy by the growing of biomass for biofuels, managing greenhouse gas levels, sustaining adequate biodiversity and providing essential amenities. In summary, we need to increase the rate of gain in food production and reverse the positions in Figure 2, intensify food production on less land and free up land for other needs. To do this, plant breeding and food production need to be supplied with a constant stream of new knowledge, tools and systems that will lead to more sustainable intensification of agriculture, just as what occurred with US corn production and Asian wheat and rice breeding during the Green Revolution [8]. The needs are urgent and the options for success are visible. It is recognised that many other factors are necessary, in addition to new varieties, for successful adoption of innovations [4,7]. They include numerous financial and policy factors, but discussion of these is outside the scope of the paper.

What is the technical basis of plant breeding?

Plant breeding involves the bringing together of new versions of genes to create plants with new properties. During the formation of eggs and pollen in plants, new gene assortments are created by existing chromosomes becoming recombined and then, as a consequence of the fertilisation of eggs by pollen, new combinations of genes from the two parents are brought together to form embryos and the new generation. There are from 30 000 to over 60 000 different genes in a plant species. Fortunately, there are also many different versions of each gene in a species, created by natural mutations, and it is the reassortment of these variants that is achieved in plant breeding. Following the creation of new combinations of gene variants the breeder grows large numbers of offspring and seeks plants that perform better than the parents and existing varieties. Because there are so many genes and variants of each, there is almost an infinite number of possible combinations that could be made. In addition, there are so many environments in which the plants need to be successful, the process of improving plants by plant breeding and demonstrating the improvements in farmers' fields is statistically very inefficient, time-consuming and relatively expensive [9].

When seeking progeny that are better than those already available, breeders have to optimise a large number of traits. Some of

TABLE 1

Traits that are commonly assessed directly or indirectly by breeders

• Architecture-height, number of leaves, tillers, branches, leaf angle, number of flowers and seeds, seed size, root structure, surface area.
• Optimum planting density.
• Flowering time and photoperiod responses.
• Growth rate regulation.
• Growth responses to light quality and quantity.
• Photosynthesis-rates and overall carbon fixed during the growing season.
• Heterosis.
• Fertility and seed production.
• Nitrogen use efficiency-uptake, translocation, storage, reduction and portioning between plant parts.
• Water use efficiency-uptake, storage, transpiration rates, loss, tolerance to chronic drought and bursts of drought.
• Disease, pest and virus resistances.
• Tolerance to heat shock and sustained heat.
• Tolerance to cold shock and sustained cold.
• Seed germination in cold.
• Tolerance to freezing.
• Tolerance to flooding.
• Tolerance to oxidative stress.
• Amounts of key nutrients in seeds, roots, leaves, fruits and stems.

these are listed in Table 1. Each of these traits is specified by many genes interacting in very complex circuits. Thus the reassorting of genes and gene variants in each breeding cycle affects almost every trait in every generation. This complexity also makes plant breeding inefficient. Where there are no genes for a particular desired trait in the species, the improvements dependent on such genes cannot be achieved, no matter how large the investment. Most traits in most crops are still suboptimal, especially resistance to pests and diseases. Shortcomings in managing all these traits in breeding programmes lead to inadequate products. What can be learnt from successful breeding programmes, past and present, that can be applied more widely? One of the most successful breeding programmes has been corn breeding in the USA [10–12]. I will use this example to make many key points in relation to knowledge and technology development for crop improvement. Other examples from rice [13] and wheat [14] could also have been chosen but even these examples do not display some of the key innovations in US corn improvement.

The sustainable intensification of US corn production

The extensive gains in yield per acre made over the past 60 years by corn breeders and farmers in the USA are well known [10–12] and are illustrated in Figure 3. What have been the innovations behind this progress? One of the most extraordinary series of innovations took place centuries earlier by the Indian enterpreneurs of Central America. They transformed an ancestral perennial species into what we now know as corn. The plants look very different and the genetic changes selected by the Indians are becoming understood from comparisons of all the genes in the ancestor and modern corn. In the US, yields stayed the same until after the 1940s (Figure 3), when innovative crosses and genetic understanding had been developed. It was discovered that if certain plants were crossed, the F1 hybrids were much more vigorous than the parents. This so-called heterosis has been the basis of many yield gains since [15]. While the plant breeders were making better and better heterotic hybrids, the use of fertilisers and herbicides helped the farmers get higher yields. The makers of farm machinery also helped by making a succession of improved machines to increase the efficiency and scale of agricultural production. In the late 1990s knowledge of how to measure genetic variation in DNA sequences at scale (see below) became

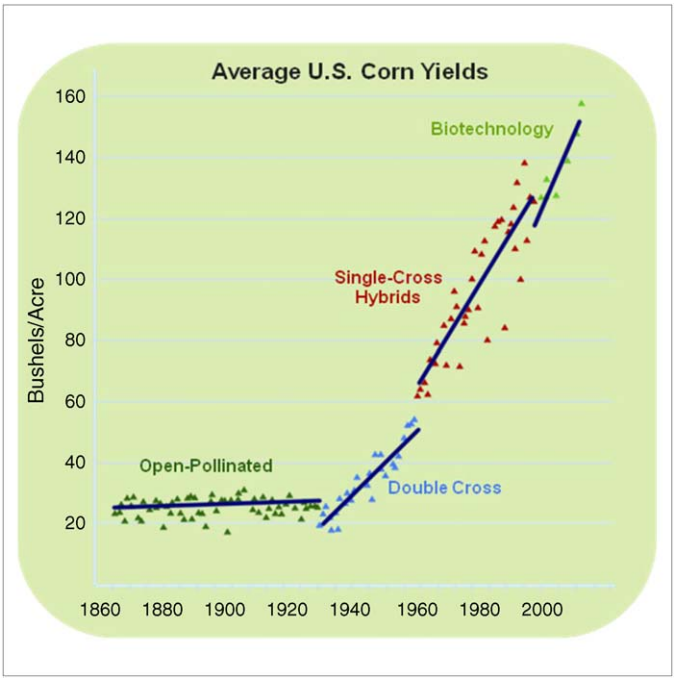

FIGURE 3

Increases in corn yields in US. The periods when open pollination and when the use of double and single cross hybrids were introduced are shown. The introduction of transgenic hybrids occurred towards the end of the 1990s when biotechnology started to make its impact.

available and this led to the commercial adoption of marker-assisted breeding. Genetic engineering emerged during the 1980s and genes were selected, purified, redesigned and then introduced into corn plants (see later) that conferred tolerance to herbicide (Roundup) and resistance to feeding insect larvae (corn stem borers). Elite transgenic lines were commercialised to be among the first transgenic row crops [16]. More recently, genes conferring resistance to root worm have been added. Today's corn lines have up to nine transgenes in them [16]. The herbicide tolerance and insect tolerance genes brought environmental benefits, because Roundup is more benign to the environment than herbicides used previously and because of the reduced use of insecticide sprays. It has also been found that protecting corn from root damage brings some drought tolerance too.

The increases in yields in Figure 3 are the results of sustained investment by government and the private sector combined with government subsidies and incentives. All these working in combination enabled and stimulated the stream of technical improvements behind breeding and the growth in production intensity. Yet over the past 15 years the farmers have applied less nitrogen, phosphate, herbicide and insecticide per bushel and adopted no-till practices to conserve water, soil structure and reduce erosion [17]. Thus, the farmers have addressed the issues of sustainability during the latter years of intensification, even while output gains have continued.

This example of the intensification of corn in the USA points the way ahead for all other crops and breeding programmes because it has both driven innovations and adopted new tools from nature, breeders and farmers as they have been developed. It has not been without its difficulties. For example, hybrids made using cytoplasmic male sterility in the late 1960s and early 1970s were susceptible to a fungus [18], but difficulties and setbacks must be expected en route to success. If all this knowledge and new technologies were incorporated into all the other breeding programmes worldwide then yield gains would be very substantial. This is emphasised by the comparative yield figures for corn in different countries shown in Figure 4. While many local features including soil and climate prevent the best yield figures being achieved everywhere, the large discrepancies revealed in Figure 4 are due to lack of sustained investments equivalent to those made in the US.

Corn Yield Trends
(Bushel Per Acre)

	1990	2000	2005
World Average	59	70	75
USA	113	137	149
Argentina	60	93	109
China	74	78	80
Brazil	33	47	54
India	23	29	31
Sub-Saharan Africa	22	24	25

FIGURE 4
Comparisons of average corn yields between countries since 1990.(Monsanto/Doane Forecast).

Comparisons of the toolkits of nature, the breeders of the past and the breeders of today and tomorrow

Progress in evolution by natural selection depends on genetic variation. This variation has its origins in genetic mistakes that survive in individuals and are inherited. They are then either selected during evolution, carried along as neutral mutations and spread in populations by accident or spread because of being linked to other favourable mutations under selection. The naturally occurring mistakes include chromosome duplications, gene loss, gene duplication, mutations in genes that change the protein or RNA products or change gene activity during plant development, and the movement of specialised gene elements, so-called transposable elements, that occur in large numbers in plant genomes and move around the genome. Such mutations become mixed in populations by sexual recombination. Occasionally, but importantly, evolution involves the rare hybridisation between different but related species to form a new hybrid. Thus the toolkit of nature is confined to the natural variation accumulated in populations during evolution and the rare hybridisation between distant individuals.

Plant breeders use this variation and recombine it as noted above, also using the processes of sexual recombination. Thus breeders use nature's toolkits, albeit augmented by other technologies. Occasionally breeders are able to force interspecies recombinations that do not occur or are inviable in nature and then select stable progeny that carry genes from both species. Breeders try this approach to introduce or improve a trait that is needed. A problem for breeders is that when seeking to add better versions of genes by making crosses between dissimilar parents that carry useful genes, they also have to import large numbers of deleterious genes. This makes improving plants in specific ways difficult, time-consuming and inefficient.

These toolkits can be contrasted with those devised by the molecular biologist. The innovations from molecular biology provide the means of isolating and defining any gene from any organism, creating any kind of mutation in any gene and designing and making new genes. The novel genes can then be inserted into any plant. Thus, with these tools and techniques, the modern breeder can overcome the serious limitations of (1) only having the mutations found in nature to solve food production and quality problems, (2) not being able to move defined genes between species to add specific traits and avoid the introduction of other deleterious traits, (3) not being able to modify varieties one step at a time and (4) not being able to track favourable and deleterious genes through breeding programmes. All of these innovations have emerged over the past 30 years. The first transgenic plants were created and bred around 1982 [19]. The technologies developed by molecular biologists when integrated into plant breeding programmes change dramatically our abilities to improve plants for agriculture. This fact should not be underestimated, but rather be the reason to make new investments and train new breeders to meet the needs of societies, especially those with poverty and hunger. The technologies bring new optimism.

Advances in gene and genome discoveries and their applications to breeding

Genome and gene sequencing

In 2000, the complete genome sequence of Arabidopsis, the first plant genome to be sequenced, was announced following a large international effort [20]. The rice genome sequence was also announced in 2002 [21,22]. Since then several plant genomes have been essentially completely sequenced, including those of corn, soybean and sorghum [23–25]. While thousands of genes were identified from the first genome sequence, their function was not understood. Also many important genes were missed in the initial interpretation of the genome sequences. Thus in 2003, there were about 5000 genes defined (by sequence and a function) in plants. In 2008, the number had grown to 200 000 and was increasing exponentially. Similarly the number of gene products (proteins) recognised was a few thousand in 1998 but is now over 1 000 000. This rapid growth in knowledge happened because of technical innovations in DNA sequencing methods and cost reductions, as a number of radically new sequencing technologies have come into commerce [26,27]. These created dramatic increases in output of DNA sequence per machine and slashed costs by miniaturising the processes and performing millions of reactions in parallel. In the year 2000, about 10 bases could be identified per dollar. In 2005, it grew to about 40 bases per dollar and in 2015 it might be 1 000 000. There is a race to deliver 'a complete (human) genome sequence for $1000'. Six or seven companies appear today to be firmly in the race. Competition in this race to capture the global market of being able to read the DNA sequence of a person at prices that individuals and healthcare systems can afford is likely to become increasingly intense. So, within the next few years, the $1000 human genome sequence will become a reality. For a plant breeder to be able to sequence the genome of a large number of potential parents and selected plants, to know the variation within them and to check his product is an extraordinary concept but is clearly almost with us [27]. It is worth noting that this innovation, perhaps the one with the largest impact for increasing commercial crop production, has come from the private sector entrepreneurs and investors in the medical and IT industries, that are not concerned with agriculture and plant science.

Cataloguing and mapping genetic variation in chromosomes using DNA markers

Plant improvement is based on, and necessarily exploits, genetic variation. Thus, being able to characterise the variation in every gene in the plants of a breeding nursery can bring powerful knowledge to the breeder, as noted above. Recombination in gamete formation in egg and pollen cells occurs only a few times per chromosome in any one generation. This results in blocks of genes being inherited together. Thus to follow which chromosome segments, and therefore constituent genes, are in a progeny plant requires only a marker for each of the chromosome regions that are inherited intact and not divided by recombination. Plant breeding is therefore greatly aided by having DNA markers for each version of the chromosome segment (haplotype) introduced via the different parents [28–30].

Finding markers today is easily achieved using the genome sequencing described above. Using these methods, the variant DNA sequences that allow the chromosomal segments to be distinguished are discovered. High throughput assays are then designed for this subset of markers. The commercial technologies for doing all this are advancing rapidly—millions of data points can be gathered in a day [28]. They are evolving in synchrony with the DNA sequencing technologies. Technical advances will arise year-on-year over the next 10 years. Therefore the goal to provide breeders with haplotype maps of essentially all the germplasm of a crop, easily accessible in databases, with full details of all the plant accessions possessing each of these haplotypes, is within reach. This too will revolutionise breeding.

As with DNA sequencing described above, the generation of large datasets of marked chromosomes needs to go hand-in-hand with IT and software innovations and development of user-friendly databases to enable the benefits of all new information to be useful to the breeders. This is a major activity by world experts and is also advancing rapidly [31].

Establishing gene–trait associations

Geneticists have long sought to define the genes that influence traits on genetic maps. The genes are embedded in quantitative trait loci or QTLs. The use of polymorphic genetic markers covering all the chromosome sets allows linkage between a chromosome segment (QTL) and a trait in populations to be sought easily when the trait is segregating [32,33]. The establishment of huge datasets of mapped sequence polymorphisms means that DNA markers need no longer be limiting. What is rate-limiting is measuring the traits. The plant breeder often needs to do this in hundreds or thousands of progeny from a large number of crosses for each species to reveal tight associations. It is also desirable to do this with plants grown in multiple environments. To measure certain traits such as those affecting disease, stresses and so on, there is the need to expose the plants to the stresses. All this adds up to an enormous task that needs considerable investment.

An alternative is to achieve gene–trait associations by comparing markers and traits in a large number of accessions of a crop that are as unrelated as possible [34–37]. If sufficient recombination events have taken place during the separate evolution of the accessions then it may be possible to infer that deviations from random linkage signify a close physical relationship between marker/gene and the trait. This newer approach of 'association mapping' is being studied in corn in detail. Nevertheless, it still leaves the necessity to measure a large range of traits (Table 1) in a large number of accessions. While it is an immense volume of work to determine gene–trait associations, they will be known for all time and this will be an enduring platform for underpinning plant breeding for ever. A different version of this approach is to find markers that correlate with selection of a given trait in breeding programmes where the genetic location of the genes is ignored [38–40]. When models built upon markers that give a high selection coefficient for the traits in question are obtained, then the markers can be deployed to drive a breeding programme, for the relevant combinations of traits. These approaches, only possible by the discovery and large-scale measuring of DNA markers, are likely to have a high impact on plant breeding in the future.

Gene–trait associations have been established extensively in Arabidopsis, corn, rice and many other crop species by mutant analysis [41] and also inferred by linking gene expression patterns

with a trait. They have also been established by QTL analysis [32,33]. All this information from multiple species can be brought together to establish hypotheses for one crop species using the results from other plant species. The future value for comparative genetics is likely to be substantial, especially where the species are closely related, for example, corn and sorghum.

Many gene–trait associations have also been established by observing the effects on traits of adding known transgenes to a plant [42,43]. Complete linkage between the added, known transgene and the new trait provides direct evidence for a gene–trait association. It remains to be seen to what extent these gene–trait associations coincide with the associations derived from genetic variation in natural populations.

Gene transformation into plants

There are two principal ways genes are introduced into plants [44,45]. The first exploits the natural process of gene transfer evolved in the soil bacterium, *Agrobacterium tumefaciens*. The second is by bombardment of plant cells capable of division with particles coated with genes. In the first, genes designed and reconstructed *in vitro* and propagated in *Escherichia coli* are transferred into agrobacteria on specifically designed plasmids that contain the DNA signals that are recognised by the bacterial transfer process. When the agrobacteria are mixed with plant cells, the gene transfer process is activated and pieces of DNA containing the genes to be transferred are passed into the plant cells and become integrated into plant chromosomes. The plant cells are stimulated to divide and those containing the new genes are selected owing to the presence of genes transferred from the bacteria that provide resistance to some chemical, such as a herbicide. When many cell divisions have taken place, then the plant cells are stimulated to differentiate into shoot and roots and so new plants are formed. In such plants, each cell should carry one or a few copies of the new genes. In the second method the genes propagated in *E. coli* are forced into plant cells and internal processes lead to the incorporation of the pieces of DNA into the plant chromosomes. Thereafter the processes adopted are similar to those in the first method.

Today any plant species can be transformed with new genes in these ways but the efficiency of regeneration of a whole plant from the initially transformed cells can vary greatly, including between lines of the same species. Where the efficiencies are low, research to increase the efficiencies is usually effective. Furthermore some transgenes have been found that increase transformation/regeneration frequencies, and these are in use commercially [46].

The ability to add new genes to a species fulfils, in principle, the dreams of most plant breeders who constantly seek to add new traits more efficiently and effectively. But, much more is emerging as the technology grows from its infancy.

Advances in plant breeding emanating from the deployment of transgenes

The combinations of genetic analyses using genomics and markers will improve plant breeding immensely, but there is substantial recognition that the deployment of transgenic technologies can achieve more far-reaching and beneficial products in agriculture. Some of these advances are listed in Table 2. They are outlined here firstly to provide some details of the technologies, but secondly to illustrate steps along a path towards a radically different kind of plant improvement that we should work towards to rid the world of the food, feed and fibre shortages and ensure the availability of land to provide other services to mankind and to manage the planet optimally.

Addition of novel traits not already in the crop species or in need of improvement

The addition of new traits, such as herbicide tolerance, insect resistance, novel omega 3 fatty acids, provitamin A and hundreds

TABLE 2

Opportunities for improvements in crop plants and breeding by the use of transgenes

• Development of a new strategy for breeding and selection of improved traits using a few, known, dominant transgenes instead of many recessive QTLs for each trait.
• The ability to substitute any allele by another using homologous recombination to optimise varieties.
• The ability to change the expression pattern of any gene by changing promoters and upstream regulatory sequences using homologous recombination.
• The ability to control the rates and places of recombination in crop chromosomes to enable new gene combinations to be produced and at much greater rates and so reduce the number of progeny that need to be produced to achieve specific kinds of products; and alternatively to reduce recombination to fix desired genotypes.
• The ability to delete unwanted transgenes by specific recombination using cre-lox or flip recombinase systems.
• The ability to control major diseases by creating novel genetic systems based on, for example, non-host resistance, pathogen recognition systems and production of downstream resistance mechanisms.
• Development of sentinels and rapid assays to reveal the health of the production crop.
• The ability to add and sustain banks of specific transgenes in one locus via a novel chromosome or chromosome segment.
• The ability to fix hybrids showing heterosis using the principles of apomixis.
• The ability to switch traits using simple reagents based on particular weather patterns and needs, such as the need for a protein rich crop as opposed to a carbohydrate crop. Switching technologies based on novel promoters that can be activated by specific chemicals are already available.
• The ability to make transformation and regeneration trivial for all crops by improvements in, for example, agrobacterium vectors and strains that include genes that stimulate regeneration, but which can be silenced or deleted when regeneration has been achieved.
• The ability to target genes to dividing cells to make regeneration more efficient.
• Optimisation of crops for their nutritional content such as provitamin A as in 'Golden Rice' and the equivalent in other crops.

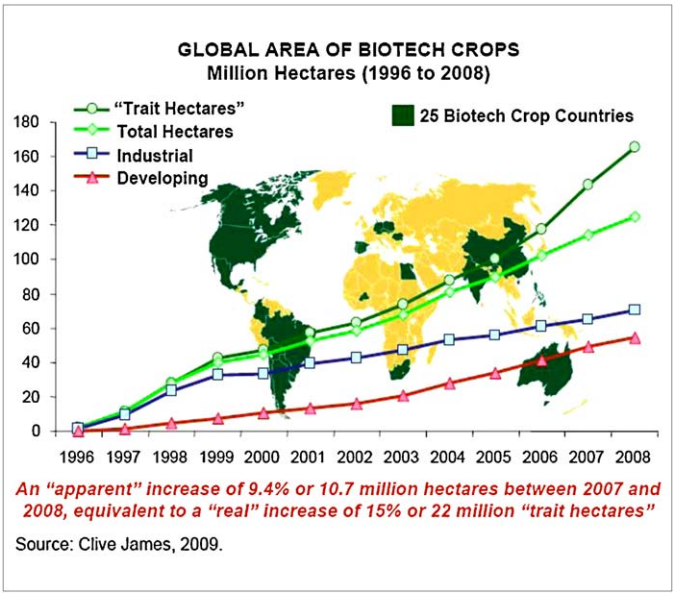

FIGURE 5

The cumulative adoption of transgenic crops into agriculture since 1996.(James, 2009).

of other valuable traits, including those listed in Table 1, will bring enormous benefits to consumers and growers of tomorrow's food [16,47]. These are already exemplified by Roundup ready and Bt soybean, corn and cotton crops. These transgenic crops are manifestations of the fastest take-up of any agricultural product (Figure 5) and over 14 million farmers are growing such crops today [16]. There are already many traits in various crops and model plants that have been 'improved' in the laboratory by the addition of transgenes. Improvements in tolerance to stresses have been a particular focus. It is likely that drought tolerance will be the first commercial product in this category [48,49]. Much research is also focused on tolerance to acid soils, better nitrogen utilisation efficiency (Figure 6) and, of course, seed yield.

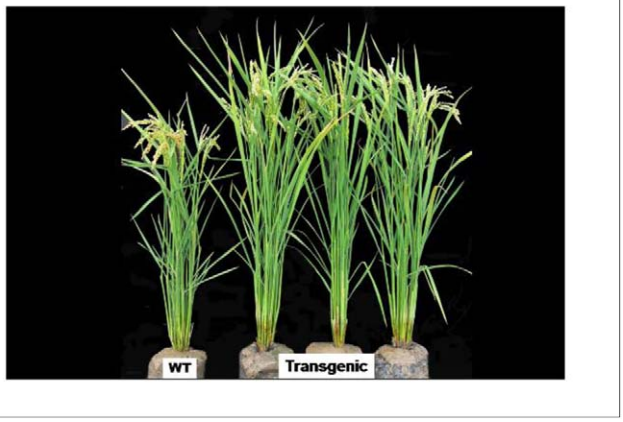

FIGURE 6

Comparison of field-grown rice plants illustrating effect of adding an Arabidopsis gene, under the control of a broadly active promoter, that stimulates height and biomass accumulation without significantly affecting flowering time (Ceres, unpublished).

Silencing and inactivation of genes in the crop

While many traits are more readily 'improved' by the addition of new functions, some improvements are made by the inactivation of existing genes and processes. This can be sometimes achieved by random mutagenesis and then seeking plants that have a particular gene inactivated. The process of 'Tilling' achieves this [50]. Large populations of mutated plants are created and then the sequence of the gene to be mutated is used to devise a polymerase chain-based assay that enables rare mutant versions of the gene to be discovered. This is a non-transgenic approach but suffers from two sorts of deficiencies. Firstly, the gene activity is lost in all cells and this can be lethal. Secondly, many genes are duplicated in plant genomes and the redundancy results in the mutation not having any effect on a trait. Often it is more desirable to down-regulate the levels of expression in particular tissues and from all copies of a gene. Here a transgene possessing sequences that match those of the gene to be down-regulated can be inserted and the RNA products of the transgene activate the RNAi pathways that result in degradation of the mRNA of the natural gene(s) [51,52]. Where gene activity is required to be down-regulated in a particular tissue, then placing the transgene under a promoter active only in that tissue should achieve the desired effect. Selection of particular transgenic events should enable the right levels of reduction to be achieved although instabilities of gene expression are difficult to manage and may change from one generation to the next. Particular genes can also be silenced by the insertion of a transgene into it as described below.

Substitution of any allele, including its promoter, by another using homologous recombination

Breeders consider the ability to replace one or a few alleles in a successful variety with another one of the most powerful additions to plant breeding. The technology would enable specific traits to be improved in the most precise way possible, using essentially the plant's own genes, without the need to either tolerate or eliminate large numbers of deleterious genes from another parent. Recent experiments and the development of novel systems to achieve homologous recombination imply that this goal is within reach and is being investigated in several crops [53,54]. The ability to replace one allele with another also provides the geneticist with the ability to compare the function of specific genes and thus prove their role. Another potentially powerful utility of this sort of technology is to change promoters and so alter the activities of resident genes in a precise way. Given that variation in gene expression is an important source of variation in breeding populations the ability to change promoters precisely is likely to have a very significant future.

Efficient homologous recombination relies on the existence of a double strand break in the chromosome. Such a break can increase the efficiency of homologous recombination several thousand-fold at that site. Thus, the challenge has been to learn how to create double strand breaks at the desired site of insertion in the defined gene. Zinc finger nucleases (ZFNs) and meganucleases are tools that have been designed to achieve this [55–57]. Zinc finger nucleases consist of a DNA-binding zinc finger domain covalently linked to the non-specific DNA cleavage domain of a restriction endonuclease. ZFNs bind as dimers to the specific DNA site and the nuclease catalyses the double strand break.

Targeting of transgenes to pre-determined sites by specific recombination systems

The sites of insertion of transgenes are not generally under the geneticist's control at present. However, transgenes can be integrated into chromosomes at particular sites using site-specific integration systems. These rely on proteins that specialise in recombining two identical, specific sequences. This enables, for example, multiple novel genes to be inserted at a target site. The so-called cre-lox recombination system from bacteriophage lambda has been used for site-specific integration of DNA into tobacco and rice [58]. Here the lox target site is inserted into the chromosome (at random) and the desired transgene is then integrated into this genomic target via recombinase-mediated site-specific integration. The cre/lox site-specific recombination system has also been used successfully in wheat and rice to target single copy insertions into lox sites placed in the genome [59]. Another system, flp-frt, involves the flippase recombinase derived from yeast. Flp recognises a pair of frt target sequences that flank a genomic region of interest. The flp recombinase system has been used in corn [60,61] for site-specific gene replacement, while the lambda and phiC31 integrases have also been used [62]. These approaches facilitate the potential to stack new traits at valuable transgenic loci in a modular fashion and can integrate new genes at a site in the genome already found to support strong constitutive expression, avoiding the disruption of existing genes and negative agronomic impacts.

Control of the rates and places of recombination in chromosomes

Progress in plant breeding depends on the recombination of different genes. How often particular genes become recombined depends on the frequency of recombination and the positions of the genes in the chromosomes in relation to the position of recombination. Given the difficulties in changing the positions of genes with respect to one another there is great appeal in being able to control the position and frequency of recombination during meiosis. This will surely become possible [63]. The ideal is that recombination can be greatly increased to generate more variation efficiently and then reduced back to current levels to maintain genetic stability and integrity. Such an advance will be brought about by the use of specific transgenes under the control of promoters that can be activated by the breeder using, for example, an externally supplied chemical.

Construction of chromosomes for stacking many transgenes in a defined order

A vision of improving plants with a large catalogue of transgenes necessarily raises the question should all the transgenes reside together to aid their regular expression and to make it easy for the breeder to select them altogether? Also should they be arranged so that individual genes can be deleted and new versions added easily? While these issues are addressed partly by development of the homologous integration systems (C and D above) other technologies may be preferable. These are being explored and evaluated in agricultural crops. A novel mini chromosome has been built for maize by combining the genes of interest with a larger piece of maize DNA that encodes satellites, retro-elements and other repeats commonly found in maize centromeres and that confer the ability of a chromosome to be divided regularly between daughter cells at mitosis and meiosis [64]. The mini chromosome, when introduced into maize cells by particle bombardment and plants regenerated containing the new chromosome, shows regular inheritance most of the time. The availability of many valuable genes for crop improvement is starting to accelerate and so there is the need to address questions of where and how to organise many genes for optimum long term utility.

Simplification of the genetic basis of traits

While the application of DNA sequencing and molecular marker technologies to plant breeding will bring about huge gains in efficiency and increases in the rate of improvement, the breeder still has to wrestle with the genetic complexities underlying the traits. It turns out frequently that variation in traits is determined by many genes and variation in each gene usually makes only a relatively small difference in the trait. Such differences are hard to measure without large-scale replication. The bringing together of many such genes by recombination and their subsequent maintenance during other breeding cycles can be very difficult. Such complexities are very hard to overcome because they are inherent in the genetic wiring of the species. If the trait could be reduced to one or a few variant genes of large effect, then such traits would be much easier to detect and manage in breeding programmes.

These issues have been a major driver for the discovery and use of single transgenes for important traits. Ceres, as well as many other laboratories, has inserted thousand of genes with high levels of expression into Arabidopsis and rice to discover single genes that make a major change in an important trait (Figure 6). When such genes are found the large trait change is inherited along with the transgene. It is then easy to track both the gene and the trait in subsequent breeding programmes. If it becomes possible to specify each of the traits listed in Table 1 by a few transgenes, then this simplification in complexity would be a huge advantage to plant breeding. Furthermore, it may be that the same or very similar genes would be able to make similar improvements in multiple crops. This would avoid the necessity to repeat the primary genetic analyses in each and every species separately, as is the case at present.

Any one of these uses of transgenes could provide extraordinary improvements in plant breeding and the quality of products, but it is the combination of these that will provide the dramatic opportunities in crop production and a rapid rise in the pace of development of new, improved varieties. Some of the technologies can be developed for application in the near term while others are high risk and it will take brilliant, inspired science to bring these about, even for the longer term. Nevertheless, since plant improvement with these crops will be needed for all time the progress envisaged here will have relevance for all time. Knowing how to improve crops and production more efficiently will never be irrelevant information.

Significant issues associated with the use of transgenes

The successful deployment of transgenes is not without its difficulties: financial, technical and social. Some of these are listed in Table 3. It is expensive to develop all the knowledge to find the relevant genes. When transgenic plants are created, they usually show variation in the expression of the trait. This is undoubtedly due to the ways in which the gene becomes modified by methylation in the cell, the chromatin configuration adopted in the chromosomes and/or the activation of RNAi protection mechan-

TABLE 3

Current issues with the deployment of transgenes
• Variable expression and instability over generations.
• Silencing of their expression.
• Desirability of removing the selectable markers.
• Inefficient transformation processes in certain genotypes.
• Consumer and political acceptance, even when improvements are valuable.
• Cost of regulation and additional time taken for these processes.
• Outcrossing to non-transgenic relatives.
• Intellectual Property and Freedom To Operate issues.
• Costs if crops have to be kept separate from non-transgenics in commercial agriculture.

isms that lead to degradation of transgene RNA or silencing of transcription [52,65,66]. Transgene expression is not always stable during generations probably for the same reasons. Any transgene for a trait will interact with the existing genes and metabolic networks in the cell. This may lead to differences in expression of the trait in different genetic backgrounds and present challenges for the breeder. Indeed all these issues are problems for the breeders but are they any more challenging that all the existing problems with improving plants? I suspect not and in any case they will be managed and overcome as more knowledge accrues.

Different sorts of problems are created by consumers and legislators who are wary of using new technologies, especially where breeding and food are concerned. While understandable in some ways, we should recognise that many of such views are the result of pressure groups against the technology who have advertised and misled societies profusely. It is the case that some of the transgenic options do have potentially far-reaching effects—that is the message of this paper. Societies are poorly equipped to evaluate them because they have insufficient knowledge of the substantial genetic changes behind selection of our current crops. The views that should prevail will surely emerge in the end from the 14 million, and increasing, farmers around the world who grow transgenic plants and the people who are eating transgenic food today. Much is said about this topic elsewhere in this volume.

Other concerns are based on the transfer of transgenes into other non-transgenic varieties by pollination. This is a complex subject with biological and legal aspects. While definitions of organic products do not allow the presence of transgenes, there will always be concerns about chance pollinations from neighbouring transgenic crops. Collection of transgenic pollen by bees and its accumulation into honey is an issue that has been fought in the courts by organic honey vendors. There are concerns about the accumulation of transgenes into wild species by pollinations from related crop plants and the consequential loss of 'clean' wild species. The concerns are often amplified where the transgenes are conferring a beneficial trait, such as drought tolerance, that could be strongly selected for in the wild species and thus increase its fitness and weediness [67]. The statistics and probabilities of pollinations, seed set and subsequent selection of new transgenic wild forms are complex and rarely addressed properly. The hazards and risks are even more rarely weighed against the benefits of boosting agricultural production levels and releasing land that can serve as a habitat for the wild species. Such issues are beyond the scope of this paper.

Many have become disturbed by the patenting of genes and generating difficulties for others to use the technologies commercially without licenses. This is addressed elsewhere in this volume.

The Future—a series of breakthroughs and radical improvements

This paper emphasises that technical advances on the frontiers that change the opportunities and processes of plant breeding are occurring rapidly. Such innovations will continue, and history tells us that numerous innovations will come along that we cannot predict at present. Would the Wright brothers, as they celebrated their success of the first flight in 1903, been able to predict that in 66 years there would be a man on the moon? Many innovations for plant breeding will come from other fields, not plant breeding, as has been mentioned several times above. Thus it is legitimate to speculate and predict that there will be additional stunning breakthroughs in the future. This is implied in Figure 7. There will be waves of discoveries involving single or small number of genes, more complex combinations of genes and entirely novel gene systems that specify extraordinary improvements in crops and production. Maybe the improvements will be novel forms of photosynthesis that harness solar energy much more efficiently [68]. Maybe they will be roots that optimise growth with less fertiliser and water, or bring nitrogen fixation into cereal crops. They will surely include understanding and exploitation of heterosis in the major crops [69,70]. They probably will enable plants to be resistant to diseases and pests. Ultimately there will surely be the creation of new crops, via synthesis of entirely new genomes, that do not suffer from the deficiencies of the species evolved in nature. Crops did not evolve to serve man. It is to be expected that many crops are not well designed for agriculture. Man must continue to seek to make the crops he needs. Such advances will enable mankind to avoid relying on natural biodiversity for food. While such advances are many, many decades away, we should believe in their potential and the contribution they will make to providing high quality food for all in sustainable ways, leaving as much land as possible for other purposes and especially for managing the survival of the planet. This scenario means we should look

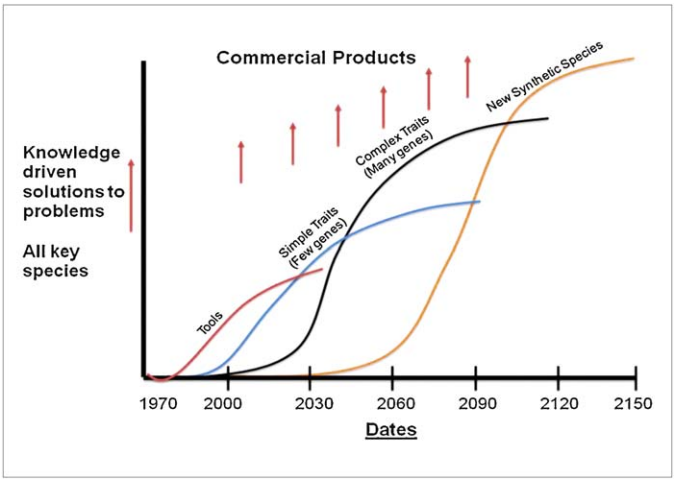

FIGURE 7

Hypothetical adoption of new technologies that provide solutions to major agricultural constraints.

at the current technologies and addition of the first few transgenes to crops as the 'tip of the iceberg'. They are the first few baby steps along a road of discovery and application. It is very important therefore not to judge the current technical achievements and difficulties in ways that undermine the future use of the technologies. This would deny mankind the benefits of huge innovations.

Can we do without the use of transgenes?

Of course we can, because we did not have commercial applications of gene transfer before the 1990s. (However, it is important to note that evolution and the development of our crops as we know them could not have taken place with transfer of genes between species over evolutionary time.) As noted earlier, if all the knowledge and kinds of non-transgenic technologies that have been deployed in US corn production, for example, were applied to cereal grain crops in the different environments around the world, then food production would be very much higher. Indeed this paper draws attention to the fact that much is starting to be achieved in increasing food production by adopting all the analytical, non-transgenic tools from molecular biology, such as molecular markers. This will undoubtedly continue, at some pace, dependent on investments and human capital. But, also as noted above, transgenic crops have already been adopted by some 14 million farmers [16] and it is naïve to believe that it will be possible to turn back the clock and withdraw these crops with their advantages. The insect resistance traits supplied by the transgenes in corn and cotton cannot be supplied by other means. To deny such traits would make many farmers poorer—in any case the farmers would surely prevent withdrawal of the crops. If societies choose not to deploy solutions involving transgenes then advances will come more slowly and some societies will lose significantly, especially where alternative solutions are not readily possible, for example, provitamin A production in rice. The losses include loss of life, sustained poverty, misery and stress and all the things that accompany poor health and reduced education. The over-riding importance of such tragedies in societies and the moral and ethical issues associated with their continuing existence prompt the necessity to change the question from 'can we do without the use of transgenes?' to 'should we do without the use of transgenes?'.

Should we do without transgenes?

The answer to this question depends on where mankind is seeking to take human existence and the planet. To me there is only one way forward and that is towards sustaining the highest quality of life for mankind consistent with sustaining the planet for all time. This means working rapidly and purposefully towards intensifying agriculture sustainably to produce the amounts and diversity of food needed using as little land as possible. This is to leave plenty of land to sustain the planet, manage greenhouse gases, provide renewable energy from biofuels, maintain adequate biological diversity and land and water for recreation and other amenities. To achieve this requires, firstly, wise decision-making from governments working together down to the smallest villages and individuals and, secondly, the deployment of safe technologies to improve food production as rapidly as possible. Nothing less is acceptable. We should not condemn future generations to more poverty and hunger or make more difficult the survival of life on the planet by not developing and using all relevant technology streams. Risks will always be with us, but the risk of not developing and deploying technologies to give better options for the future is the biggest risk. This means accelerating investments in training, education and the dissemination of valuable proven technologies in societies.

Concluding comments

From all that is written above, it should be clear that our responsibilities are much more obvious now, because we know what previous generations did not know. We now know every gene in the major crop plants and have the ability to learn them for any new plant. We know how genes have evolved in nature and what gene systems breeders have selected to adapt our crops to our uses and fields across the world. We know how to speed up rates of improvement, create improvements where none were possible before and produce more on less land. We can describe this information in great detail and are beginning to design improvements. With all this knowledge our responsibilities have become sharpened. Of course there are risks in deploying any technology but to employ the precautionary principle routinely in agriculture where so many are hungry and enveloped in poverty is condemning societies to even greater misery and possibly compromising the ability to manage the planet in beneficial ways for ever. Fortunately agriculture is practised by many millions of farmers all over the world and so experiments involving new technologies are being adjudicated year-on-year millions of time. This puts a huge quality control into the system. All should recognise this. The fast growing global wireless communication systems will increasingly enable farmers and consumers, rich and poor, to know what works well and what does not, what is available elsewhere and what should be adopted. May the farmers, knowledge generators and entrepreneurs of the world teach us all, and especially disconnected decision-makers and citizens, how to overcome our current challenges, decade by decade and create the sustainable promised land for 9 billion people.

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