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Traditional and Modern Plant Breeding Methods with Examples in Rice (*Oryza sativa* L.)

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ABSTRACT: Plant breeding can be broadly defined as alterations caused in plants as a result of their use by humans, ranging from unintentional changes resulting from the advent of agriculture to the application of molecular tools for precision breeding. The vast diversity of breeding methods can be simplified into three categories: (i) plant breeding based on observed variation by selection of plants based on natural variants appearing in nature or within traditional varieties; (ii) plant breeding based on controlled mating by selection of plants presenting recombination of desirable genes from different parents; and (iii) plant breeding based on monitored recombination by selection of specific genes or marker profiles, using molecular tools for tracking within-genome variation. The continuous application of traditional breeding methods in a given species could lead to the narrowing of the gene pool from which cultivars are drawn, rendering crops vulnerable to biotic and abiotic stresses and hampering future progress. Several methods have been devised for introducing exotic variation into elite germplasm without undesirable effects. Cases in rice are given to illustrate the potential and limitations of different breeding approaches.

KEYWORDS: *plant breeding history, Oryza sativa, crop domestication, genetic improvement, marker-assisted selection*

INTRODUCTION

Plant breeding can be considered a coevolutionary process between humans and edible plants. People caused changes in the plants that were used for agriculture and, in turn, those new plant types allowed changes in human populations to take place. Plants yielding more generous harvests freed some of the people's time for developing art, handcrafting, and science, eventually leading to modern human life as we know it. Civilization could not exist without agriculture, and agriculture could not sustain the civilized world without modern crop varieties.¹ From this point of view, it becomes clear that plant breeding is one of the main foundations of civilization.

In industrialized countries, only a small portion of the population is engaged in agriculture. The vast majority of people rely on a tacit social pact for their survival, which assures that someone will provide food in exchange for some service or good. This pact is so basic to modern life that people take for granted that food is available in the nearest supermarket. However, agriculture failure could cause a disruption of this pact, leaving people in a situation of food insecurity. Thus, protecting agriculture means warranting the foundation pact of modern civilization.

The core of plant breeding is the selection of better types among variants, in terms of yield and quality of edible parts; ease of cultivation, harvest, and processing; tolerance to environmental stresses; and resistance against pests. Each of these aspects of agronomic or food value can be dissected in many specific traits, each presenting its own range of variation. Manipulating a single trait, disregarding all others, is relatively straightforward; however, this is unlikely to result in a useful variety.

The challenge of plant breeding resides in improving all of the traits of interest simultaneously, a task made more difficult by the genetic correlations between different traits, which may be due to genes with *pleiotropic effects*, to physical linkage between genes in the chromosomes, or to *population genetic structure*.² Selecting for one trait will change correlated traits, sometimes in the desired direction, other times in an unfavorable way.³ For this reason, selection can lead to unanticipated changes, which are normally within the range that is normally observed in the crop and thus assumed to pose no risk to consumers or the environment. Whether this assumption is reasonable or not is a matter of debate.⁴

The objective of this paper is to discuss plant breeding methods as an evolving technology, considering the increasing levels of knowledge of the underlying mechanisms and the control of the process of generating and selecting superior plant types. In this context, three main eras of plant breeding can be identified: (i) plant breeding based on the selection of observed variants, disregarding their origin; (ii) generation and selection of expanded variation by controlled mating; and (iii) monitoring the inheritance of within-genome variation and selection of specific recombinants. The fourth stage of plant breeding, which is not discussed in this paper, can be considered the creation and introduction of novel variation

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into genomes through genetic engineering. The varieties resulting from the methods presented in this paper can be considered a reference against which transgenic plants are compared with regard to their food safety.

■ PLANT BREEDING BASED ON OBSERVED VARIATION

The most primitive form of plant breeding was the selection of naturally occurring variants in the wild and, later, in cultivated fields. Genetic variation was continuously submitted to the selection pressure of food gathering or planting—harvesting cycles. In some cases, this process resulted in deep changes in plant phenotypes, as exemplified by the derivation of maize from teosinte.⁵ This early phase of plant breeding spans the period from the origin of agriculture until the first hybridization experiments carried out by Kölreuter in the 1760s.⁶ With the discovery of the laws of heredity, in the turn from the 19th to the 20th century, the importance of hybridization in plant breeding became widely recognized.⁷ Today, almost all plant breeding programs involve some use of hybridization.⁸

Plant Domestication: The Origin of Crops. For a given gene, mutations are rare events, but considering the large numbers of plants in a field and of genes in a plant, mutations are quite frequent events in a population.⁹ Most mutations are unfavorable for survival in the wild, being eliminated from the population in a few generations, as a consequence of natural selection. However, some of these mutations may result in more favorable phenotypes either in terms of cultivation or in terms of food quality. Some of those mutants were rescued by ancient farmers, who protected them against competition and established with those otherwise disabled plants a relationship of symbiosis. Unlike wild habitats, cultivated fields were environments in which those mutations conferred a selective advantage, thus becoming the predominant type through human selection. The accumulation of this type of mutation is the major cause of the domestication syndrome, a set of characteristics that made many cultivated species irreversibly dependent on humans for their survival.¹⁰

The molecular variability in domesticated plants tends to be smaller than in related wild species, as a consequence of the *founder effect* during domestication. By strongly selecting for the rare mutant plants adapted to cultivation, early farmers dropped most of the variation present in the wild populations from which cultivated forms arose. It is now clear that many valuable genes, especially those related to resistance to pests, were left out of the cultivated gene pool.¹¹ Incorporating those genes into modern cultivars, without losing ground in terms of yield and product quality, is one of the challenges of modern plant breeding and one of the most relevant applications of molecular tools in breeding programs.

Intuitive Farmer Selection: The Origin of Landraces. Landraces are populations of plants that have been cultivated for many generations in a certain region, being shaped by biotic and abiotic stresses, crop management, seed handling, and eating preferences. They are dynamic genetic entities: continuously changing as a consequence of intentional and unintentional selection, seed mixture, and pollen exchange. Landraces are shaped by a balance between *stabilizing selection*, which keeps the identity of the landrace in a given region, and mild *directional selection*, leading to slow adjustments to environmental changes. In some cases, quick changes can take place, especially when the landrace is taken to a different region or when new materials are cultivated in close proximity

with the original landrace. Landraces can still nowadays derive from modern cultivars, if certified seed production is discontinued and farmer-saved seeds are planted recurrently, without care for isolation against seed or pollen contamination.

The major characteristics of landraces are¹² (i) high levels of genetic diversity within populations, characterized by a limited range of variation between individuals, with distinctive traits that make the landrace identifiable; (ii) adaptation to soil and climate conditions typical of the region, combined with resistance to common pests; (iii) edible parts that are valued by local people, normally shaping and being shaped by the local cuisine; and (iv) modest but stable yield, conferring food security to the local community under normal environmental variation.

Intuitive farmer selection has the virtue of shaping varieties for the actual and specific environment of use and for the local food preferences, serving well the case of subsistence agriculture, where most of the production is locally consumed. However, when farmers select for one trait, genetic correlations may result in undesirable changes in other traits. For example, cereal landraces are normally tall plants, prone to lodging and presenting low *harvest index*, probably as a result of human selection for large edible parts (panicles, ears, spikes).

Nevertheless, for their wealth in genetic variability and adaptability to different environments, landraces are the most valuable genetic resources for long-term plant breeding programs and also prime targets for germplasm collections. Seed banks around the world keep thousands of samples of landraces under “ex situ” conservation. In some countries, there are efforts to devise regulatory mechanisms and financial incentives for traditional communities to keep growing their heritage varieties, aiming for their “in situ” conservation. New systems of germplasm conservation have been built on social networks connecting people interested in the subject as a hobby (e.g., seedsavers.org). Those networks take advantage of modern communication tools to replicate on a global scale what used to happen through personal contact in traditional communities.

Notwithstanding, a large part of the variability that once existed in cultivated fields of annual plants may have been irreversibly lost during the introduction of modern, high-yielding cultivars. In this sense, the same modern cultivars that saved millions from starving may have wiped out varieties that were the result of centuries of local intuitive selection by farmers and a valuable resource for future genetic improvement.

Pure Line Selection and Mass Selection: The Origin of Cultivars. The earliest method of plant breeding based on an elementary knowledge of the laws of inheritance has been the selection of plants within landraces, based on the assumption that the progenies of the best individuals are expected to be superior to the progeny of a random sample of the population. This method was formally proposed by Louis de Vilmorin in 1856, although there are mentions of the use of its principles by some farmers earlier in the 19th century.¹³ This realization can be considered as the origin of the paradigm of homogeneity that dominates breeding, and agriculture as a whole, until today. From this point on, within-field heterogeneity was considered to be undesirable and both plant breeding and agronomy developed methods to achieve maximum spatial homogeneity (e.g., “precision agriculture”).

In self-pollinating species, such as rice and wheat, landraces can be thought of as a mixture of pure lines, including some heterozygous individuals derived from a low frequency of cross-

pollination. In this type of population, selecting single plants and deriving inbred progenies invariably result in some lines that outperform the original landrace for a given growing condition. However, this superiority comes at a cost, because pure lines are normally less stable than diverse populations in the face of stresses, especially diseases, and have no capacity for long-term adaptation, because it is monomorphic for most genes.

In the case of open-pollinated species, such as maize, landraces are populations of random mating individuals, approximating the *Hardy–Weinberg equilibrium* (HWE), with some deviations due to mild selection. Mass selection and recombination of the top-performing portion of the population result in a gradual increase in the frequency of favorable alleles. Successive generations of selection on maize landraces resulted in improved open-pollinated varieties, which were the basis of corn production until the advent of hybrid maize.

■ PLANT BREEDING BASED ON CONTROLLED MATING

Despite the great spontaneous diversity that can be found in the landraces, simply applying selection on preexisting diversity is an eroding process that eventually comes to a limit. The true creative power of plant breeding resides in promoting recombination for shuffling favorable alleles.¹⁴ The combination of different alleles in many loci results in a virtually infinite number of genotypes. One could conceivably start a commercial breeding program from a dozen well-adapted founding parents, with a clear focus on a specific target environment and evaluating large segregating progenies. Injection of novel variability might become necessary in the case of a significant change of the target environment, such as the emergence of new pests for which the founder materials had no resistance.

Given the myriad of possible genotypes resulting from crossing diverse parents, the limitation for genetic gains becomes the capacity of the breeding program to evaluate a large number of plants, derived from a large number of crosses. For this reason, plant breeding is frequently dubbed a numbers game, and large competitive programs in commodities invest heavily in high-throughput methods for seed handling, planting, evaluating, and harvesting. As genetic gains accumulate, the bar is gradually raised, and increasingly higher investments are required to keep a steady rate of genetic progress. The limit of this escalation is the financial viability of returns in the seed market and associated business. The main methods developed for efficient use of resources in breeding programs are discussed next.

Pedigree Breeding: Playing with Parents. The vast majority of the released cultivars of self-pollinating species have been developed through the pedigree method. Pedigree breeding consists of crossing parents and generating segregating populations, which are conducted through generations of self-pollination and selection, until a set of derived lines that combines the good characteristics of both parents is obtained. Because it is based on the complementation of traits, this method is efficient for breeding for qualitative traits, such as disease resistance, or easily classifiable traits, such as plant architecture or the color or shape of plant parts. The pedigree method is appealing to breeders because it allows building better varieties by putting together, in the same plant, good characteristics that were present in different materials. Because

all crossings are controlled, it is possible to know the genealogy of each cultivar.

The main weakness of the pedigree method resides in the fact that yield is evaluated efficiently only at the end of the process, on inbred lines, when seed is available for replicated trials. At this point, though, unless a large number of lines have been advanced, there is little room for improvement of yield potential. Consequently, the rate of progress for yield resulting from the pedigree method is normally modest, rarely exceeding 1% per year.¹⁵

Ideotype Breeding: Playing with Traits. The ideotype breeding approach can be regarded as a strategy to improve the capacity of the pedigree method to promote gains for *quantitative traits*, especially yield. It is based on the hypothesis that one can improve complex traits by changing simpler traits that are positively correlated with them.¹⁶ The advantage of this method is that, if the underlying hypothesis proves correct, one could promote significant gain for yield, even with a small breeding program, taking the “smart” approach, as opposed to the “numbers game” approach of large-scale breeding programs. Additionally, it is scientifically attractive to breeders, because they have a chance of changing paradigms in their favorite crop. However, it is important to keep in mind that unfavorable genetic correlations can offset the advantage brought by the traits that make up the ideotype.¹⁷

Changing the ideotype of a crop often requires looking for variation beyond the boundaries of elite germplasm, which is normally of the current plant type. However, using landraces as parents in breeding programs normally results in marked reduction in yield. For this reason, backcross toward the elite materials is necessary to recover a competitive progeny.¹⁸ If the donor of the new trait is a wild relative, problems of sexual compatibility, flower sterility, grain quality, or seed dispersal (i.e., grain shattering) may persist for several generations.

To avoid undesirable effects of direct introduction of exotic materials into elite breeding populations, those materials are normally used first in a phase of prebreeding, when breeders try to break the association between useful and undesirable traits. Once the new trait needed to assemble the ideotype is inserted into an elite background, those vector lines can be transferred to the elite breeding program.

Population Breeding: Playing with Genetic Variance. Although all breeding methods imply the application of selection pressure on a variable population, the term “population breeding” indicates a method designed to improve the phenotypic performance of an intermating population by increasing the frequency of favorable alleles controlling traits of interest. The simplest version of population breeding is the mass selection method applied to cross-pollinated species, in which the improved population is directly used as a cultivar. Later, more sophisticated schemes of population breeding have been designed, providing the framework for the development of the quantitative genetics theory. In modern population breeding, the objective is to increase the value of the population as a source of elite lines. Improving the mean quality of the population, while preserving the variation within it, results in top individuals that outperform previously existing lines. Those lines can be used as cultivars, in the case of self-pollinated species, or as parents of hybrids, in the case of cross-pollinated species.

Population breeding is an open-ended scheme of consecutive rounds of selection and recombination, thus being also known as recurrent selection breeding. Recurrent selection requires an

efficient crossing system, which can be a limitation for self-pollinating species. This scheme, when applied repeatedly on small populations, leads to depletion of genetic variation and slowing of genetic gains.¹⁹ For this reason, a large effective population size must be kept throughout or else *genetic drift* will override the other forces acting on the population, hindering genetic improvement.²⁰

However, even moderate population sizes (e.g., 50 plants) appear to be sufficient to avoid rapid depletion of genetic variation.²¹ This is probably due to the fact that, whereas selection leads to the loss of diversity in some genes, recombination breaks linkage blocks into smaller pieces, allowing many more combinations of genes than was possible with large DNA segments. This phenomenon releases hidden variation in latter generations down the process of recurrent selection, compensating for the loss of variation due to selection.²²

Breeders create new populations by intercrossing several lines, chosen as sources of favorable alleles for one or more traits. These synthetic populations under recurrent selection mimic the genetic events that used to take place in the landraces in the hands of traditional farmers, with the difference that the whole process is monitored and controlled, and selection pressure is intensified for faster gains. The rate of genetic gain per unit of time can be increased by speeding up the selection–recombination cycles, by intensifying the selection pressure, by improving the evaluation precision (thus increasing the heritability), or by any combination of those.

The general scheme of population breeding is very flexible, allowing customization to specific needs and objectives of different species and breeding programs. The system can be formatted for rapid, short-term results, normally by applying strong selection pressure on genetically narrow-based populations, or for sustained, long-term results, by applying moderate selection pressure on genetically broad-based populations. Population breeding can also be used as a prebreeding scheme, because its frequent crossing events promote recombination between exotic and elite genomes, purging unfavorable exotic genes from the population.

Hybrid Breeding: Playing with Heterosis. Heterosis is the superiority of hybrid individuals compared to inbred individuals.²³ Within certain limits, the more divergent are the parents, the higher is the heterosis on their offspring.^{24,25} Hybrid vigor decays rapidly through generations of inbreeding, indicating that, whatever is the mechanism underlying heterosis, it is due to the presence of heterozygous loci. For this reason, maize breeding programs nowadays are focused on developing competitive *F1* hybrids, in which heterozygosity is at its maximum.

Two challenges are present in hybrid breeding programs: (i) the need to improve at least two populations toward agronomic adaptation, while keeping them genetically distant enough to express strong heterosis, and (ii) developing efficient seed production of selected hybrids, such that the cost of seed production does not offset the value of the additional yield resulting from heterosis. In maize, the first problem led to the concept of *heterotic groups*,²⁶ splitting the elite gene pool into subsets, within which population breeding is applied. The second problem was solved by the improvement of parental lines, resulting in higher yield in seed production fields, and by the use of male sterility genes and mechanical emasculation (“detasselling”) to avoid self-pollination.

On the other hand, hybrids present great advantages from the business perspective. The seeds produced by hybrid plants are genetically heterogeneous due to the segregation of thousands of genes. If planted, the resulting crop would present a large variation in agronomic traits, plant architecture, and cycle duration, thus reducing yield and grain quality. Hence, farmers must buy new seeds every year, resulting in a constant demand for hybrid seeds. Additionally, with the advent of the transgenics, hybrid seeds became preferential carriers of those valuable proprietary traits because they allow a better control of the event by the owner.

■ PLANT BREEDING BASED ON MONITORED RECOMBINATION

Traditional breeding methods were based on the complementarity between parental characteristics. However, little or nothing was known about which part of the genome came from each parent. This situation changed with the advent and dissemination of molecular marker technologies, which made it possible to monitor the transmission of chromosome segments in the progeny.²⁷

Virtually any sequence variation between individuals can be used to design a marker that will allow the identification of the parent that contributed a specific segment of the chromosome in a recombinant line. Until recently, the most popular markers were the simple sequence repeats (SSRs), also known as microsatellites. Those markers were superseded by the single nucleotide polymorphisms (SNPs), which are more abundant in the genome and more amenable to high-throughput genotyping.

Molecular markers are essential tools for studying the genetic control of any trait of interest, eventually leading to the identification of the genes underlying the trait and the metabolic chains involved. This venue can be broadly defined as molecular biology, which dominated the field of biological sciences in the past decades. However, in this paper we limit the discussion to the application of molecular markers as tools for plant breeding.

Mapping Genes of Interest: Finding Needles in the Haystack. When many molecular markers are genotyped in a set of plants derived from a single cross, the frequency of recombination between them can be used to infer their order and relative distance in the chromosomes, resulting in a genetic map. If those plants, or their progeny, are evaluated for a quantitative trait, a statistical model can be built in which part of the phenotypic variance can be explained by some of the markers, which implies that those markers should be linked to the genes underlying the trait. This approach results in *quantitative trait loci* (QTL) maps, which are normally the first step toward understanding the genetic control of a quantitative trait.²⁸ From a QTL map, a plant breeder can get an insight into the number of loci controlling a trait, their relative importance, and their approximate position in the genome. In the case of large-effect QTLs, it may be possible to jump directly into marker-assisted selection, using the closest markers available in the map. However, in most cases a step of fine mapping, involving a larger population and denser marker spacing in the target genomic region, is necessary for developing useful selection tools. The ultimate result of this approach is the identification of the gene, and the polymorphism in its nucleotide sequence, responsible for the observed phenotypic differences.

The QTL mapping approach is effective for explaining contrasts between two parents, but is inefficient for exploring the wider genetic diversity for a trait in the germplasm. The association mapping approach offers a shortcut in this path and for this reason has strong appeal to breeders.^{29,30} In this approach, a panel of genetically diverse lines is densely genotyped and carefully evaluated for a phenotypic character, with the aim of identifying associations between marker alleles and traits. Because the genealogical distance between the materials in the panel is large, those associations will remain significant only if the marker is tightly linked to the causative gene or if factors related to population genetics (population structure) create associations between unlinked loci. For the latter case, estimating population structure and taking it into account explicitly in the statistical model can avoid the detection of false associations.³¹ When correctly applied, association analysis can result in the detection of genomic regions related to the trait of interest and, simultaneously, in the identification of donor lines of favorable alleles from a wider germplasm.³²

Gene banks harbor thousands of accessions that are potentially useful for plant breeding. Those accessions include wild crop relatives and obsolete landraces. Although presenting poor agronomic value, compared to modern cultivars, those materials are believed to have useful genes³³ that have not been captured from wild species in the process of domestication or from landraces in the early phases of scientific breeding. Rescuing those useful genes that were “left behind” is a difficult task, because the genes of interest may be tightly linked to unfavorable genes, which would be dragged along into the breeding population. Understanding the genetic diversity in germplasm collections is the first step toward better use of a broader gene pool in breeding programs. Marker-assisted backcrossing allows identifying the rare recombinants in the vicinity of the introduced gene, trimming the chromosome segment as close as possible to the target gene.³⁴ Additionally, by identifying individuals with the cleanest genome (with the least residue from the donor parent), it is possible to reduce the number of backcrosses needed for full recovery of the elite phenotype.³⁵

Collections of chromosome segment substitution lines (CSSL libraries) are a set of lines derived from an elite variety, in which each line has one chromosome segment replaced by the corresponding segment in the wild species of interest.¹¹ The CSSL library carries the whole genome of the wild relative split in bins. The phenotypic effect of the set of genes in each bin can be evaluated against the original elite line, used as a check. Those genetic stocks can facilitate the identification and introgression of genes from wild relatives into elite germplasm. Superior CSSLs can be used for fine mapping and simultaneous development of an improved version of the elite variety.³⁶

Marker-Assisted Selection: Building Tailored Genotypes. Whereas in the case of marker-assisted gene introgression the breeder will normally tag only one gene, a full-featured marker-assisted selection scheme would monitor several genes simultaneously. Knowledge of the allele borne by each plant in each major locus of interest creates the means for building specific allele combinations that would maximize the agronomic value of the line. However, when one is dealing with several genes, it is important to keep in mind that the interaction between genes (epistatic effect), and not only the additive effects of the genes, defines the expression of a trait.

A typical application of marker-assisted selection is resistance gene pyramiding. When one is dealing with diseases caused by pathogens presenting high variability, a single resistance gene may confer complete resistance for a few plant generations until it is overcome by strains of the pathogen. Accumulating several resistance genes in a variety can confer durable resistance, because the pathogen would have to beat all of the genes simultaneously, which drastically reduces the odds of resistance breakdown.³⁷ Resistance gene pyramiding by classical breeding methods is almost impossible, because the effect of a gene could be visible only in the absence of other resistance genes. Marker-tagged resistance genes can be easily combined without the need for phenotypic screening. A similar strategy can be used for preventively breeding resistance to pathogens that are currently absent from a region, because in the absence of the pathogens no phenotypic screening of resistance would be possible.

Molecular markers of interest for breeders of a given species can be combined in optimized sets for simultaneous genotyping (“multiplexed”), allowing high-throughput application in applied breeding programs, especially those dealing with a broader genetic base.³⁸ The implementation of such a marker set depends on a large body of previous research. For each of the traits of interest, several steps of research are required, normally starting with mapping quantitative trait loci (QTL), followed by fine mapping and eventually positional cloning. Once the causative gene is identified, based on a pair of contrasting alleles, “mining” novel alleles in diverse germplasm may expand the range of variation of the trait or the toolbox to deal with specific stresses.³⁹

Genomic Selection: Speeding up Genetic Progress. Recent advancements in genotyping technology sharply reduced the cost of genotyping, creating the possibility of scoring thousands of markers in populations of plants under selection. The several steps of genetic analysis required to identify the relationship of each of those markers with the phenotype could not keep pace with that. In this scenario, the breeder can have a large amount of genotypic information with unknown relationship with the traits under selection. The genomic selection approach⁴⁰ proposes that knowledge of the relationship between specific markers and specific genes is not necessary in the breeding context. Instead, the breeder can use the information available for all markers in a plant to predict its breeding value, without effectively evaluating its phenotype, based on previous statistical models built for those markers using a “training population” for which all genotypes and phenotypes have been scored. Markers are considered as random factors, in the framework of mixed model analysis, because the number of markers used in genomic selection is normally superior to the number of individuals in the training population, such that estimating the effect of each marker would not be possible due to the lack of degrees of freedom. Simulation studies demonstrated that genomic selection can accelerate the genetic progress of quantitative traits in population breeding.⁴¹

Genomic selection can be seen as a method to maximize the return from the phenotypic evaluation step in the breeding cycle.⁴² The information drawn from one round of proper phenotypic evaluation can be propagated to subsequent generations. Once models are built for different environments, the unique marker profile for a given individual can be used to predict its performance in each of these environments, improving considerably the inference made on each individual

performance and largely reducing evaluation costs. The potential use of this approach in choosing parents of new breeding populations is also promising. However, the predictability of the phenotype from the marker profile fades out along generations, and new rounds of phenotypic data must be input to update the statistical model. This approach will be especially useful in cases when phenotypic evaluation is difficult, expensive, or conditioned to uncertain events, such as the occurrence of a pest or specific weather condition.

Genomic selection can be used to accelerate breeding for yield potential, which is frequently considered as the most difficult trait for marker-assisted selection. In highly competitive seed industries, such as the cases of maize and soybean, any increase in the rate of genetic gain could make a significant difference in market share. For this reason, large seed companies are investing heavily in high-throughput DNA analysis and bioinformatics for seamless integration of genomic selection in breeding programs.⁴³ Seeds can be genotyped before planting, such that only preselected individuals will use expensive plots in the field. Full application of genomic selection should happen more easily in large private companies, when compared to small seed companies, public institutions, and universities, considering the amount of investment required for setting and keeping up-to-date the genotyping pipeline, the short turnaround time for DNA analysis, data processing and selection, and the well-trained team required.

CONCLUSION

The raw material of natural genetic diversity was carved by human selection during plant domestication, resulting in profound changes in plant phenotypes. Intentional or unintentional selection through millennia of traditional farming resulted in a wealth of genetic diversity, adapted to different human needs. However, this process worked at a speed that no longer matches the demands of modern society. The advent of scientific plant breeding accelerated the rhythm of varietal improvement, and at this point it is difficult to predict the limits of this approach. Molecular tools now permit monitoring the dynamics of genomic recombination, making possible a gene-by-gene breeding approach. The impact of those new methods in farmer's fields is just in the beginning, but the expectations for plant breeding to help meet the challenges related to food supply, environmental sustainability, and even fossil fuel replacement are huge. The most modern methods will be necessary for going back to what remains from the raw material where our ancestors found their sources of food, to find the genes that will be the building blocks of the cultivars that will solve the problems of the 21st century.

EXAMPLES OF CHANGES IN RICE DUE TO BREEDING

Rice Domestication. There are two species of cultivated rice: *Oryza sativa*, originated from *Oryza rufipogon* in Asia, and *Oryza glaberrima*, originated from *Oryza barthii* in Africa.⁴⁴ The South American wild species *Oryza glumaepatula*, although similar to *O. rufipogon*, has not been domesticated (Figure 1).

O. sativa accounts for almost the entirety of global rice production, whereas *O. glaberrima* is planted only in some parts of Africa, but is being displaced by more productive *O. sativa* varieties, some of them with introgressions of *O. glaberrima* genes.⁴⁵ The history of rice domestication is highly complex and still not completely understood. *O. rufipogon* is the most



Figure 1. American A-genome wild rice *Oryza glumaepatula*. Abundant and wide angle tillering, open and shattering panicles, and small grains with awns are typical of wild rice species.

widespread and diverse of those species, which makes it the most likely source of the original domestication event. However, unlike cultivated rice, this species is perennial and predominantly outcrossing, whereas *Oryza nivara* is annual and self-pollinating, so it would take a lesser change to originate the cultivated type.⁴⁶ It is probable that both species contributed genes for the early forms of cultivated rice.⁴⁷

A suite of differences are observed between wild and cultivated rice.⁴⁴ Wild rice presents seed dispersal mechanisms, including seed shattering, long awns, and hairy hulls. Cultivated rice lost those characteristics, partially or completely, with nonshattering being the key trait of domestication. Seeds of wild rice present long dormancy, which increases the odds of perpetuation in uncertain wild environments, but is disadvantageous in cultivated fields, where the farmer provides adequate conditions for germination in every growing season. The change in those traits may have resulted simply from harvesting, when the majority of the seeds of shattering plants were lost, and from sowing, when plants derived from nondormant seeds dominated the field. Dark hulls and red pericarp are also typical traits of wild rice that have been eliminated in cultivated materials, possibly by intentional human selection of straw-colored hulls and white pericarp.

O. sativa presents two subspecies, *indica* and *japonica*. It is uncertain if each type derives from an independent domestication event or if a single event later split into two subtypes.⁴⁸ In favor of the independent domestication hypothesis is the fact that the level of divergence observed between those two groups projects the separation before the origin of agriculture;⁴⁹ furthermore, wild rice populations present some degree of population structure in line with the differences between *indica* and *japonica* rice.⁵⁰ Nevertheless, the fact that the mutation causing the loss of shattering in both *indica* and *japonica* is the same⁵¹ is strong evidence in favor of a single domestication event. Trying to reconcile those two conflicting pieces of evidence, Vaughan et al.⁵² offered an interesting explanation for the observation of similar differentiation in wild rice populations: the two rice types under cultivation would have frequently hybridized with wild rice populations, transferring part of the genetic structuring to

natural populations. If this hypothesis holds true, what we see today in the wild would be a consequence of cultivated types, as much as the opposite. This implies that plant breeding reshapes not only the plant under cultivation but also, to some degree, its relatives in the wild.

Understanding the domestication history of a species is important to estimate the potential of wild relatives in breeding. QTL mapping studies showed that *O. rufipogon* can be used to improve the agronomic performance of *O. sativa*.^{53,54} Understanding the genetic relationship between germplasm accessions, through high-throughput marker technology, will allow deep understanding of thousands of rice accessions in seed banks around the world.⁵⁵

Rice Ideotype Breeding. Since the semidwarf rice plant, grown under higher N fertilization, brought a dramatic increase in yield in the period of the Green Revolution (1960s), rice breeders search for other traits, or set of traits, that could promote another leap forward in rice productivity. Although there have been yield gains, conventional breeding was more successful in improving disease resistance, grain quality, and earliness than yield potential.⁵⁶

On the basis of simulation studies,⁵⁷ scientists from the International Rice Research Institute (IRRI) designed a hypothetical ideotype that should push the limits of rice grain yield upward. This model plant, known as the rice “new plant type”, had few tillers, but all of them bore a large panicle with more than 200 grains on top of thick, lodging-resistant stems. Leaves were thick and erect, with intense green color.⁵⁸ Those traits, which are relatively easy to select in the breeding program, when combined should result in increased harvest index and yield (Figure 2).



Figure 2. Comparison of schematic rice plants representing typical traditional varieties, modern semidwarf cultivars, and the proposed new plant type. Reprinted with permission from IRRI Images.

Although based on reasonable premises, the new plant type failed to yield more than the best varieties with the typical, modern plant type. Reduced tillering resulted in low canopy biomass, which, combined with incomplete grain filling, offset the gain in harvest index on a plant basis. A second generation of new plant type lines, with larger proportion of *indica* genotype, performed better but still failed to beat the best checks.⁵⁸

China's national breeding program also pursued an ideotype for achieving unprecedented rice productivity. The Chinese ideotype is similar to IRRI's ideotype, with the main difference of having the panicles lower in the canopy, below the height of

the top three leaves, which should remain productive until the grain-filling period. By adding those key traits and taking the hybrid breeding approach, the Chinese program had greater success.⁵⁹ Hybrid breeding represented a shortcut toward construction of improved ideotypes, because instead of having to introduce all of the ideotype traits into a single inbred line, it was possible to breed two complementary parents that, when crossed, completed the ideotype, on top of which, heterosis added additional yield potential.⁶⁰

Direct Selection of Cloned Genes in Rice. With the recent advances of molecular knowledge of genes and trait control, the possibility of doing plant breeding by picking specific genes and building planned combinations is gradually becoming real. Soon it may be possible to model complete molecular ideotypes, which would be hypothesized optimal combinations of alleles at many major genes, according to the target environment of the crop.

With a small genome size, rich genetic diversity, and other experimental conveniences, rice became the model plant among crops. Reference genome sequencing has been completed, both for *japonica*⁶¹ and for *indica*⁶² types, an invaluable resource for gene cloning. Upon this body of information, rice genes are being cloned in laboratories around the world.⁶³ Classical breeding programs will benefit from the knowledge of major genes with significant effect on important traits. Specific primers can be made to detect the favorable alleles, making sure they are present in the elite lines.

One of the genes of high interest to breeders is *Ghd7*, which simultaneously affects grain number per panicle, plant height, and heading date.⁶⁴ This locus alone has a large influence on adaptation to long growing seasons, with high yield potential, or short growing seasons, with lower yield. Controlling this locus can help to bring about the use of exotic germplasm in breeding programs in different geographic regions.

Submergence tolerance is a trait better handled molecularly than phenotypically. As an erratic natural disaster, testing plants for flooding tolerance in the field is difficult and expensive. Scientists from IRRI identified a large effect QTL for flooding tolerance (Figure 3), in which the favorable allele conferred tolerance to several days of complete submergence.⁶⁵ Further investigation led to the underlying gene, named *Sub1A*, which played a role in the response of the plant to ethylene.⁶⁶ Marker-



Figure 3. Experimental plots showing the contrast between lines with and without the allele conferring submergence tolerance of the *Sub1A* gene. Plots without the tolerance gene have been almost completely eliminated after 15 days of flooding. Reprinted with permission from IRRI Images.

assisted selection was used for developing submergence-tolerant versions of important rice cultivars.⁶⁷

Soils with low P content are common in the tropics and limit rice yield. Rice varieties differ significantly for adaptation to low-P soils, and part of those differences is due to a major QTL on chromosome 12, named *Pup1*.⁶⁸ Several traditional upland varieties present the favorable allele at this locus, whereas modern varieties appear to lack it, probably because selection is often done in well-fertilized experimental fields. Additional studies identified the underlying gene, named *Pstol1*, which promotes root growth.⁶⁹ Larger roots explain not only the better P uptake of some traditional rice varieties but also their superior drought tolerance. Marker-assisted selection of the *Pup1* locus will facilitate reintroducing the favorable allele into modern cultivars, without yield penalty.⁷⁰

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Notes

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GLOSSARY

directional selection: selection favoring outstanding individuals in the population; this kind of selection operates favoring individuals with trait values at one of the extremes of the character distribution

F1 hybrid: first generation of plants derived from a cross between two inbred lines

founder effect: populations originating from a small number of individuals tend to differ significantly from the population from which those individuals originated, due to sampling error of alleles

genetic drift: unintentional changes in allele frequencies due to sampling variance inherent to small samples

Hardy–Weinberg equilibrium (HWE): theoretical condition reached by infinite panmictic populations that are not under mutation, migration, or selection; under HWE, the frequencies of homozygotes and heterozygotes are those expected by chance, which are solely a function of allele frequencies

harvest index: ratio between grain mass and total biomass, in single-plant or canopy basis

heterotic group: group of genotypes that display similar combining ability and heterotic response when crossed with genotypes from other germplasm groups

pleiotropic effect: phenomenon relative to one gene affecting more than one trait

population genetic structure: differences in the genetic constitution of subpopulations that are part of a larger reference population

quantitative trait locus: position in the genome that contributes to the determination of a phenotypic trait with continuous distribution

quantitative traits: traits presenting a continuous variation, normally resulting from the simultaneous influence of many genes and the environment

stabilizing selection: selection favoring typical individuals in the population; this kind of selection operates against individuals with extreme trait values

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