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MORE FOOD: ROAD TO SURVIVAL

Editors:
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FOREWORD

In a recent seminar on agriculture sustainability, an old Professor of agronomy, present in the audience, commented that all what was said was correct, but that he was grown in an age where the increase of agriculture productivity was a central dogma, today, apparently, correctly sacrificed in favour of a better interaction between environment and food production. His feeling is probably motivated by robust reasons that several agronomists and geneticists, me included, share: we feel almost out of place in a cultural landscape which has forgotten promises and achievements of the green revolution, effective in nourishing a planet at a time of rapid population growth, a condition persisting still today. In 1960 the global yield of cereal grains was around 10 billions tons, while in the next 40 years it doubled. The green revolution had to do with the creation of high yielding plant varieties capable to efficiently utilize increasing agro-technical inputs. This was achieved by a rational use of genetics to recombine in single genotypes the resistance to pests, insects and abiotic stresses. In Italy, genetic and agronomic progress based on improved varieties was impressive: from 1945 to 1995 average yields increased from 0.68 to 2.57 t/ha for durum wheat, from 1.04 to 4.51 for common wheat, from 1.14 to 9.01 for maize, from 3.68 to 5.74 for rice and from 22.9 to 47.2 t/ha for sugar beet.

One can ask what are the reasonings behind the adoption of new paradigms and priorities by scientists and institutions dedicated to the future of agriculture. The first consideration is that while the south of the planet still has to solve problems of true rural development, in the western societies the social implications of agriculture are part of the more general problem of finding a balance between food and feed production and care of the environment [1]. As a consequence, the theme is debated with contributions of social scientists and politicians not familiar with agricultural specificities; as a result, their genuine good intentions frequently ignore the technical consequences of the solutions they suggest. The second point to be raised concerns our poor capacity to adopt proper parameters defining agriculture sustainability, particularly in terms of maintenance of soil fertility. On the issue, two contrasting visions have emerged. The first supports the adoption of mild systems with decreased productivity, a *wildlife-friendly* agriculture reducing its impact on the environment while minimizing the negative effects of fertilizers and pesticides. The second suggests more *intensive* agricultural systems avoiding the necessity to plogh new virgin soils which, frequently, for climatic and edaphic conditions are marginal lands hosting peculiar sources of biodiversity.

In any case, a general agreement exists on the need to consider with priority the problem of future sustainability of agriculture. A possible evolution is that agriculture will remain intensive, but being based on methods and principles derived more from biology than from chemistry. Along this line of thought, new suggestions are currently emerging concerning the radical modifications of our agricultural systems [2]. This will imply that defining a possible future should reconsider the difficulties inherent both to the practical use of the biology of the living components of agricultural processes, and to the need that such components will be properly managed in terms of sustainability. This is the core issue of this volume dedicated to the breeding of tomorrow crops.

The first group of contributions introduces macro-agronomic and economic topics, related also to the comparison between industrial and subsistence agriculture. This part includes a discussion on the role and impact of genetics in support of future yield gains. The next four chapters take into account the biological-genetic components responsible for the interaction among plants and the environment: seed germination and plant nutrition; plant development; photosynthesis. Four contributions follow, grouped under the title *Tools*. This is the most

evocative part of the volume: it illustrates the methodological revolution linking genomic resources and the capacity to predict plant phenotype and behaviour based on molecular markers; the adoption of new crops adapted to sustainable agricultural systems (one example is perennial cereal grains); molecular approaches to heterosis and apomixis; the role of epigenetics in determining the yield capacity of superior varieties. The volume ends with a chapter on quality and security of field-produced commodities and with a discussion on the state of art of the breeding of minor cereal grains.

The consideration of what the volume offers, allows to anticipate, at different levels, a vision on principles, methods and conclusions on the future sustainability of food production. A first level is the attention here dedicated to reappraise relevance and role of genetics in the sustainability context. Particularly in terms of resources dedicated, the possibility of future food crises should, in fact, suggest to stress the central role of the breeding of conventional and future-tailored varieties, once the social role of this activity is recognized, as done in the past with the peace Nobel prize assigned to Norman Borlaug.

A different level of discussion sees the future as interpreted in terms of targets to be assigned to plant breeding. Two cases are topical. The first regards the hybrid varieties in terms of contribution to yield increase. The adoption of hybrid crops as a final outcome of genetic selection, indeed, is becoming obligatory even for plants where autogamy does not favour an easy production of hybrid seeds. In this respect, molecular breeding, boosted by genomics, has contributed to bring again the phenomenon of heterosis to the attention to plant breeders, considering the possibility of revealing its molecular bases and of using effective prediction methods of hybrid value [3]. The second case has to do with perennialism. Compared to annual plants, perennials reduce the need of energy and agrochemicals, as well as of soil and nitrogen losses and of irrigation water.

A last consideration is proper to mitigate the impression that in the future food production may represent a problem of difficult solution. In the past plant breeders have successfully used genetics, but their approach to yield increase was essentially empirical. The incoming century, however, has already shown that varieties resistant to biotic and abiotic stresses can be developed using rational predictive methods based on molecular markers and exploiting genomics and transgenosis [4]. More recent molecular technologies allow to generate mutations, with positive phenotypic effects, at very precise nucleotide positions in genes with a known sequence. It can be concluded that the road to survival will be largely dependent on the accumulation of knowledge and on the evolution of methods capable to meet our future food needs.

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PREFACE

The need for more food is rapidly increasing as the world population is growing at a fast pace. The scientific community involved in crop production and its improvement is being called upon to find solutions to the expanding global demand for crop plants and their products. Two options are available to increase yields: the first consists of an increase in the areas under production, and the second, an improvement of productivity on existing farmland. Of the two options the second seems preferable, as it avoids the disruption of existing ecosystems as well as an increase in greenhouse gas emissions. In both cases two strategies can be adopted, one classical, of extensive industrial agriculture largely adopted in developed countries to produce major crops like corn, rice, wheat and soybean and another one, known as sustainable agriculture, characterized by a production more respectful of the ecosystem. The two strategies are not mutually exclusive and which one of the two should be adopted depends on the characteristics of local production methods and on economic and political considerations, as well as the choice of global *versus* local food production and consumption. In this context, to really improve the situation, we should focus our efforts on the areas of the world where the nutrition of the population should be improved, like Africa, India, Bangladesh, Pakistan, China and some regions of South America. In these areas the solutions to solve the problem of hunger should be local and specific, related to the real needs of the population and respectful of local traditions. The knowledge, experience and know-how available to the western world could be invaluable tools for improving their agricultural production. Application of our model of industrial agriculture should be avoided. Only by trying to understand what are the needs of these populations and exporting our knowledge to improve their situation can we hope to contribute to solve their problems. These considerations are developed in the first two chapters of the book. The following chapter will deal with genetic variability as an essential source of plant improvement. The following chapters will analyse basic physiological processes which represent bottlenecks for productivity and the efforts that could be directed to increase the efficiency of these processes. The topics analysed will be the genetic control of seed size; germination and seedling elongation, representing crucial steps in plant development; photomorphogenesis and the effects of light on aspects related to yield, such as photoperiod and shade avoidance, photosynthesis and the sink-source flux; and mineral nutrition. These topics will be covered in chapters 5 to 9. We will concentrate on factors that are directly related to yield, omitting those indirectly affecting productivity like herbicide- and pest-resistance, drought tolerance and cold resistance. In the last part of the book, attention will be given to some of the tools available to the researcher to achieve plant improvement. We will focus attention on available tools such as molecularly assisted breeding, gene editing, domestication of new species, heterosis and apomixis.

IMPORTANCE OF THE FIELD

The importance of increasing productivity of the major crops to meet the demand of an expanding population is self-evident. What is not so obvious is how to achieve a significant improvement in a short time, and what tools we can rely upon to accomplish a second

"green revolution". The great majority of the contributors to the chapters of the book are teachers of advanced courses to graduate students in Biotechnology or to post-graduate students in Ph.D. programs and they feel that this book could be of interest for their students.

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CHAPTER 1

The Yield in the Context of Industrial *Versus* Sustainable Agriculture

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Abstract: During the Green Revolution both the yield and the global production significantly increased. The yield increase was achieved, for some main crops, thanks to the so called high yielding varieties. Higher global production was also due to the increase of the crop production surface which took place especially in some areas of the planet. In the current scenario of rapid human population increase, with a sharp increase of livestock, the challenge is to achieve efficient, productive, sustainable and resilient land use, while conserving biodiversity and assuring, everywhere, food security inside a framework of sustainable diets. The paper, after a discussion on the meanings of such concepts as yield, yield gap, production and global production describes some of the main issues related to increased intensification of food security and global productivity in the current discussions on the potential of the Green Revolution approach and the agro-ecological paradigm.

Keywords: Agro-food system, Diet gap, Food security, Global production, Sustainability, Yield, Yield gap.

BASIC TERMS

The issue related to crop yield, despite its fundamental importance for our future, even though extensively studied, has been poorly defined and discussed on a sufficiently broad time-space scale.

The role of the technology in yield change has often been confounded by other influences [1].

During the Green Revolution the crop yield has been the main, if not the only, goal to be considered and the farms have been viewed for decades as industries where input is converted in output thanks to an industrial-like production process.

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Few studies have been carried out by referring to theoretical and practical analysis influences of the Green Revolution approach on the innovation in agriculture, including both the positive and negative consequences on the natural resources.

Crop yield is the weight of the so called economic products (*i.e.* grain, root vegetables, and fruits, *etc.*) at standard moisture content, referred to unit of land area cultivated per crop, conventionally and generally referred to in metric tons per hectare (t/ha). Energy, protein, oil, vitamin, micronutrients contents in the total weight are of fundamental importance in yield analysis taking into account the influence on the final utilization of the value chain (human diet and nutrition) when the nutritive, energetic, economic balances, also have to be considered.

As underlined by Fischer *et al.* [1] the energy contents reflect the cost of biosynthesis of the major constituents of the product. Cereals for examples are characterized by a total energy content of around 15 MJ/kg, whereas soybean contains about 24 MJ/kg, the comparison of the yield obtained from these crops must consider these different energy costs.

For agriculture the main figure is average yield in terms of t/ha, not only referred to field and farm, but also to different levels of the territorial systems *i.e.* districts, regional, and national. **Farm Yield** (FY), reported from yield measurements, or more often from surveys, are part of the local and national statistics annually collected without considering the cases where, for various reasons, the district is not planted to its full potential.

The possibility is not always considered, such as in warm climates, to have different crops/harvests, per year, in the same field. Nevertheless this FY is generally indicated as **Real Yield**, different from the so-called **Potential Yield** (PY), which is the uppermost end of the yield scale, which is reached with the combination of some important factors. When the most appropriate varieties are cultivated with the best agronomic management, there are no manageable abiotic and biotic stresses [2].

"PY defines what might be obtained for particular plants species when not limited in technology, *i.e.* when the best cultivars, fertilizer, machinery, labor, and knowledge are all available and applied in the best possible ways" [3].

The concept is close to the so-called **Attainable Yield** corresponding to the best yields achieved through skillful use of available technology. It is usually achieved in experiment centers or by the best farmers [3]. This simple theoretical definition does not have an easy method that actually measures it. The sowing date can be a complication. The optimal sowing date may be constrained in a multiple cropping system [4]. PY is usually determined with direct measurements or indirect

estimates in plots, in two types of experiment: comparative variety ones and in plot/field experiments carried out by crop physiologists or agronomists. In this type of PY determination sampling errors occur. Crop modeling can be used to predict PY in different environments and their accuracy has significantly improved. Integrated methods, *i.e.* direct measurements, modeling and expert opinion can be used [5]. The integrated methods are particularly useful when the so-called water-limited potential yield (PYw) has to be determined. The crop yield depends on the quantity of available water and the PYw is generally calculated as a linear function of the water supply, but variation in rainfall during the development stages can create a more complex picture and modify this linearity.

Current yield in a given agricultural area is usually a poor indicator of potential performance, falling on a continuum between crop failure and potential yield. FAO defines **Actual Yield** (AY) as the average yield of a district.

The concepts of the actual attainable potential yields are useful for defining the agronomic concept of **intensification** of the farming system: where actual yields are close to the projected attainable ones. The farming system and the agriculture of the area can be described as intensive. The **intensification** of farming systems increases when the available technology is appropriately adopted and as the proportion of time in crop is relative to fallow increases. The yield can be referred to both the total biomass obtained from the growth/development process and the part of this biomass. The term biomass indicates the total dry biomass accumulated by the crop, where the term **Economic Yield** (**EY** or simply **yield**) indicates the portion useful to humans as food/fiber/fuel or as feed. The fraction yield/total biomass is defined as coefficient of economic yield, the **Harvest Index** (HI) is calculated as the useful fractions/above-ground biomass.

If we compare PY, AY (or FY), EY and calculate the differences (*i.e.* (PY-AY) we have a better knowledge on what is defined as **Yield Gap** (YG). It can be expressed in percentage on PY or on FY. The latter is more appropriate since it indicates how much is the possible, desirable increase in actual grain yields that is achievable by farmers. Scientific literature supports the notion of a minimum yield gap (FY equals EY depending strongly on prices). If the future prices will be favorable for the farmers it is suggested [1] that the minimum yield gap is 30% of FY; that is to say EY is 23% below PY [4]. The yield gap across 40 agricultural regions around the world was calculated to range between 25 and 400%. (For more information and more recent data refer to both [4] and http://www.yield-gap.org/). Many of the countries with the highest YG have the poorest access to technology, infrastructure and capital required for the model of Green Revolution agricultural development.

CHAPTER 2

Increasing Plant Breeding Efficiency through Evolutionary-Participatory Programs

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Abstract: One fundamental problem in plant breeding is the relationships between selection and target environments. Selection theory shows that response to selection (genetic gains) depends on this relationship because of genotype x environment interactions. Therefore, response to selection can be increased by making the selection environment as similar as possible to the target environment (decentralized breeding). However, this does not yet guarantee farmers' acceptance of the new variety, which we argue is a more correct way of measuring plant breeding efficiency than variety release as usually done by public breeding programs. Using selection theory, the chapter shows that the probability that a new variety is accepted by farmers, thus impacting their livelihood, increases by selecting in the target environment (decentralized selection) in collaboration with farmers. Decentralized-participatory plant breeding also increases agrobiodiversity and makes plant breeding more cost-effective. The proclaimed efficiency of private breeding program, which can claim a wide farmers' adoption, is actually driven by a seed market monopoly, which severely limits farmers' choice of which seed to buy. However, the weak point of decentralized-participatory plant breeding is the unreliability and unpredictability of Institutional participation. Evolutionary-participatory plant breeding may overcome the limitations of participatory plant breeding, because farmers can handle evolutionary populations independently from Institution, yet without excluding them from participating. Because in evolutionary-participatory plant breeding the unit of selection becomes the individual plant rather than a plot, a much higher selection intensity is possible, thus increasing even further the efficiency.

Keywords: Biodiversity, Climate change, Efficiency, Evolutionary plant breeding, Genetic gains, Genomic selection, Genotype x environment interaction, Human health, Participation, Response to selection, Seed.

INTRODUCTION

Biodiversity decline, climate change, hunger and malnutrition, poverty, water and the increased frequency of a number of diet related diseases such as diabetes as

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well as diseases associated with overweight and obesity are currently major global problems. All of these problems are related to seed.

Seed is related to water because, at the global level, agriculture uses 70% of the total water consumption, and the development of crops that can produce an economic yield with less water, will make more water available for human use. Seed is related to poverty through malnutrition: poor nutrition in the first 1000 days of life does affect the mental development of children [1]. Seed is related to climate change because farmers will need seed of crop varieties better adapted to the climate of the future. This is a particularly intriguing problem because of the uncertainty of the expected changes in temperature and rainfall [2, 3]. Therefore, plant breeding programs aiming at improving crop adaptation to climate change are actually addressing a moving target and probably a different target in different areas [3, 4]. Breeding for adaptation to climate change implies also breeding for resistance to new insect pest and diseases, which have been shown to have altered their latitudinal ranges in response to global warming [5]. An additional effect of climate change is on malnutrition, as the increase of CO_2 in the atmosphere is expected to decrease in C3 crops the content of iron and zinc whose deficiency is already causing the loss of 63 million lives annually [6].

Hunger and food security continue to be staggering challenges as about 800 million people are still undernourished and about half of the world population is lacking one or more essential nutrients [6, 7]. Whether the problem is insufficient agricultural production (but 30% of agricultural production is wasted annually) or non-equitable distribution of available food [8], seed, and the way in which it is produced, is central.

Therefore, talking about seed is not only talking about the major global problems but also about our health because most of our food comes from seeds, and food affects our health. A number of modern diseases are associated with food, such as the well-known case of celiac disease [9, 10], and the decrease of diversity is possibly related with the increased frequency of inflammatory diseases [11]. Overweight and obesity, largely associated with diet, have become a major global health challenge [12]; similarly mortality rates due to diabetes have increased [13] and non-alcoholic steatohepatitis (NASH) is becoming epidemic [14].

WHERE THE SEED COMES FROM?

Plant breeding, and the way in which it evolved from the way in which it was practiced by farmers for millennia to modern or “scientific” breeding, offers an understanding of how the problems discussed above developed and how they can be solved.

Over the years and before harvesting, farmers have selected the best plants to obtain the seed for the next cropping season, and this was done individually in each farmer's field: in other words they selected for millennia for specific adaptation producing what today we call ancient, old or heirloom varieties.

When plant breeding started to be done on scientific basis, there was a shift from selecting from specific adaptation to selecting for wide adaptation. This was done at the global level by the Green Revolution, which developed varieties able to make full use of fertilizers, pesticides, irrigation water and mechanization. If some elements of the package were missing, the varieties alone did not have any specific advantage over those that the farmers already had. On one hand, the Green Revolution averted the danger of extensive famine, but, on the other hand, it had a number of negative consequences [15 - 17]; eventually the poorest farmers could not benefit because they were not able to afford some of the components of the package [18]. GMOs, cannot be the solution to these problems because they ignore the Fundamental Theorem of Natural Selection (FTNS) by which the organisms to control evolve resistance [19]. The resistance to antibiotics, a phenomenon that is becoming widespread, and that can be very rapid [20], is based on the same fundamental biological principle. An agro ecological model of agriculture, such as different forms of organic agriculture, could be a solution, but is considered unable to produce enough food to feed a growing population, raising doubts on whether food security and food safety can be compatible objectives. The argument that organic conditions are associated with lower yields is biased by the fact that many of the meta-analysis used varieties not specifically selected for organic conditions.

In addition, the type of plant breeding, which has emerged with the Green Revolution and which is still largely followed today, particularly in public breeding, is not even the most efficient.

THE EFFICIENCY OF PLANT BREEDING

In public Institutions such as Ministries of Agriculture and the Centers of the CGIAR, the number of varieties released is the most common way of measuring plant breeding efficiency. A more scientific measure of a breeding programmes efficiency is the selection or genetic gain (or response to selection) obtained at the end of a breeding cycle [21]. Another measure of efficiency is the ratio between benefice and cost; this has been used by economists [22], but almost never by breeders. The number of varieties released is used as measure of plant breeding efficiency because is easy to measure; however, this measure ignores that a variety generated by plant breeders produces benefits only when it is accepted and grown by the farmers [22, 23]. The number of varieties released is also one of the

CHAPTER 3

Genetic Tools for Crop Improvement: Past, Present, and Future

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Abstract: To respond to the contemporary increasing demand for food, feed, and feedstocks for bioenergy and bio-factory applications, there is an urgent need to improve agricultural plant production and quality-related features. Genetics and plant breeding are powerful strategies for increasing crop productivity. The objectives of this chapter are devoted to summarize i) historical developments of applied plant genetics or plant breeding, ii) fundamental principles affecting the current methods of molecular plant breeding, and iii) key factors that will affect the use of molecular breeding in crop improvement procedures. Additionally, the chapter takes a close look at the current exploitation of molecular plant breeding for the discovery of genes and their functions. These topics would disclose new perspectives for crucial plant biology research that will be beneficial to ensure food security to the rapidly growing world population and to sustainable agricultural systems. Moreover, they would open new doors to improve feedstocks to sustain non-food applications for the synthesis of high-added value products.

Keywords: Applied plant genetics, Biotech crops, Crop improvement, Genetic and genomic tools, Genetic engineering, Green revolution, Plant breeding.

INTRODUCTION

A recent report on the world population prospect, elaborated by the United Nations, indicates that people is projected to advance, on a global scale, from the current 7.2 billion to 9.6 billion by the middle of this century [1] (Fig. **1**). This trend will approach 11.0 billion by 2100.

To accommodate the additional demand for food and changing diets in developing nations, it is needed to supplement the global agricultural performance by approximately 100% by mid-century: namely doubling crop yields by 2050 [2]. The increased demand for crop and land derives not exclusively from the growing population and wealth, but also for ensuring non-food applications -*e.g.* energy

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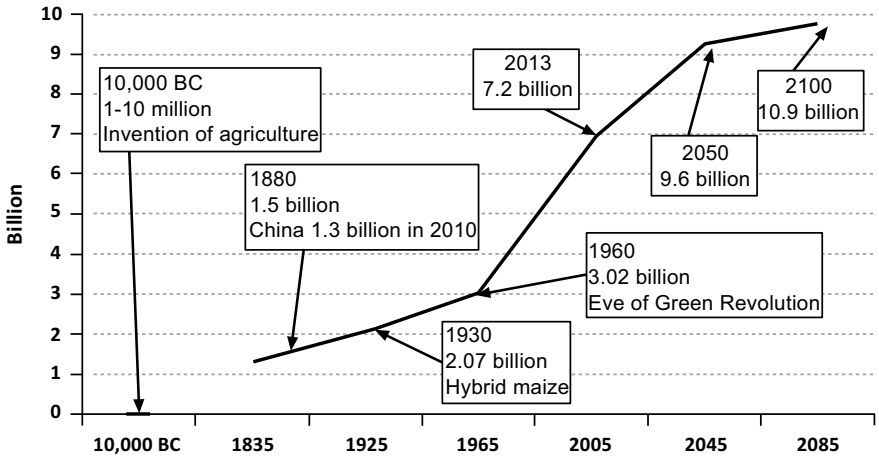

Fig. (1). U.N estimations of human population growth to 2100. Notably, from 1960 to presently, the human population has more than doubled to reach 7.2 billion people; by 2050, the population is forecasted to increase to approximately 9.6 billion and reach 10.9 billion by 2100. (Modified from Popp *et al.*).

access and chemicals- and climate changes. Moreover, it is worth noting that globally 805 million people are currently persistently starved and 2 billion are malnourished [3]. To double food yield by 2050, Ray and coworkers [4], have estimated that crop yield should increase at a rate of 2.4% per year. However the contemporary growth rates in global crop productivity of all major crops (*i.e.* maize, wheat, rice, soybean, roots and tubers) is declining from 2.5% and higher, since the mid-1990s, to less than 1.5% today [5]. This trend points out that the population growth rates have globally bypassed the linear rate of increases in food. In this scenario, raising crop yields to meet the expansion of food demands, rather than extend land for agriculture uses, has been highlighted as the most sustainable solution to meet this objective [4]. As a result, there is a pressing requirement to amplify agricultural productivity. It is also clear, at the same time, the necessity to diminish the contradictory influence of agriculture on the ecosystem stability: shortly a more sustainable and environmental friendly farming systems that will increase production [6, 7]. This may also be accomplished by considering, simultaneously, the tolerance to abiotic and biotic stress factors, the efficiency of nutrient and water use inputs, and the development of crops and cultivars having a broad range of end uses. The vast scientific progress in the last decades may help to accelerate these new challenges. In particular, genetic improvement of crops may assist to give a solution to this multifaceted target [8]. Accordingly, the production and diffusion of high-yielding varieties (HYVs) and the best achievements of biotechnology projects (*e.g.* pertinent Genetic Modified, GM, and/non-GM features), cultivated on the 1.5 billion hectares of arable land worldwide, will provide, in the near future, a

fundamental contribution to sustainable agriculture. In addition, genetic crop interventions are an appealing goal. This approach is particularly ascribable to the potential of fast dissemination of the improved crops- that is seed distribution- and to their contribution in solving diverse features of contemporary crop biology. In this chapter I have briefly summarized i) historical developments of applied plant genetics or plant breeding, ii) fundamental principles affecting the current methods of molecular plant breeding, and iii) key factors that would sway the application of molecular breeding in crop improvement procedures. Additionally, in this chapter I have highlighted how the exploitation of molecular plant breeding is currently contributing to the discovery of genes and their functions. It is thereby predicted that these findings will open new perspectives in contemporary plant biological sciences.

CLASSICAL PLANT BREEDING

There are several approaches for increasing food production. These include: expanding the arable cropland, improving agronomic methods, extending the use of mechanization, as well as making perfect the supply of chemical fertilizers, and pesticides. However, the simple most agreeable factor in enhancing plant productivity is the genetic improvement of the crop plants themselves [9]. Specifically, plant breeding has had a dominant role to increase and safeguard potential, harvestable, and commercial productions. This scope was accomplished, *via* selecting for yield *per se*, addressing crop quality attributes, and developing plants exhibiting tolerance/resistance to disease and pest agents. It has also contributed timely to farmers by affording the best seeds of new developed HYVs. This has been implemented, in addition to higher yield, with other profitable features, able to upgrade farming incomes and sustainability. In the following sections, it is given a brief overview of genetic improvement from agricultural invention to present. Even though plant breeding emerged around 10-12,000 years ago, over the course of the human history, the general perception of genetic technology concepts is a relatively recent phenomenon (Fig. **2**).

Domestication and Empirical Plant Breeding

For 10-12,000 years, humankind has modified the genetic architecture of crop plants, initially *via* simple domestication (*i.e.* selection of plants having desirable traits to humans) and, in the last 2-300 years, employing more refined procedures.

For the domestication process, different plant species were selected for peculiar characteristics to satisfy human needs: cereals and pulses for their seeds, other plants for tubers, fruits or leaves. Evidence indicates that domestication or its syndrome incorporate i) combinations of various distinct attributes including: seed retention (non-shattering), enhanced fruit or seed size, alterations in branching

CHAPTER 4

Role of Epigenetics in Crop Improvement

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Abstract: It is time to start to take into consideration the role of epigenetics in breeding programs. So far, various authors have reported hereditable gene silencing phenomena generally affecting qualitative traits. Usually the genes involved in these phenomena determine characters which can easily be scored by visual inspection, such as modifications in the plant architecture and pigment accumulation, or characters like the loss of antibiotic resistance. But we have to take into account that the majority of genes are involved in the determination of quantitative characters, and the phenotypic modifications caused by QTL silencing will result in subtle variations which are difficult to detect. For this reason it would be very hard to find silencing phenomena involving quantitative traits. Therefore, assuming that epigenetics concerns not only the qualitative but also the quantitative traits, this phenomenon must be taken into account in breeding programs. In particular, the transcriptional state of the different epialleles should be considered. This chapter will start by defining what we mean by epigenetics, as numerous definitions are now used, starting from the original definition of Waddington and adapting the definition to the different fields of study. We will then describe the epigenetics marks, before going into more detail of the epigenomic studies on two model plants, arabidopsis and rice. Then we will present data concerning the interaction of epigenetics and the environment and the role of the epigenetic phenomena on crops and in particular, on yield improvement. A brief paragraph on the epigenetic phenomenon called paramutation will conclude the chapter.

Keywords: Crop improvement, Epiallele, Epigenetics, Gene silencing, Paramutation.

INTRODUCTION

The fine modulation of genome expression is essential to cell specialization in complex organisms. In fact, all the cells in a multicellular organism have the same genome, but, just as obviously, cells that are different at morphological, develop-

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mental and functional level, need different information and thus the activation of different subsets of genes [1].

Since the early studies on the inheritance of traits, it was clear that not all the changes in gene function segregated in accordance with Mendelian laws: among these exceptions are the epigenetic phenomena.

The term of epigenotype was first introduced by Waddington to define the "complex of developmental processes connecting genotype and phenotype" [2]. Over the years, new definitions have emphasized the meanings acquired by the term epigenetics with the increase of studies in this field [3]. In particular the definition of Russo, Martienssen and Riggs, currently one of the most used and accepted, revised the definition of epigenetics as "the study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence" [4], thus defining new levels of gene regulation.

An important breakthrough in the evolution of the initial definition of epigenetics was the work of Nanney who focused his studies on the concepts of stability and heritability of the expression states [5]. These concepts were also included in the definitions of Holliday ("study of the changes in gene expression, which occur in organisms with differentiated cells, and the mitotic inheritance of given patterns of gene expression" and "nuclear inheritance, which is not based on differences on DNA sequences") [6] and in the previously reported definition of Riggs and colleagues [4].

These more recent definitions fitted in particular with the genetic studies focused on the conservation of expression patterns through mitosis and meiosis, while studies of ecology and physiology, more concerned with gene-environment interactions, refer to the earlier definition of Waddington [3].

Epigenetics Marks

The studies of the molecular mechanisms responsible for the epigenetic variations disclosed the importance of two main type of DNA modification: DNA methylation and chromatin/histone modification. The covalent modifications of nucleotides, among which the most common is the methylation of cytosine, can be seen as a guide for a correct organization of the subsequent epigenetic modifications, *i.e.* the post translational modifications of the histone proteins [7].

These epigenetic marks, modifying the DNA packaging into nucleosomes, promote changes in chromatin structure, and thus DNA accessibility, regulate gene expression and gene function, and thus phenotypes, and furthermore because

these modifications are enzyme-mediated, they are inherited even though they are not alterations of the nucleotide sequence [8].

Cytosine methylation can be in three possible contexts: CG, CHG and CHH (H: A, C, or T). The enzymes necessary for these modifications are maintenance DNA methyltransferases and *de novo* methyltransferase. In Arabidopsis the enzyme responsible for the *de novo* methylation, is the methyltransferase DNRM2 (DOMAINS REARRANGED METHYLTRANFERASE 2) [9].

With regard to the maintenance methyltransferases, MET1 (DNA METHYL-TRANSFERASE1), is responsible for maintaining the GC methylation [10, 11], the methyltransferase CMT3 (CHROMOMETHYLASE3) acts together with the histone methyltransferase KYP (KRYPTONITE) to maintain the CHG methylation [12, 13], and the CHH methylation is maintained by DRM2 (DOMAINS REARRANGED METHYLTRANSFERASE2) that is targeted on DNA by the 24-nucleotide small interfering RNAs bound by AGO4 (ARGONAUTE) [8]. Among the higher plants a high level of conservation of methyltransferases has been found, suggesting the evolutionary conservation of their function [13].

The mechanism used by the maintenance cytosine methyltranferases is based on the recognition of sequences containing hemimethylated cytosine, this is the signal allowing the methylation of the cytosine on the newly synthesized DNA sequence.

A similar mechanism is used to maintain histone modification during DNA replication, the existing modifications are the signal for the modification of the newly assembled nucleosomes, and this is possible also thanks to the association of the enzymes required for histone modification (histone methyltransferases and histone demethylases) with the DNA replication machinery [8].

The complex regulation of the propagation of the epigenetic marks has to satisfy two requirements: to allow the transmission of the epigenetic information *via* both mitosis (to daughter cells) or meiosis (to the next generation) but also to allow the meiotic resetting of the epigenetic information to enable the genome plasticity necessary to cope with environmental and developmental changes.

The propagation through mitotic divisions of the epigenetic marks is guaranteed by *de nov*o methyl transferases and by the histone modification machinery, activated by small RNA molecules and sequence-specific binding factors [7].

With regard to the meiotic transmission of the epigenetic marks, there is much experimental evidence for the meiotic or developmental erasure of epigenetic

CHAPTER 5

Enhancing Photosynthesis: Different Strategies to Improve the Process at the Basis of Life on Earth

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Abstract: Doubling agricultural production will be essential by 2050 to satisfy the demand of food of a constantly growing population, but climate change brings a lot of uncertainty and complexity to this challenge for agriculture. One of the most important changes that must be addressed is the increase in atmospheric [CO_2], which has increased from approximately 280 ppm in pre-industrial times to about 400 ppm nowadays and will further increase to values of 470–570 ppm by 2050 depending on the climate scenario (IPCC Synthesis report, Climate Change 2007). Although this increase in [CO_2] is expected to have a positive and significant effect on C_3 crops production, it is counteracted by the rise in temperature and the higher evaporative demand, with the increased risks for drought and heat likely to be progressive in all regions of our planet. As a matter of fact, the average stimulation of C_3 leaf photosynthesis under field conditions at elevated [CO_2] has been reported to be only 14% on average across FACE (550–600 ppm in Free Air CO_2 Enrichment) experiments, much lower than the expected increase of 38%.

Down-regulation of photosynthesis can be ascribe to multiple factors. These include the limited sink strength of the plants and the consequent accumulation of inhibitory photo-assimilates, the "hysterical" behavior of photosynthetic organisms to excess illumination, by either triggering EED (Excess Energy Dissipation) beyond the level effective for photo-protection or retaining a relevant fraction of quenching for extended periods after return to limiting light conditions, and the complex and multi-factorial network that controls CO_2 fixation and carbon allocation.

Here we describe the genetic constraints that limit yield potential and prevent it from being realized on the farm, in order to improve the understanding of plant responses under elevated [CO_2], and provide tentative biotechnological solutions to overcome the crop yield limitations.

It is worth noting that the huge improvements in agricultural production gained during the 'Green Revolution' were not directly related to manipulation of photosynthesis, therefore its modification remains an unexplored target for crop improvement.

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Keywords: Dark phase efficiency, Improvement strategies, Light phase efficiency, Photosynthesis, Yield potential.

INTRODUCTION

A steady and reliable supply of food is an essential factor in ensuring the survival of humankind and the stability of human societies. However, it is expected that the global population will increase from the current 7 billion to 9. 3 billion by mid-century before stabilizing at the end of the century [1, 2]. In addition to population growth, other factors are expected to put a strong pressure on agricultural production, such as the growing economic prosperity of the developing countries, which results in the formation of a middle class with increased demands for goods, and a strong growth in urban population. The final outcome will be an enlarged demand for processed foods and high-quality animal products, like dairy products and meat. Furthermore, taking in consideration the rapidly decreasing reserves of fossil fuels, such as oil, coal, and natural gas, the use of plants as a source of energy (biofuel) and as a commodity for industrial uses is expected to grow, making a substantial increase in agricultural output even more important and necessary. It is estimated that to meet these rising demands global agricultural production needs at least to be doubled by the year 2050, and this while adapting to climate change and adopting a global sustainable model of agricultural production, in order to take in consideration also the equally crucial needs for environmental protection [3].

Doubling yields will require considerable efforts. If in the coming years the rates of crop yield increase will simply be maintained at the current level, by the middle of this century we will be unable to meet the demand of food. For instance, it has been estimated that wheat, rice, maize and soybean, the most important global crops, have current yield improvements far below those deemed necessary to double the production by 2050 (0.9-1.6% per year, *versus* the required 2.4%) [4] (Fig. **1**).

Furthermore, the rate of yield improvement in some areas of the world is stagnating if not decreasing. Of the most important cereals, only maize maintained steady growth rates [2, 5]. The yields per hectare of rice in the most important producing countries (China, India and Indonesia) strongly increased between 1970 and 1990 but then they gradually downsized during the following years, despite continued genetic improvement [6] (Fig. **2**).

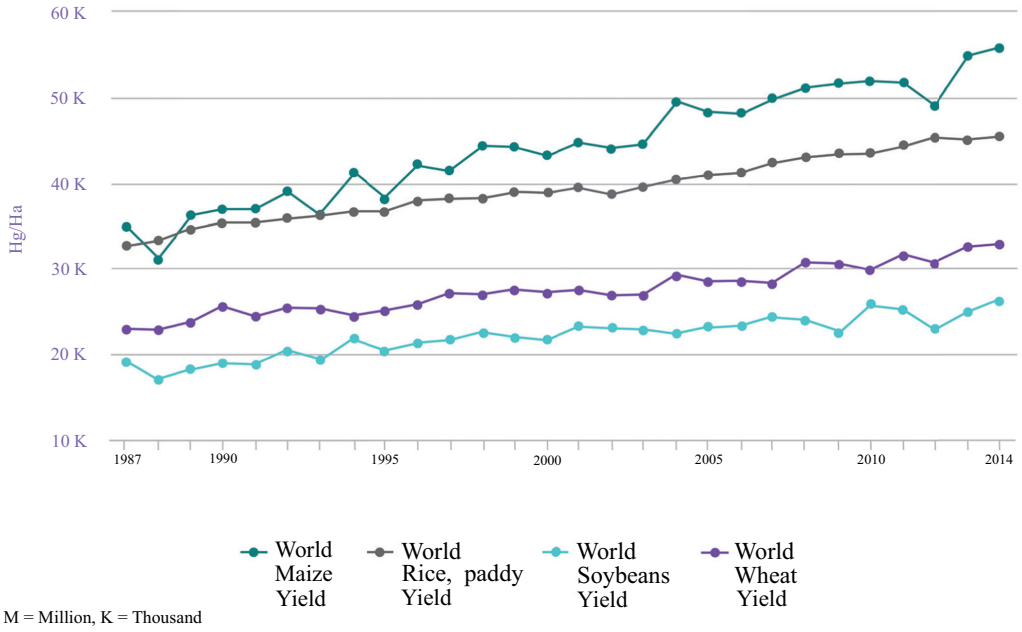

Fig. (1). Annual average global yields of the most important crops top (wheat, rice, maize, and soybean) from 1987 to 2014 (Source: Food and Agriculture Organization of the United Nations, FAOSTAT, 2015).

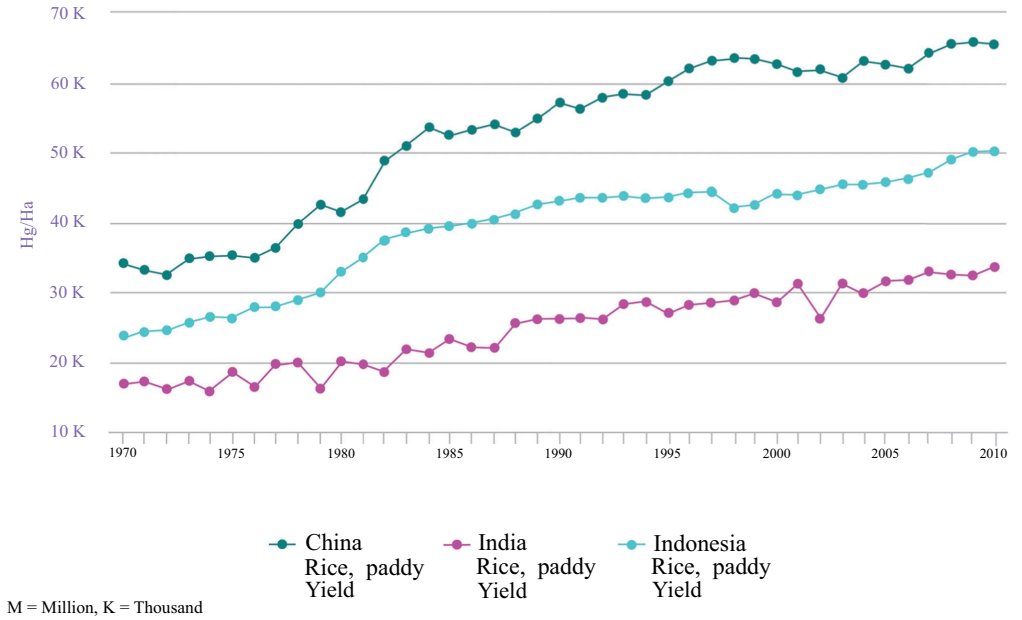

Fig. (2). Annual average yields of rice in China, India, and Indonesia from 1970 to 2010 (Source: Food and Agriculture Organization of the United Nations, FAOSTAT, 2015).

CHAPTER 6

Seed Size: an Important Yield Component

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Abstract: Grain yield in cereals is considered one of the most important traits in the perspective of global food supply. It largely depends on several components including grain size and grain number. Several studies have been conducted to understand the genetic and molecular basis of these two traits. In this article we review the information so far obtained on the mechanisms governing seed size in crop plants particularly in cereals and discuss the potential uses of this information to improve the productivity of seeds in cereal crops.

Keywords: *Arabidopsis*, Cryptic variation, Grain weight, Hormones, Maize, Seed size, Tillers.

INTRODUCTION

Small seeds are a prerogative of wild species. Because of the small size they can be produced in high numbers and are easily dispersed. These traits have presumably increased the fitness of the plant and for this reason they have been a target of natural selection. On the other hand, starting from domestication, cultivated plants have been manipulated to improve seed size. The interest in studying seed size relies on the fact that this trait is an important yield component, particularly in cereals. Functional analysis of single genes affecting seed development and in particular seed size is fundamental for unraveling the complex genetic network underlying the processes of seed formation. It may also be instrumental for establishing classical as well as advanced breeding programs aimed at improving cereal productivity. Recently the advent of new technologies allowing the precise manipulation of single genes has renewed interest in understanding the role of genetic factors underlying these complex processes.

Genes that retain key functions will become the object of this approach since they will enable us to obtain gene variants with better performance with respect to

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grain yield, which is the main trait of agronomic importance in cereal improvement.

In this review we will concentrate on the analysis of factors, acting at the genetic, epigenetic and physiological level, involved in the control of seed size as outlined through several studies. We also discuss future perspectives in improving this character.

Yield Improvement By Seed-Size Selection

Farm-saved seed accounts for the greatest proportion of seeds used by farmers especially in low-income countries. Farmers have been able to improve the quality of the farm-saved seed by applying seed selection at harvest time. As a result of seed selection, yield increases have been reported in wheat, up to 50%, 24% in rice, 15.3% in okra and 13% in maize. In maize the selection is based on the choice of kernel size and ear dimension. In an experiment reported by Msuya and Stefano [1], selection of the larger seeds located in the basal portion of the ear and exclusion of the smaller ones, in the apical region, resulted in improved germination and seedling vigor, and yielded larger seeds in the progeny. Even though the authors did not analyze heritability of the improvement in seed size we assume that the observed gain in seed size is inherited since the selection applied is the one traditionally used by farmers to improve this crop. In an accurate study Odhiambo and Compton [2] by subjecting the Krug maize variety to 20 cycles of mass selection for seed size, obtained two populations with large and small seed sizes confirming the heritability of the trait selected.

Origin Of The Seed

The seed in flowering plants represents a crucial phase of the plant cycle, ensuring the reproduction of the plant and, through its dispersal, its spreading around over the surrounding soil surface. Its formation requires a double event of fertilization of the female gametophyte, the embryo sac, by the two sperms of the male gametophyte, the pollen grain. One of the two sperms unites with the egg cell, and the ensuing zygote, carrying one paternal and one maternal genome, undergoes several cell divisions resulting in the formation of the embryo, the progenitor of a plant which will form the next generation. The second sperm unites with the central cell of the embryo sac obtained by the fusion of two identical maternal genomes, giving rise to a triploid cell which is the precursor of the endosperm. Embryogenesis is characterized by different morphological stages, corresponding to the acquisition and development of new functions. After several cycles of cell divisions, the embryo acquires polarity followed by a morphogenetic program leading to the formation of root and shoot primordia at the two poles of the embryonic axis followed by a maturation phase, dehydration and quiescence. The

endosperm shows four developmental phases: syncytium formation, cellularization, differentiation and cell death. The mature endosperm consists of four domains: the starchy endosperm, the single aleurone layer, the embryo surrounding region and the basal endosperm transfer layer [3, 4]. Since the embryo sac develops within the ovule, a sporophytic organ, the seed is surrounded by maternal sporophytic tissues (Fig. **1**). So the seed consists of two main compartments, firstly the embryo and the endosperm with 2n and 3n ploidy levels respectively, and secondly the seed coats with a 2n maternal genotype (Fig. **2**).

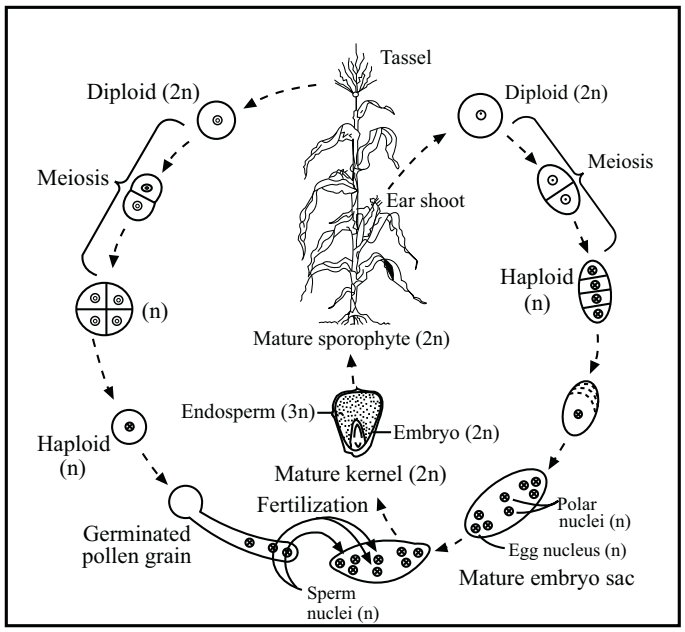

Fig. (1). Life cycle of *Zea mays* L. (adapted from Mc Clintock, 1983).

Seed development implies the formation of embryo and endosperm as well as interaction between the two and between the developing seed and the maternal tissues. Several factors influence seed size: ecogeographical factors such as photoperiod, edaphic conditions, precipitations, biotic and abiotic stresses [5]; the coordinated growth of the different seed components (embryo, endosperm and pericarp) [6] and hormones (auxin, cytokinin and brassinosteroids). In studying it we should be aware that seed size is the end result of the interplay of several factors. A comprehensive view of the mechanisms underlying seed size requires an analysis not only of the effect of one or a few genes at a time but also the application of modern-scale genomic approaches.

CHAPTER 7

Genetic Variability as a Means to Improve Seedling Emergence and Early Developmental Phases in Crop Plants

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Abstract: Embryogenesis, germination and the early phases of seedling growth represent critical phases in the plant life cycle and are probably the most important events in determining the success of an annual plant. In the perspective of a more sustainable agriculture, we aim to achieve a robust seedling phase with improved resistance to abiotic as well as biotic environmental stress. Genetic improvement has forced the developmental pathway of crop plants toward the realization of highly productive species in which resource allocation processes are optimized at the expense of defense processes. In this context the discovery of key factors underlying the developmental process and at the same time playing an important role in the interplay between the young individuals and the environment is crucial for designing future gene manipulation approaches. Among the different aspects affecting seedling development, the two that will be analyzed in this chapter also play an important role in the interplay with the environment. Hormones are endogenous signals governing seedling growth and architecture establishment but at the same time are able to induce plant responses to environmental stress. Wax deposition is required for determining correct embryo and seedling development, and provides, besides that, a protective barrier that plants produce in their early developmental phases to defend themselves from pathogens as well as from variation in environmental abiotic components, such as temperature and water availability. We will explore the genetic, biochemical and physiological factors implicated and highlight the most significant aspects that might be taken into consideration in future breeding programs.

Keywords: Abiotic stress, Biotic stress, Cuticle, Cuticular wax, Embryogenesis, Germination, Heterosis, Phytohormones, Seedling growth, Transgenic plants.

INTRODUCTION

Embryogenesis, germination and early phases of seedling growth represent criti-

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cal phases in the plant life cycle and are probably the most important events in determining the success of an annual plant. Embryogenesis can be seen as the first phase of a continuous process, only temporarily interrupted by dormancy [1]. During the succeeding phase, germination, the embryo becomes the seedling. Thus, proper embryo development is a prerequisite for successful seedling emergence.

Germination and seedling emergence occur when environmental conditions for establishing a new plant generation are likely to be suitable. A rapid and robust emergence positively influences the capacity of the plant to take advantage of the growth-favouring environment and to compete with its neighbors. From an agronomic point of view, synchrony in germination and seedling emergence of a crop is a desirable character for a cultivated plant since it will allow the optimization of weed control practices. The possibility of predicting and synchronizing the time of emergence will reduce chemical use and will allow this strategy to be combined with more sustainable approaches, such as biological and physical weed control [2]. In the perspective of a more sustainable agriculture, certain specific characters are envisaged for a crop seedling, such as resistance to environmental critical abiotic as well as biotic factors. For these reasons, key factors controlling subtending plant developmental processes and contributing to the achievement of a productive and robust plant have to be searched for within the genetic network that controls embryo and seedling development.

There is evidence that heterosis is already determined at early developmental stages. Various studies on heterosis have shown that some traits related to this phenomenon are already evident during early phases of plant development, as clearly described for young maize roots [3, 4]. It has also been reported, in Arabidopsis and in crops, that F_1 seedlings are already larger than their parents [5, 6]. Greater cell number is the main determinant of the larger size of organs in heterotic plants [7]. It is conceivable that the rate of cell division, which is defined very early during embryogenesis, is higher in the progeny than in parental lines. The level of heterosis is most probably set very early and conditions the final organ size and numbers. On this basis the study of the mechanisms governing early phases of plant development, *i.e.* embryogenesis and seedling development, are appealing not only for investigating the molecular and genetic network underlying these processes, but also for the identification of genetic tools that might be of interest in breeding programs.

Maize embryogenesis leads to the formation of two main structures, a well differentiated embryo axis and a storage organ, the scutellum. The mature embryo axis comprises at the two poles the embryonic primary root and the embryonic shoot, separated by the scutellar node. They are both enclosed in protective structures,

respectively the coleorhiza for the root and the coleoptile for the shoot. The shoot stem comprises a first internode, called the mesocotyl, that is located between the scutellar node and the coleoptilar node and five or six short internodes, depending on the genetic background, located above the coleoptilar node, with a leaf primordium attached to each node. Each leaf is rolled up inside those which enclose it, thus forming a cone shaped structure which encloses the shoot apical meristem (SAM). During embryogenesis, the coleoptile develops as a sheathing structure and envelops the stem tip and the embryonic seedling leaves [8, 9].

The scutellum, a massive organ, in which mainly lipids and proteins are accumulated, is attached to the scutellar node. For its functional equivalence, it is considered to be the single cotyledon in the embryo of monocotyledons. The epidermis of the scutellum differentiates in two regions. On the side facing the endosperm a scutellar epithelium is produced, while on the side adjacent to the coleoptile, scutellum cell walls develop a heavy cuticle [10].

Both coleoptile and the first set of seedling leaf primordia are initiated during maize embryogenesis at about 12-14 days after pollination [8] but, as shown by morphological analysis [9], they retain a different origin. The coleoptile arises as a ring of cells on the surface of the scutellum, whereas the first leaf is initiated at the basal face of the shoot apical meristem (SAM), where the coleoptilar ring closes, from the SAM cell population. This observation is also supported by the expression pattern analysis of different marker genes [11]. For instances the knotted gene is specifically expressed early in development on the anterior side of the embryo in two groups of cells that will give rise to shoot and root meristems, whereas its expression has not been found in the coleoptile founder cells that are visible in the scutellum [12]. Another example is the ZmWOX3A/B, the expression of which is specifically confined to a ring of peripheral cells, which marks the recruitment of cells to establish firm the P_0 primordium [13].

In maize, the coleoptile is the first organ that is produced when seed germination occurs. It appears as a cone shaped structure that elongates and pierces through the soil, enclosing and thus protecting the young leaves and the shoot apex, which comprises the shoot apical meristem (SAM), until they reach the above ground level. In this initial phase, first leaf elongation keeps pace with that of the coleoptile. Later on the coleoptile elongates and opens at its apex. The first leaf continues to grow and emerges from a coleoptile gap that is initiated at the top. The second and following leaves appear subsequently in a sequential manner, soon after the coleoptile dies.

Among the different aspects affecting seedling development, the two that will be analyzed in this chapter also play an important role also in the interplay with the

CHAPTER 8

Natural Genetic Diversity and Crop Improvement

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Abstract: With the human population expected to exceed 9 billion by 2050, food production will need to increase significantly in the coming years. In particular the forecast doubling in cereal demands requires improvement of yields of the top four cereal crops, maize, rice, wheat and barley. This goal is made more challenging by global environmental changes and the connected abiotic and biotic stresses. In this chapter we briefly discuss two different breeding strategies to increase cereal yield: the heterosis approach in maize and the ideotype approach based on knowledge of the genes controlling yield components in rice. We further discuss the importance of crop genetic diversity in connection to studies of the domestication history of maize, rice, wheat and barley. We present examples of how crop genetic resources including landraces and wild relatives have been used in genetic improvement of yield and adaptation to biotic and abiotic stresses. More extensive deployment of such resources to face future challenges is now empowered by new genomic tools enabling efficient exploration of genetic variability and innovative approaches to direct collection and conservation of genetic resources for food and agriculture.

Keywords: Adaptation, Barley, Breeding, Conservation, Crop genetic resources, Genetic diversity, Grain yield, Maize, Rice, Wheat.

INTRODUCTION

Out of the estimated 298.000 plant species present on earth [1], only 353 are domesticated food crops [2]. Cereals are the primary source of daily energy for humans and animals (FAO, 2013; http://faostat.fao.org/site/345/default.aspx). The first four cereals at the global level are grasses of the Poaceae family, maize

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# These authors contributed equally to this work.

(*Zea mays*), wheat (*Triticum aestivum*), rice (*Oryza sativa*) and barley (*Hordeum vulgare*) with a total annual production of 2.4 billion tons (FAOSTAT, Fig. **1**). Maize, wheat and rice together are estimated to provide nearly half of the world's food production. The consumption of these four major cereals varies based on region; maize is mainly used in central America, Mexico and Southern and Eastern Africa, wheat is preferred in south and north America, central and west Asia, and Europe, while rice is widely used in Asia [3].

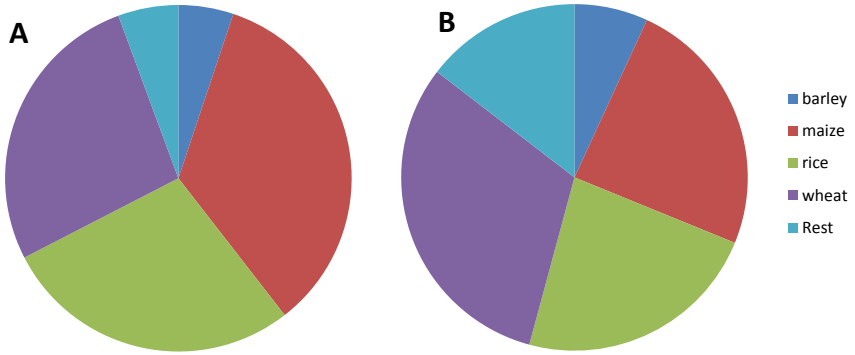

Fig. (1). Production (**A**) and areas harvested (**B**) of the four most important cereals in the world (FAOSTAT data http://faostat.fao.org/).

Maize

Maize is not cold tolerant, therefore it is cultivated only in temperate zones during spring or summer, it is also intolerant to water and nutrient deficiency. The top three maize-producing countries in the world are USA, China, and Brazil, producing ca. 563 of the 717 million tons/year [3]. Maize contains mainly starch that includes approximately 72% as well as 10% protein, and 4% fat, providing an energy density of 365 Kcal/100 g [4]. However, it has lower protein content compared to rice and wheat. In addition, maize provides essential minerals, many of the B vitamins, and fiber, while it is a modest source of calcium, iron and folate, and lacks vitamin B12 and vitamin C. Maize grains are mainly processed into flour and meal production for human uses and animal feed, and then for industrial products such as starch, oil, sweeteners, beverages, ethanol, and glue [3].

Genetically, maize is a diploid (2n=2x=20) and open-pollinated crop. Its genome was first completely sequenced in 2009 (inbred line B73). The maize genome is 2.3 Gb, 85% of which is composed of transposons and it harbors 32,540 genes [5].

Wheat

Wheat is an important staple food in both developing and developed countries worldwide. Bread wheat provides about 20% of total caloric requirement of humankind and is a primary source of protein, vitamins and minerals (World Agricultural Supply and Demand Estimates, WASDE, http://www.usda.gov/oce /commodity/wasde/). Wheat contains mainly carbohydrates, around 14% protein and 2.5% fat, supplying approximately an energy density of 327 kcal/100g. Besides being an important source of carbohydrates, wheat supplies ca. 20% of the protein for more than half of the world's population, and it also contains fiber, B vitamins, folic acid, magnesium, manganese, phosphorus and niacin, antioxidants and phytochemicals. Wheat can be used as human food in various ways such as bread, biscuits, pastry and cakes *etc*. Bran and straw are also used as animal feed. Moreover, it is sometimes used in preparation of paper, glue and some washing powders. The top three wheat-producing countries in the world are China, India and USA.

Genetically, bread wheat is an allopolyploid (2n=6x=42) and self-pollinated crop. Its genome was first sequenced with low coverage on a variety named Chinese Spring and published in 2012. The wheat genome is 17 Gb in size, with 94,000-96,000 predicted genes [6].

Rice

Rice is the major staple for about half of the human population [7]. Rice was traditionally grown in tropical and semitropical regions, and then it was rapidly dispersed in a wide range of environments.

The majority of production of rice is consumed as food for humans as a source of energy. The quality of rice protein is nutritionally ranked high among cereals, however its quantity is modest, the grain also contains vitamins, minerals, and fiber.

Genetically, rice is a diploid (2n=2x=12) and self-pollinated crop and it was the first crop whose genome was sequenced: draft genome sequences were first published in 2002 for both subspecies, japonica [8] and indica [9], and high quality complete genome was released in 2005 [10]. The rice genome is 389 Mb including 37,544 predicted genes [10].

Barley

Barley ranks in fourth position among cereals in terms of harvested area and production (http://faostat.fao.org). It is among the world's earliest domesticated

CHAPTER 9

Domestication of New Species

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Abstract: Domestication is the process through which a wild plant becomes a crop. The process is the result of the selection, either deliberate or as a byproduct of agricultural practices, of characteristics favorable to human beings. The sum of such characteristics is usually described as the 'domestication syndrome' because the types of traits selected are often shared among many different species. The most commonly selected traits are loss of seed dispersal, reduced seed dormancy, changes in growth habit, flowering time, and gigantism, all of which have an impact on morphology, reproductive strategies and, most importantly, production (yield and harvest index). Depending on the plant and its use, other traits could be selected, such as reduction or loss of toxic compounds, winter hardiness, nutritional quality, *etc*. Most domestication took place in ancient times, but there are a few examples of recent and accelerated domestication, for instance sugar beet. It is now possible to achieve the domestication of new species, based on the deliberate induction and combination of traits, using a set of approaches: classical plant breeding *via* hybridization and selection (including wide area crosses, hybrid seeds and plant cell culture), coupled with molecular tools such as Marker Assisted Selection, transgenesis, and site directed mutagenesis. Examples of interesting traits as well as candidate crops are discussed. Thus, we have the means to repeat the achievements of the early domestication wave and do even better, but this requires drastic changes in international and national regulations impacting on plant biotechnology and novel breeding techniques.

Keywords: Agriculture, Biofuel, Biomass, Biotechnology, Crop, Genetic engineering, Genetic resources, Genome, Harvest index, Marker assisted selection, Natural pesticides, Secondary metabolism, Seed dispersal, Seed dormancy.

INTRODUCTION

Agriculture has been an incredibly important invention in human history. The increased food production and reliability allowed the creation of stable settlements and denser societies. Agriculture does not rely on wild plants, but on

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plants which have been adapted to specific environments created by humans. Such 'domesticated' plants, usually called crops, present a set of specific characteristics which differentiate them from the wild counterparts. In order to replicate on new crops the achievements connected to the invention of agriculture, or to further improve existing crops, it is helpful to reflect on the nature of the domestication process and of its consequences on the organisms. Part of this chapter is devoted to these reflections, while the rest describes desirable traits, the methods to obtain them and suggestions of potentially interesting and promising crops.

1. WHAT IS DOMESTICATION? TRAITS OF THE DOMESTICATION SYNDROME

Domestication is the process driven by human selection by virtue of which a wild, spontaneous plant is turned into a crop. The concept applies also to animals, but these will not be dealt with in this chapter and we refer the reader to specific reviews [1 - 3]. The process of domestication implies the stable acquisition (and therefore the inheritance by the progeny) of a suite of traits, which are collectively defined as the 'domestication syndrome', which mark the difference between the crop and its wild ancestor(s) [4 - 7]. It is important to stress that the word syndrome (literally: running together) refers to "a group of symptoms that together are characteristic of a specific disorder, disease, or the like" (*Random House Kernerman Webster's College Dictionary)* and therefore domestication is seen as a complex illness from the point of view of the wild plant. When looked upon from the side of human beings, crops are marvelous organism on which we ultimately depend, or have depended, for most of our history, for food, feed, fiber, flower, fuel and fun (consider beverages such as wine, beer, tea or coffee, for instance).

Two Crucial Traits

These morphological and physiological differences are known since a long time, but the molecular details started to become available in the past two decades and several reviews appeared covering this subject [8 - 14]. For cereals, which represent the major and most studied group of crop plants because of their importance as a food/feed source, several genes have been identified and cloned [14]. The first crucial trait in the domestication of cereals and other grain crops is the loss of seed shattering (Fig. **1**), whose benefit is easily understood even by pre-school children, while the second most important trait is the reduction in seed dormancy.

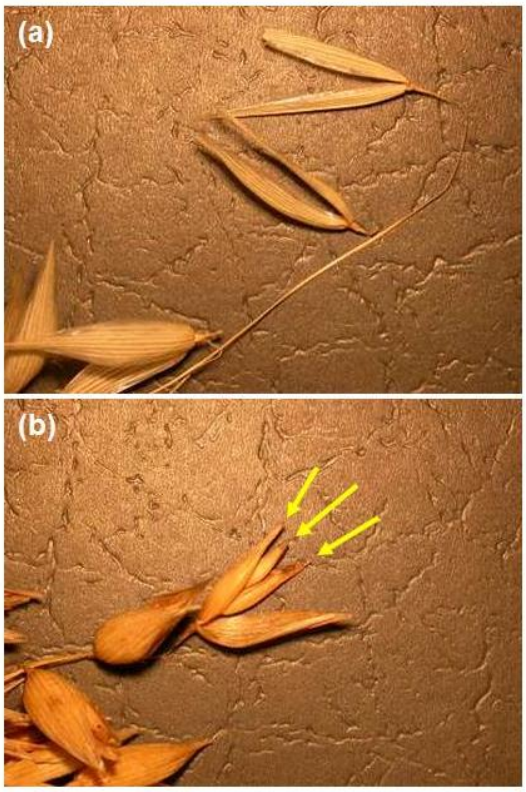

Fig. (1). Shattering behavior in wild and cultivated oat. (**a**) Wild oat spikelets at maturity are empty. (**b**) Cultivated oat spikelets retain seeds (highlighted by arrows) and only a vigorous treatment (threshing) is capable of releasing them.

The first mutations for shattering identified in molecular terms are qSH1 [15] and SH4 [16] in rice. It is remarkable that in several cases a single nucleotide change is responsible for the non-shattering trait. In the case of qSH1, the change localizes almost 12,000 bp upstream of the coding region and it is likely to affect the binding of transcription factors responsible for the activation of the gene in the pedicels, which is necessary to create the abscission layer [15]. In the case of SH4, the mutation hits a conserved Lysine residue in the DNA binding domain [16]. Interestingly, identical regulatory point mutations occurred in quite different plants [17]. Another gene (SHAT1 [18]), was identified as directly regulated by SH4 in rice and these two together influence the expression of qSH1, which, on turn, maintains SHAT1 and SH4 expression in the abscission zone. In sorghum, three independent mutations at the main shattering locus Sh1 [19] prevent seed dispersal. This gene is homologous to SH1, a minor rice QTL for shattering. In barley, the deletion of 1 or 11 bp in either of two tightly linked genes (Non-brittle

CHAPTER 10

Breeding for Drought Stress Resistance in Plants

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Abstract: Most cropping environments worldwide are suboptimal for plant growth and reproduction. Unfavorable environmental conditions prevent crops from attaining their full yield potential. Abiotic stresses including heat, cold, drought, salinity, and flooding have a major impact on world agriculture, reducing by 50% the average yield for most crop plants. Among these, water scarcity is the major factor limiting the expansion of agriculture and the single leading cause of crop losses worldwide. With the impact of climate change and demographic growth looming, stress-tolerant varieties and climate-resilient crops emerge as relevant and necessary targets to ensure global food security and to improve sustainability in agriculture. Breeding of crops with enhanced stresses tolerance has been particularly compelling, as the related agronomic traits are complex and quantitative in nature, often associated to several loci exhibiting additive effects. Advances in plant genomics have greatly contributed to dissect such complex traits, unraveling the mechanisms underlying the plant response to stress, and opening unprecedented avenues in breeding improved varieties against unfavorable environmental conditions. This chapter will focus on recent successful stories in molecular breeding and biotechnological strategies for crop improvement against abiotic stress, with particular emphasis on drought tolerance.

Keywords: Climate change, Crop biotechnology, Crop breeding, Crop yield, Drought, Drought-resistance, Food security.

INTRODUCTION

Plant growth and productivity are constantly challenged by stressful conditions originating from the interaction with pathogenic insects and microorganisms (biotic stress) and from the physical environment (abiotic stress). Altogether abiotic factors, including drought, salinity, heat, cold, flood and heavy metals, account for up to 80% of annual yield losses in agriculture [1]. Among them, drought is thus far the single leading cause of crop losses worldwide [2]. Nearly 40% of the world's land surface experience precipitation that is largely insufficient for agricultural practices. Areas subjected to predictable 'dry seasons',

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as many developing regions in Africa and Asia, can suffer annual yield loss up to 50% or more [3]. Importantly, high yielding cropping environments can also endure periods of water scarcity of variable duration. According to the "US Department of Agriculture" (USDA), the drought encountered in the Corn Belt region in the summer of 2012, reduced maize yields of 21% compared with the 2009-2011 mean levels (http://quickstats.nass.usda.gov/). Ongoing climatic changes are expected to further limit the availability of water for agricultural needs. Global warming will enhance evapotranspiration losses and decrease rainfall in many agricultural contexts, severely affecting staple crops that are essential to food-security [4, 5]. The increased frequency of concurrent dry and warm conditions will also threat food production in the most developed regions of the planet, as demonstrated by the acute environmental, agricultural and economic impacts associated with the 2012–2014 drought in California [6].

In this scenario, access to irrigation is becoming increasingly important to sustain crop productivity and food production. In the period 1998-2002, irrigated cereals yielded 442 Mg km^{-2} on average, whereas rainfed cereals only attained 266 Mg km^{-2} [7]. Data indicate that "without irrigation, global cereal production on irrigated land is anticipated to decrease by 47%, corresponding to a 20% loss of total cereal production" [7]. Currently, agriculture uses over 70% of the total human consumption of fresh water, with an increase of over 6-fold in the past 100 years [8]. Following this pattern, agricultural demands for fresh water are predicted to double by 2030 [9]. Yet, sustainability issues question any additional expansion of crop irrigation. Globally, we experience severe shortages of freshwater, lowering of underground water reservoirs and competition for dwindling resources [10]. Further, with increasing irrigation comes the threat of salinization of field soil, a problem that is already affecting crop productivity in many regions of the world [11].

Under rainfed conditions, the adoption of suitable water management strategies can significantly improve agricultural yields, especially where water limitations seem to be strongest. For instance, the introduction of relatively inexpensive small-scale water harvesting methods (*i.e.* terracing, dams and ditches), together with conservation tillage in several parts of Africa, has increased yield stability and raised plant productivity from 1 ton per hectare to 3–4 tons [12]. Improved management coupled with a better deployment of existing crop varieties is expected to help in closing yield gaps across many developing regions of the planet. Concurrently, the continued development of high yielding/stress-tolerant varieties and of climate resilient crops is an urgent and essential target to reduce crop losses and increase potential yields into the future. Indeed, breeding approaches to select crop plants with enhanced yield under stress have thus far met with limited success. This is in part due to the quantitative nature of tolerant

traits, with low heritability and significant genotype-by-environment (G×E) interaction [13]. Additionally, breeding for drought resistance is hindered by the plant phenology (*e.g.* reproductive phases are generally more sensitive to drought compared with vegetative development) and by the simultaneous exposure of the plant to concurrent stress conditions (*e.g.* drought often occurs in conjunction with high temperatures and high irradiance) [14].

This chapter will critically address the main questions that challenge the breeding of stress-tolerant traits in crops, while providing examples of success stories in delivering drought-resistant varieties to farmers worldwide.

WHAT IS "DROUGHT"? WHAT IS "DROUGHT RESISTANCE"?

The definitions of "drought" and "drought resistance" are not necessarily univocal. The perception of drought varies substantially among agronomists, meteorologists, hydrologists and economists (see Table **1** for definition of drought and drought-related traits). What is relevant in a cropping environment is the amount of moisture present in the soil, readily accessible to plants at a particular developmental stage. Drought develops when soil moisture is insufficient to cope with the requirement of the crop. In natural environments, drought resistance is essentially associated with the ability of plants to survive dry conditions, regardless of their capability to bear fruits and seeds. Clearly, plant survival *per se* is a trait of little value in an agricultural context. Rather, drought resistance in crops must be considered in terms of **yield** in relation to a limiting water supply.

Even brief periods of moderate drought can significantly affect crop yields, as most land plants, crops persistently lose water from their surfaces due to disequilibrium with the atmosphere. Under fully hydrated conditions, plant water potential (Ψw) for mesophyte vegetation, ranges from -0:3 to -0:5 MPa [15]. Only at very high relative humidity (RH=99.6%) the air water potential (Ψwv=-0.54 MPa) is in equilibrium with plant tissues. Minor reductions in RH result in significant decreases in Ψwv. Even at 99% RH, Ψwv is far below equilibrium, reaching -1:36 MPa. At 90% RH, Ψwv matches-14:2 MPa, whereas at RH below 50% it drops to less than -93:6 MPa [16]. As evident, even under the most favorable cropping conditions plants are constantly exposed to an exceptionally water-demanding environment. When this demand is not met by adequate supply from the soil, plants undergo the net loss of water to the atmosphere with the consequent decline of Ψw and relative water contents (RWC). Under these conditions, the closure of the stomatal pores drastically reduces transpiration and CO_2 uptake and growth is rapidly inhibited. If the water deficit persists, plants experience premature leaf senescence, wilting, desiccation and ultimately death [17].

CHAPTER 11

Genetic Strategies to Improve Resistance to Biotic Stresses in Plants

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[2] *Fondazione Istituto Tecnico Superiore per le nuove Tecnologie della Vita, Bergamo, Italy*

Abstract: The long-term target of improving crops resistance to biotic stresses is a familiar goal for breeders. Plants ought to constantly protect themselves *versus* aggressions from a wide spectrum of organisms that include viruses, bacteria, oomycetes, fungi, insects and other herbivores, and weeds. In this chapter attention will be given to depict a picture on the genetic and molecular mechanisms that plants have promoted to recognize and react to invasion by numerous parasites (pathogens and pests). These topics include non-host resistance, constitutive barriers, and race-specific resistance. The chapter also examines current progresses in clarifying the structure and molecular devices developed by plants to neutralize pathogen and pest aggressions. Moreover, it takes a look with aspects experienced in breeding for resistance to relevant biotic stress factors. Major considerations in breeding for resistance to pathogens, insect pests, and weeds, traditional sources of resistance or other possible strategies, such as mutation breeding, genetic manipulations, and molecular strategies to develop crops more resistant to parasites are also explored.

Keywords: Defense mechanisms, Genetic basis of resistance, Pathogenesis related proteins, Signal transduction network, Transgenic plants.

INTRODUCTION

Biotic stresses, the damage caused by plant pathogen, insect, and weed pests, have a negative impact on productivity of our crops by reducing yield and quality [1]. It is estimated that 35% of crop production, on a global scale, is annually lost to pre-harvest biotic stresses, with an additional 6 to 20% of losses due to post-harvest events [2]. A survey of the potential and actual yield injuries attributable to biotic constraints in important crop plants is shown in Table **1**. This information indicate that there is remarkable deficit between potential and realized crop productions.

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Table 1. Survey of deficit between potential and realized crop yields due to fungal and bacterial pathogens, viruses, animal pests, and weeds including the efficacy of the used pest control activities in various crops (*e.g.* maize, wheat, rice, barley, potatoes, soybean, sugar beet, and cotton). Modified from Oerke and Dehne [3].

	Pests and pathogens				
	Fungi and bacteria	**Viruses**	**Animal pests**	**Weeds**	**Total**
Loss potential(%)[a]	14.9	3.1	17.6	31.8	67.4
Actual losses (%)[a]	9.9	2.7	10.1	9.4	32.0
Efficacy(%)[b]	33.8	12.9	42.4	70.6	52.5

[a] As percentage of attainable yields; [b]As a percentage of potential prevented yields loss

Plant protection has a cardinal function in assuring crop yield performances against plant pathogens, animal pests, and weeds. This relationship is illustrated by a 15–20-fold annual increase in the volume of chemicals (pesticides) employed on a global scale [3]. However, plants are able to counteract parasite damages by several genetically inherited mechanisms, acting at the morphological, physiological, biochemical, cellular, and molecular levels. Therefore, the introduction of genetic resistance or tolerance into plants to the plethora of biotic stresses that severely damage our crops, is an important goal for scientists. Besides, this strategy has showed up relevant consequences for both growers and the seed and agrochemical industrial sectors [4]. Notably, genetically resistant or tolerant crops able to neutralize pest attacks have various benefits over the employment of pesticides or additional procedures to manage biotic stresses. These advantages are reflected by the following arguments: i) economic savings of the costs of pesticide treatments, ii) seed of resistant varieties generally costs to growers no more that the susceptible varieties, iii) although the resistance does not fully protect the crop, partial resistance may conduct to a sizeable decrease in the amount of pesticides needed to provide a tolerable control. Evidence suggests that these benefits depend on simple genetic stability, insignificant expenses after varieties are produced, and a remarkable effectiveness. The principal drawback of genetic resistance in plants to neutralize biotic stress factors is due to the issue that selection pressure is focused on parasite populations: the development of individuals with inherited mechanisms to breakdown plant resistance are favored within these populations. Thereby, it is obvious that this occurrence is restricting the temporal length of resistance performance in crops.

Typically, microbial organisms causing diseases are referred as pathogens, and herbivorous insects, mammals, and birds are termed pests. In this chapter we take a close look at the importance of genetic, biochemical, and molecular processes by which plants protect themselves from diseases and damages caused by plant

pathogens, insects, and weed pests. Additionally, breeding strategies devoted to the development of tolerant or resistant plants are also highlighted.

PLANT PATHOGENS

The many organisms that cause infectious diseases and damages in plants include fungi, oomycetes, bacteria, viruses, and nematodes. A comprehensive description of individual diseases and the methods used in their control is outside the objectives of this chapter. A number of books and reviews have been written in this field to which the reader is addressed for a more detailed illustration [5]. For the sake of brevity, specific pathogens or the diseases and damages they cause are herein mentioned without further explanation.

The main findings emerging from those publications that may worth noting indicate that:

i. Several pathogens are specialized to growth on a specific plant species and cannot strike and produce disease in other plants. Others can devastate numerous, frequently unrelated, plant species. To exploit a distinct species as nutriment, a pathogen must be competent to defeat the species defense systems. Nearly all plant species are resistant to the majority of pathogens.
ii. Pathogens can enter into plants through several routes, such as direct penetration *via* intact surfaces, entry *via* natural opening (*e.g.* stomata), or *via* opportunist entry represented by existing wounds or cracks on the plant surface.
iii. After the pathogens have entered into the plant, three major colonization tactics are used by these organisms to take advantage of the host plant as a nutritional substrate for their growth and development. Essentially, either these organisms parasitize the vital plant to pick up nourishments (biotrophic lifestyle) or they destroy the plant tissues that are infected and use up nutrients from the non-longer alive tissues (necrotrophic lifestyle). Hemibiotrophs embrace both lifestyles, shifting from a biotrophic stage at the starting of the infection to a necrotrophic lifestyle as pathogenesis advances.
iv. Pathogenesis describes the series of phases concerning host and pathogen interaction (*e.g.* infection, colonization and plant pathogen reproduction) to the progress of the whole syndrome.
v. Recent evidence indicates that fungal pathogen employed sex pheromone receptors for perceiving chemotropically host plant signals in an intricate environment medium like the soil [6].
vi. A pathogen race that induces disease is named virulent. Its favorable outcome may depend from different elements that include: i) very quick and elevated rate of reproduction throughout the central growing season for plants; ii) high performance dispersal system and long-standing survival ability; (iii) great

CHAPTER 12

Harnessing Apomixis to Improve Crops

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Abstract: Apomixis is a naturally occurring reproduction mode in flowering plants that results in embryo formation in absence of both meiosis and eggcell fertilization. Apomixis results from the combination of two processes: apomeiosis (unreduced embryosac formation due to diplospory or apospory) and parthenogenesis (development of an embryo without fertilization). Seed-derived progenies of an apomictic plant are genetically identical to the maternal parent, *i.e.*, they are clonal in origin. The impact on agriculture of the introgression of apomixis into sexual crops, will be revolutionary. In fact, apomixis will allow clonal seed production and thus enable efficient and consistent yields of high quality seeds, fruits and vegetables at lower costs. The development of apomixis technology will reduce cost and breeding time also avoiding the complications typical of sexual reproduction (*e.g.,* incompatibility barriers) and vegetative propagation (*e.g.,* viral transfer). Progresses in the search for apomixis genes obtained by several groups could allow the manipulation of apomixis and its transfer to crop species where the apomixis system is not present and to revolutionize modern agriculture. Moreover, when coupled with male-sterility systems, apomictic reproduction (with no need for male contribution) could help in addressing issues related to transgene escape from GM crops to organic or conventional crops, and thereby allow for better coexistence systems.

Keywords: Apomeiosis, Apomixis, Asexual reproduction, Fertilization, Hybrid vigor, Inbreeding depression, Meiosis, Parthenogenesis, Plant reproduction, Sexual reproduction.

APOMIXIS IN PLANTS

In modern agriculture the tremendous yield increase achieved by coupling high-yield varieties with high-input agronomic systems (Green revolution) has been one of the major successes. Plant breeders are now trying to extend this by intensifying the selection, develop more hybrids in greater number of crops, increase the range of plant functions through mutagenesis and advanced genetic

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improvement. Therefore, breeding will continue to play a crucial role in crop improvement because the needs are varied, the techniques are continuously expanding, the possible new genetic combinations are limitless and the successes achieved in the past are a good omen for future.

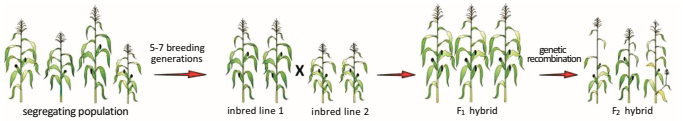
The problem for cross-pollinated species is that alleles segregate in progenies and, therefore, the optimal genotype is lost together with the phenotypic evidence of the trait of interest (Fig. **1A**). In nature, a trait, termed apomixis, allows the clonal propagation of a plant through seeds by avoiding the process of genetic recombination connected to meiosis and fertilization.

Apomixis could represent a must in modern agriculture but its introgression from wild relatives in crop species and transformation of sexual genotypes in apomictic genotypes were in long persued by plant breeding (Fig. **1**). The impact of apomictic crops in agriculture would be enormous; it is estimated, for example, that the hybrid rice production could lead to economic benefits in excess of 1.8 billion Euros per year [1, 2]. If applied to clonally propagated crops apomixis could allow the production of disease-free material more easily storable and transportable [3]. Apomixis could make the true potato seed an attractive option for plant breeders and farmers with possible economic benefits estimated up to 2.3 billion Euros per year [1]. But, so far, the exploitation of apomixis has been unsuccessfull in all major agricultural crops where it was attemped and only some features of it have been engineered in model species.

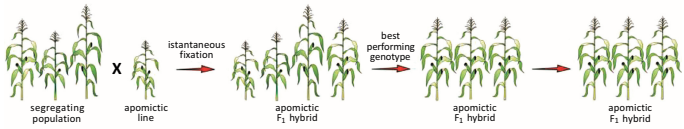
Moreover, apomixis could produce extremely rapid breeding results responsive to specific micro-environments, cropping conditions and markets. This would stimulate diverse strategies of breeding aimed at more sustainable crop management with deep implications in modern agricultural research systems. In simple terms the technique could also facilitate the farmers in avoiding the need to buy seed each year thus reducing the production costs. This will also have positive economic impact for consumers due to the decrease of the expenses for the food supply purchase.

The development of apomixis-based technology will require a deep understanding of the genetic control of the trait. Our molecular understanding of apomixis would be greatly increased if it was possible to identify the genes differentially expressed during the development of embryo and embryo sac. Chaudhury and Peacock [4] hypothesized that genes isolated in model species such as *Arabidopsis thaliana* would be important for the study of apomixis. Analysis of mutations in plants belonging to sexually reproducing species that resulted in phenotypes displaying one or more components of apomixis led to the isolation of a number of candidate genes (reviewed in Bicknell and Catanach 2014) [5]. The

major result obtained by this approach has been the creation of a sort of synthetic form of apomixis. In fact several mutations were identified and combined into two Arabidopsis lines, which, when crossed, gave rise to a significant proportion of strictly maternal origin [6 - 8].



(A) traditional breeding for hybrid production



(B) Exploiting apomixis in hybrid production

(C) seed production in traditional breeding

(D) seed production using apomixis

Fig. (1). Conventional *vs* apomixis-mediated breeding as reported in Barcaccia and Albertini [3]. Considering, for example, the conventional maize breeding, the best plants are selected within a segregating population, normally a F_2, and after several generations of selfing and phenotypic selection, their progenies are evaluated for the specific combining ability to identify those which can be used as parental inbred lines in the synthesis of F_1 hybrid varieties able to show heterosis. The best inbred lines are then maintained, multiplied in isolation and crossed each other in pairs in order to give uniform F_1 hybrids vigorous and highly productive. This breeding scheme, however, requires a complex procedure to obtain hybrid seed: the two inbred lines must be kept in isolation in separate fields; then, in order to produce hybrid seed, it is necessary to organize seed production fields where about a quarter of the plants are used as pollinators (*i.e.*, pollen donor inbred) while the F_1 hybrid seed is collected on the seed-bearing plants (*i.e.* maternal inbred) which covers only three quarters of the surface.. Farmers cannot reuse the seeds produced by F_1 hybrids that would give rise to crops characterized by high variability due to recombination and segregation. Using apomictic imbred lines, this problem would not arise. After the best inbred lines to be used as maternal plants are selected, these could be crossed with pollen donor clonal lines carrying the gene for apomixis; this will lead to hybrid seeds characterized by highly heterozygous genotypes. From this moment on, each hybrid F_1 could be maintained as such for many generations in a state of fixed heterosis.

CHAPTER 13

Molecular-Assisted Breeding

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Abstract: The steadily increasing world population and the concurrent reduction in cultivated lands are two major threats to food security, especially in the under-developed countries. Among the many strategies that can mitigate this situation modern biotechnologies play a central role. Many tools are available to scientists to face the challenges of increased production needs and sustainable agriculture. Among these, genetic modification and molecular breeding appear to be the most promising. Both of these approaches require the combined use of technologies such as genome sequencing, genotyping and phenotyping and large-scale data analysis and mining to determine genes and functions amenable to manipulation in the target species. Molecular markers, used in both target gene studies and assisted breeding, represent a powerful tool. This chapter deals with their nature and applications, and also describes some case studies where their use in marker-assisted selection has positively affected some target crop species.

Keywords: Breeding, Cassava, Database, Food security, Genetics, Genome-wide association studies (GWAS), Genomic selection, Genomics, Grapevine, Manihot, Mapping, Marker-assisted selection (MAS), Molecular markers, Nutrition, Pathogen resistance, Polymerase chain reaction (PCR), Quantitative Trait locus (QTL), Tomato, World wide web (WWW).

THE ROLE OF MOLECULAR MARKERS IN MODERN GENETICS AND BREEDING

According to the UN, the world population will be more than 9 billion by 2050 and over 11 billion within the next century. That means that farmers around the world will have to produce increasing amounts of nutrient-rich foods. During a high-level Expert Forum in Rome, held in 2009, the Food and Agriculture Organization of the United Nations (FAO) discussed food needs around the planet. The Forum estimated that to sustain over 9 billion people in 2050 would

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require increasing overall food production by some 70 percent. Production in the developing countries would almost need to double [1 - 5].

Modern biotechnologies are expected to play a steadily growing role in this scenario as the extent of cultivated land will be reduced by the demands of the expanding world population.

In the biotechnological toolbox, molecular markers and marker-assisted breeding (MAB) represent a sector of great potential applied to plants and animals that can improve characters of interest (productivity, resistance to abiotic and biotic stress), through the use of morphological, biochemical and genetic markers and by exploiting the natural potential of recombination. Often these characters are determined by areas of the genome called QTL (quantitative trait loci) whose mapping and characterization represents a major obstacle in the MAB approach. This technique is different from transgenesis, that leads to the production of genetically modified organisms (GMOs). Unlike transgenesis, the final products of marker assisted selection (MAS) are not obtained through genome manipulation and the insertion of foreign pieces of DNA into the species in question, but they derive from a guided selection of naturally occurring recombinations between individuals bearing contrasting characters. Therefore, marker-assisted selection is a technologically-improved version of traditional breeding in both plants and animals.

The availability of increasingly sophisticated and powerful equipment allows us to generate data in increasing quantities and at steadily decreasing costs.

The activity of developing new varieties can last several years. However, today's biotechnological tools have significantly shortened the time to six to ten years for new varieties of crops to get to the market. One of the tools that can make it fast and easy for scientists to select the desired traits is marker-assisted selection or breeding. Some features, such as the color of flowers or kernels, can be determined by a single gene. More complex features, however, such as starch content, crop yield, plant height or drought resistance can be controlled by many genes. Producers have traditionally selected plant species according to their visible or measurable characteristics, known as the phenotype. Such process is difficult, slow to undertake, is affected by the environment and expensive, not only in the development phase, but also uneconomical, as farmers, in the meantime, continue to suffer crop losses.

The molecular markers are identifiable genetic sequences which mark the DNA sequence of the trait under study and are passed on following the laws of inheritance at each generation. As markers are close to genes on the same chromosome, they normally move together, at each new generation (genetic

linkage). This connection can be used to predict whether an individual will carry the desired gene. If it is possible to find a marker or markers for the gene, it means that the desired gene itself is probably present. The distance of a marker from its target gene is important to determine its usefulness and to predict the final phenotype as a result of a genetic (marker) state. The ultimate marker, when the physical correlation between marker and genetic trait is total, is represented by the gene itself.

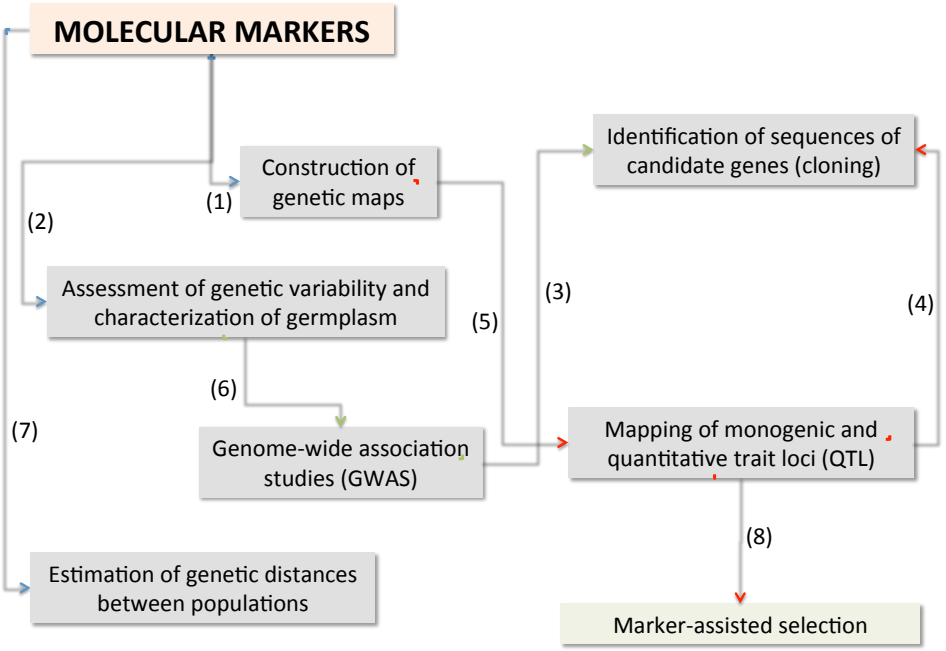

Fig. (1). The main applications of molecular markers in genetic studies and breeding. Blue arrows show the direct applications of molecular markers while red and green arrows mark the downstream logical relations between upper-level applications. For example "Construction of genetic maps" is a prerequisite to "Mapping of monogenic or polygenic (QTL) loci" (link 5). In turn, the latter is a prerequisite to "Identification of sequences of candidate genes (cloning)" (link 4) and to "Marker-assisted selection (link 8). An alternative route to "Identification of sequences of candidate genes (cloning)" passes through "Assessment of genetic variability and characterization of germplasm" and "Genome-wide association studies (GWAS)" (link 6).

Once markers locations on chromosomes and their distance from specific genes are known, it is possible to make a genetic linkage map. In this way it is possible to produce detailed maps in only one generation of plant crossing. The various direct and indirect applications that molecular markers have for genetic studies and breeding are summarized in Fig. (**1**). However, it is important to note that breeding by MAS has some limitations compared to genetic engineering, for the following reasons: 1) its efficacy is restricted to traits already present in a crop; 2) its advantages are limited when breeding species with long generation times (*e.g.*

CHAPTER 14

Genetic Engineering for Crop Yield

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Abstract: By genetic engineering, one or a few genes are typically introduced into plants, so major direct improvements on complex quantitative traits such as yield are not easily realized. However, yield can be, and has been indirectly increased by reducing the gap between potential and actual crop yield, introducing resistance to diseases, pests, abiotic stresses, or herbicides, alone or combined. New traits are appearing on the market and many others are in the pipeline, which promise to contribute to global food production, such as tolerance to drought, to acid or saline soil, and nutrient use efficiency. The possibility to boost the photosynthetic potential by genetic engineering is also attractive. In this chapter, after an brief presentation of plant engineering techniques, I provide an overview of the actual contribution and potentialities of genetic engineering for enhancing crop yield.

Keywords: Acid soils, Agriculture, Biotechnology, Crop yield, Drought, Genetic engineering, GMO, Herbicide tolerance, Insect resistance, Photosynthesis, Salinity, Transformation, Transgenic plants, Virus resistance.

INTRODUCTION

Genetic engineering (GE) is the modification of the genome of an organism by means of the recombinant DNA techniques. Besides introducing new genes, it is possible to shut off the expression of an endogenous gene (gene silencing), by introducing sequences that interfere with its expression.

Transferring genes between species is regarded by many as potentially dangerous, but routine use of GE microorganisms to produce drugs and vaccines and almost 20 years of large scale cultivation of GE plants (more than 180 million hectares in 2014) [1] have demonstrated that GE does not pose intrinsic risks [2].

Yield is a complex, quantitative trait affected by many genes, each gene exerting a small effect. Since GE manipulates one or a few genes at a time, large direct

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improvements of yield are difficult to realize. Progress in the dissection of quantitative traits by QTL mapping and cloning will certainly bear fruit (for example [3],) and the molecular dissection of key developmental steps may open ways to manipulate plant architecture and productive potential (for example [4],). In particular, the elucidation of the role of transcription factors in regulating plant growth and development may open the way to direct improvement of yield. For example, the expression of a truncated *Arabidopsis thaliana* HD-Zip transcription factor (ATHB17) in maize results in increased ear biomass at the early reproductive stage, which can brought about yield improvements [5]. Until now, however, the impact of plant GE on yield has been an indirect one.

The *potential yield* of a crop is the yield that can be obtained under optimal agronomic, environmental and management condition. Potential yield can be increased by breeding more efficient genotypes and by improving agricultural practices. *Actual yield* is what a crop actually produces, and can be increased both by increasing potential yield and, often more effectively, by reducing yield losses caused by biotic and abiotic stresses. So far, GE has been successful in making actual yield closer to potential yield, that is, in filling the "yield gap", by reducing the negative effects of weeds, insect pests and viruses; recently, drought tolerance has been tackled by GE with some success (see below). Acid or saline soil stress are major limiting factors for agriculture worldwide because large agricultural land areas are affected by these problems in many parts of the world [6]; GE holds the promise to make these soils capable to sustain crop production. Nutrient use efficiency is another key factor directly affecting yields and sustainability of agriculture [7].

It is important to consider that food production is strictly connected with environmental sustainability: only if agriculture is sustainable it will continue to produce food in the long term. Disease and stress resistance, efficiency in the use of water and nutrients can make crops more sustainable. In this chapter, I will show how GE has already, and will continue to play a positive role to increase food production and agricultural sustainability.

THE PLANT GENETIC ENGINEERING TECHNOLOGY IN BRIEF

When a breeding objective cannot be easily pursued with classic crossing and selection, it is possible to recur to GE, which allows to source genes from any organism, or even to use artificially synthesized genes.

Genetic Constructs

The genes that are to be introduced in a crop plant must be designed for optimal expression in the host organism by choosing suitable control sequences (promoter,

terminator, targeting sequences) which must be assembled in the lab into a functional gene construct (Fig. **1**). The elements of the construct are briefly described.

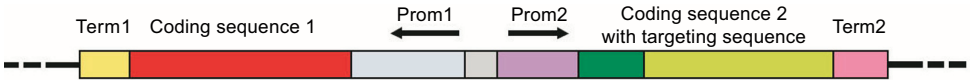

Fig. (1). Schematic representation of a typical genetic construct for plant transformation. Here, two genes were cloned so that their transcription is divergent.

Coding sequence. A gene coding sequence spans from the start to the stop codon. Eucariotic genes are usually cloned from their cDNAs, thus excluding introns from genetic constructs. An important factor for optimal gene expression is codon choice, or codon usage. Up to six different codons specify a single aminoacid but generally one of them is preferred by each species, which can be different in different species. If in the introduced gene the preferred codon for a given aminoacid is scarcely used in the host organism, the expression of the introduced gene could be inefficient, due to scarcity of the tRNA for that codon. The problem can be fixed by replacing the codons of the gene with those preferred by the host. Codon optimization has been shown to increase gene expression in plants in many cases, and is provided by several companies that synthesize genes.

Targeting sequences. These are portions of the proteins, usually the amino terminus, that codes for peptides that allow for the transport of the protein to the correct cell compartment. If, for example, a bacterial protein must be targeted to the plastid, a DNA sequence encoding a chloroplast transit peptide should be added to the 5' of the coding sequence. When the protein reaches its destination, the targeting sequence is removed by proteases.

Promoter and terminator. The promoter is the key component of a genetic construct, because it regulates the timing, the site and the rate of transcription. The promoters is located upstream (5') of the coding sequence and contains the sequences recognized by transcription factors, proteins that control binding and activation of RNA polymerase. Promoters vary widely in length, generally from a few hundred to a few thousands bp. A wealth of information is available on gene sequence and expression, allowing for a fine regulation of transgene expression by choosing a promoter with the desired characteristics. Constitutive promoters are preferred when a high transcription level is desired in all cells. Among these, the promoter of the 35S RNA gene of the cauliflower mosaic virus (*CaMV35S*) is by far the most popular. Other plant-derived constitutive promoters are taken from actin or ubiquitin genes. Promoters of storage protein genes such as glutelin (from cereals) or napin (oilseed rape) or patatin (potato tuber) are useful to accumulate the gene products in harvested organs. In other cases, fine, tissue specific gene

CHAPTER 15

Plant Breeding and Next Generation Sequencing (NGS)

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Abstract: Next generation sequencing (NGS) refers to a set of technologies based on massive parallel sequencing. NGS technologies can be included in three main groups: sequencing by synthesis, by ligation, and single-molecule sequencing. NGS technologies are improving at a fast pace and the cost of sequencing per base is decreasing, thus allowing to use NGS to approach a variety of biological questions as impossible before. In plants, tools based on genome decoding, building on NGS, contribute to increase the rate of genetic gain during selection and the precision when choosing superior genotypes. The tools used to facilitate the process leading to genomes drafts is described. The chapter also presents new methods, such as genomic selection or genome-wide association mapping, which are based on NGS to disclose an unprecedented amount of genetic variability made available to plant breeding. In addition, the use of NGS to decode the epigenome and the transcriptome is reported, as well as its power, when combined with appropriate genetic designs, to map and clone quantitative trait loci. It is out of scope of this review to provide a comprehensive repertory of sequencing efforts and techniques that are rapidly evolving and quickly becoming obsolete. The paper, moreover, does not provide a comprehensive list of all the too numerous experiments conducted in the field, but rather it describes rationales and examples of possible applications of plant genome sequencing.

Keywords: Breeding, Breeding by genotyping, Crop improvement, Genome sequencing, Genome wide association studies, Genomic selection, Next generation sequencing, Plant breeding, Pyrosequencing, QTL-seq, RNA-seq.

SEQUENCING TECHNIQUES

In the past few years, sequencing techniques witnessed an impressive increase of technical advancement. Improvement in sequencing contributed to the so-called "next generation sequencing" (NGS) protocols based in most cases on massive

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parallel sequencing of single isolated DNA molecules. Technological advances are mirrored by a diminishing cost per base sequenced: the first human genome was fully sequenced in 2003 at the cost of USD 300 million, and involving dozens of corporations and institutions. In 2007, the genome of two persons (C. Venter and J. Watson) was sequenced at USD 1 million each. A year later, the genome of a human female was sequenced in 1 month at roughly USD 60,000. The target, also valid for plant genomes, is to cut every 1.9 years [1] half of the cost of sequencing of a single DNA base pair.

The output of the sequencing efforts are DNA reads, which are available in different formats:

1. Twenty-250 bp sequences, ready to be attributed to a single location in the genome;
2. Sequences originating from ends of DNA templates that have been circularized so that distant ends are physically ligated and read together ("mate-paired" fragments);
3. Paired-end, when the sequences originate from each end of a DNA template.

One of the current limits of NGS is the reading length. This is hindering the possibility of an accurate assembling the highly repetitive plant genomes. Sanger sequencing takes care of small repeats, at least of those shorter than the 700 bp. Moreover, sequencing of clones ranging in size from two to 150 kb covers even long repeats, also common in plants. Methods to capture more structural information useful in genome assembling using NGS technologies are being developed. In the following section, we will shortly review sequencing techniques. For more detailed reviews the readers may consult [2 - 4].

Sanger Sequencing

The Sanger technique has been used for more than 30 years, including its adoption in the sequencing of the first decoded plant genomes. The protocol is based on the cloning of DNA fragments in bacterial vectors (such as bacterial artificial chromosomes, BAC) and, following an amplification step, on direct sequencing of shorter fragments. Dideoxynucleotides (ddNTPs) are mixed with DNA resident dNTPs. ddNTPs contain a hydrogen group on the 3'-carbon, instead of a hydroxyl group. The incorporated ddNTP prevents the addition of further nucleotides and the DNA chain terminates. ddNTP can be labeled with different fluorescent dyes, thus allowing the detection of all terminated sequence products through electrophoresis based on size separation. The reactions need to be separated by size of products using electrophoresis, which can be done on capillary sequencers.

This protocol produces high quality read length of more than 1 kb [5]. The drawbacks of the technique include the laborious separation step and the need to prepare clonal populations of DNA in *E. coli*, a procedure that is costly and not adequate for large-scale operations.

The Arabidopsis [6] and the rice [7] genomes, completed respectively in 2000 and 2004, were produced using the Sanger sequencing approach.

Next Generation Sequencing (NGS)

The term next generation sequencing (NGS) relates to technologies other than the Sanger one. NGS used at first a massive parallel sequencing where hundreds of millions of reactions are detected per instrument run. The protocol eliminates the need for the bacterial cloning step while introducing the amplification of single DNA molecules and their subsequent massive and parallel analysis. In a second generation of NGS, millions of single-stranded DNA molecules are immobilized on a solid surface such as a glass slide or beads. In the third generation, the length of DNA fragments to be sequenced is much larger and the running time and costs are reduced due to multiplexing. More in details, the second generation requires the amplification of the template molecules prior to sequencing, while the third generation relies on sequencing directly molecules of DNA extracted from plant tissues.

Currently, six NGS technologies are in use and a seventh is in development. Most platforms require a short template DNA (<1000 bp) and each template contains forward and reverse primer binding sites (*i.e.* a library of templates is needed) [8]. Steps in common to all NGS platforms are DNA shearing, ends polishing, and custom DNA linkers (adapters) ligation. The fragments of the library are then amplified on a solid support that provides DNA sequences hybridizing to the library adapters. The amplification step can occur either by emulsion PCR, *in situ* polonies or bridge PCR [2]. The PCR steps introduce several potential risks of inaccuracy: PCR fragments can be preferentially amplified, or false positive artifacts derive from polymerase errors. Artifacts due to unbalanced G/C content are particularly pronounced in the case of bridge amplification.

The detection of the nucleotide incorporated by each amplified library fragment set occurs step-by-step and is mediated by a polymerase or a ligase. The reaction is monitored by image acquisition, typically of fluorescence signals.

Based on the type of molecular representation, NGS technologies can be divided in two groups:

1. Amplification-dependent. Needs the amplification of the target by PCR;

CHAPTER 16

Genome Editing in Crop Species

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Abstract: The possibility to feed an increasing world population will largely depend on the capacity to increase yield and the nutritional value of crops. Breeding has played a pivotal role so far but more is to be done to meet the challenging objective of feeding 9 billion people by 2050. Applied plant genetics is facing the dawn of a new era, in which novel genome editing technologies are opening unexpected horizons in basic and applied research. The study of DNA nucleases that can be engineered to land on specific loci of the DNA and alter its sequence is providing incredible tools for plant geneticists. This Chapter will describe genome editing technologies and the molecular bases that govern their function. Application to plant species is recent but advancing fast. Several traits of interest have already been successfully introduced or modified in crops and the first applications are starting to leave the lab and enter the path leading to commercial approval. This process is raising issues related to the regulation of genome-edited crops that governments all over the world will soon be called to rule.

Keywords: CRISPR/Cas9, Crop genetics, DNA repair, Double strand break, Genome editing, GMO regulation, Meganuclease, Oligonucleotide mediated mutagenesis, TALEN, Zinc finger nuclease.

INTRODUCTION

The world population has tremendously increased during the last decades, going from 3 to 7 billions in less than 50 years. This trend is predicted to be maintained for the near future and the growth rates will be higher in developing countries, putting pressure on communities and governments for access to resources and energy [1]. Feeding an increasing world population becomes a necessity and represents a multi-faceted problem that can be addressed in different ways.

From an agricultural perspective, increased productions of food and feed would be desirable but will have to be obtained using fewer inputs (land, fertilizers and

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pesticides), as intensive farming has limitations for sustainable production increases [2].

During the past century, genetics has greatly helped to address some of the challenges mentioned above, increasing food production and securing yields, also thanks to the introduction of novel wheat, barley and rice varieties during the Green Revolution [3]. Classical breeding can still provide solutions to many problems but the scientific community is calling for a Green Revolution 2.0, in which the contribution of biotechnology has to be thoroughly considered [4]. Most crops are being improved to be more resistant to pathogens and abiotic stresses (*e.g.* water scarcity is an issue in many areas of the globe), to be more resilient to climate changes (often hitting locally and unpredictably) and to be prone to cultivation for biomass rather than for a specific edible part of the plant. Time is a major issue that classical breeding has to deal with, because problems can arise abruptly and require a rapid response, often incompatible with the time required to produce and validate novel crop varieties [5].

The success of most varieties has depended upon the modification of the genetic information of a species, in which selection has guided the choice of a plethora of desired traits. Variation at the DNA level is a prerequisite to be able to direct such modification in any crop species. Genetic variation is often present in nature, for example in the form of rare alleles that can be exploited by breeders when identified, passed onto superior strains and used to confer specific characteristics. For example, a crucial step during crop domestication has been the selection of mutations that reduced seed shattering, an essential trait for offspring propagation in nature, but the major cause of yield loss for ancient farmers [6]. In several polymorphism and not polymorphisms species, a single gene modification, often a single nucleotide polymorphisms (SNP), allowed to pass from shattering wild plants to domesticated, non-shattering crops [7, 8].

When genetic variation is low or absent, it can be induced in a genome using chemical or physical treatments to introduce heritable changes in the DNA such as SNPs, insertions and deletions (Indels), whole genome rearrangements, including translocations, duplications or even polyploidizations [9]. The effects of these treatments on the genome of a plant are severe and in most cases unpredictable, because they occur randomly on the DNA sequence. However, the ultimate effect is on the underlying genetic information of the organism.

The use of transgenesis (the transfer of genetic information from an organism to another by means of molecular technologies) has paved the way to introduction of novel genetic information, not previously encoded in the DNA of the species. However, genetically modified organisms (GMOs) did not meet wide acceptance

and their cultivation is banned in many countries because of ethical or political issues.

The discovery of novel technologies for targeted genome editing opens novel perspectives and incredible potential for plant geneticists and crop improvement [10]. Traits of interest can be introduced or modified by acting directly at specific loci of the genome and can quickly lead to yield and agricultural productivity increases. Hundreds of plant varieties have been developed using conventional mutagenesis, often leading to random mutations in the genome and producing plants with multiple and unspecific genetic changes [11, 12]. On the contrary, genome editing technologies can be used to produce site-specific modifications in an efficient and precise manner, with little disturbance on the rest of the genome. The time required to engineer a plant genome to meet the needs of a breeding program can be strongly reduced, because the time-consuming cycles of crosses and selection could be shortened. The purity of the genetic background would be safeguarded by the targeted nature of the approach, while deleterious mutations would not be an issue. Finally, the type of genetic modification introduced in most instances would be indistinguishable from that induced by conventional mutagenesis. This issue is important from a regulatory perspective as it might allow the scientific community to move forward from a stalled debate on the use of GM plants in agriculture, while calling for a thorough reconsideration of national and international regulations governing registration, safety assessments and cultivation of such novel plant types.

A Primer On Genome Editing Technologies

Genome editing technologies collectively comprise a diverse set of molecular tools that allow the targeted modification of a DNA sequence within a genome. Precise genome modification starts by inducing double strand breaks (DSBs) in the DNA, exploiting engineered site-specific nucleases that cleave the target locus at the desired position and trigger the activation of repair pathways.

Plant cells can use two types of endogenous repair systems: non-homologous end joining (NHEJ), which involves rejoining of the broken ends, and homology-directed repair (HDR), which uses homologous DNA sequences as template for reparation [13] (Fig. **1A**). The term “non-homologous” indicates a feature of this reparation pathway in which ligation of free chromosome ends takes place without the need for a homologous template [14]. Two distinct types of NHEJ mechanisms have been described, including a classical one (cNHEJ) and an alternative one (aNHEJ) [15, 16] (Fig. **1A**). In cNHEJ the double-stranded ends are bound by ku70-ku80 heterodimers, which prevent DNA damage and recruit DNA ligase and its cofactors at the broken ends [17 - 19]. This repair pathway can

CHAPTER 17

Progress in Small Grain Cereals: A Case Study

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Abstract: Small grain cereals (here including durum wheat, bread wheat, barley and oats) have been crucial to the development of mankind providing a regular staple source of food compounds – carbohydrates, proteins, fat and secondary metabolites – since their domestication 10,000 years ago. Historically, genetic studies have their foundations in Mendelian mutants, characterized by altered physiology and/or morphology. In this regard there are examples of morphological mutations described in the past for which the gene/genes responsible have been recently cloned, characterized and used. An example is the Rht-B1b gene that controls plant height in wheat, which induces semidwarf plants due to the effect of a single nucleotide mutation capable of converting the majority of sugar into grain starch. With this model the source-sink relationship has been studied in depth and new varieties based on the concept of "Improved Harvest Index" have been released with an impressive grain yield enhancement in a wide range of environments. The question is: "Can we produce and supply sufficient food in the next 40 years without consuming more land?" On the basis of modern plant science, the answer is positive. Selection is specifically directed to create highly tolerant and/or resistant genotypes to increase the "High Yield Potential and Stability of Yield" and to reduce the gap between high yield potential and the actual yield also in very poor small farms (low or zero input). In fact the interaction between private and public pre-breeding-/breeding programmes, allowing the introduction of modern varieties which are very well adapted in fertile as well as in severe stress conditions, represents the modern vision to improve not only grain yield but even the quality of life of all farmers.

Keywords: Barley, Breeding progress, Genomic revolution, Grain Yield, Oats, Plant ideotype, Wheats.

HISTORICAL CEREAL BREEDING PROGRESS

Cereals have been and are important because they provide two-thirds of the calories and half of the protein in the diets of humans and they are major feed-

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stuffs for both monogastric and ruminant livestock. In 2015, world cereal production was estimated at 2,525 Million tonnes (FAOSTAT). Small grain cereals (here including durum wheat, bread wheat, barley and oats) have been crucial to the development of mankind, providing a regular staple source of food compounds – carbohydrates, proteins, fat, fibre, minerals, and secondary metabolites. Their history as crops began in the 'Fertile Crescent', a region including parts of Iran, Iraq, Turkey, Syria, Jordan, Lebanon and Israel. The domestication of the wild ancestors, *Hordeum spontaneum* (L.), diploid wheat and later oats, which are still to be found in the Middle East, dates to the Neolithic. Human intervention has been a decisive factor in the breeding of these species down through the millennia, the cultivars currently available manifesting completely different traits from those found in either the original parents or in those populations that have evolved in the wild. The first, and perhaps most outstanding development, was domestication itself – that moment in which man stood before an immense number of different species and chose cereals. In these species the new farmer saw the genetic variants most responsive to his/her needs [1]. He invented by copying from mother nature the earliest rudimentary agronomic techniques: seed and soil selection, ploughing, sowing, manual weed control to protect the crops, seed gathering and storage. With experience, the best plants were likely earmarked for the following year's seed supply rather than for direct consumption, incidentally giving rise to 'mass selection'.

Yet why, we might well wonder, among the thousands of species available, were cereals the first crops bred and continuously cultivated down through the ages? At the dawn of agricultural civilization humans realized that the grain cereals could ensure their survival, an intuition which initiated an instinctive selection, generation after generation, of the genotypes best suited to the farmer’s needs. One example of this is the discovery of plants with non-fragile spikes. This was indubitably a spark in the impulse towards cereals cultivation, being in all likelihood the first criterion of selection in the history of plant breeding. Such alterations in the rachis were the pre-requisite for the harvest of the whole ear. Brittle rachis genes are present in all wild species of small grain cereals and very well described. The trait itself, *e.g.* in barley, is actually controlled by the two complementary genes *Btr1* and *Btr2* [2]. The change may seem a simple one to us, yet for Neolithic or late Palaeolithic man it very likely improved his chances of survival. The discovery too of naked seed represented another important milestone on the road to the spread of cereals as a food staple. This selection process was necessarily focused on phenotypes distinguishable for simple traits controlled by one or not more than a few genes - a method that was empirically pursued right up to the end of the nineteenth century.

Another step forward in newborn agriculture was the conservation for the subsequent year's sowing (rather than for direct consumption) of the seeds produced by those plants deemed the best performing. The progress this practice engendered evidently had to be extraordinary in terms of quantitative and qualitative increase (large kernels), given the subsequent Middle Eastern civilizations that sprang up and developed around these plants. This Neolithic model of agricultural technology was exported from the Middle East at a rate of one kilometre per year and it is from here that the species set out first to colonize the Mediterranean Basin and then, following the great river and sea migration routes of the time, fanned out to central and northern Europe and the Far East. The perfecting of crop management techniques (*e.g.* the advent of rotation and the practice of fallowing), in particular during the period of Roman dominion, contributed to the spread of cereals in the fertile as well as in the poorer soils of the Empire. As is well-known, cereal breeding has been developed during three revolutions, beginning with the Neolithic, during which, unconsciously a good gene – non- fragile spike - was discovered and used. The humans learned to sow and to harvest and they worked hard to develop new equipment to improve quantity and quality of food production. It was the birth of agriculture. The second revolution was the Mendelian, during which theoretical and practical goals have been reached. The work of G. Mendel was re-discovered at the beginning of 1900 when the modern age of plant breeding began. Until that time, the increase in food production was obtained simply by bringing new land into cultivation. In fact, the yield per unit of land remained stable from the Roman period to the beginning of the 20th Century. The application of Mendel's laws, the development of modern varieties and the introduction of new agricultural practices gave strong contributions towards improving grain yield around the world. However, more rapid increases in grain production were attained when semi-dwarf varieties were introduced into cultivation. In fact the new varieties increased grain yield by reducing height at the expense of straw biomass, and as a consequence became more resistant to lodging. The increase of the grain-to-straw ratio resulted in improving the Harvest Index (HI). In 1964 CIMMYT varieties were introduced in Asia, the Near East, Latin America, Australia and Europe. Grain production in many cases doubled and countries from importers became exporters. In 1970 the so-called "Green Revolution" was realised. Farmers rapidly adopted the new varieties and new agronomic techniques were developed. The technique of ideotype breeding was born. How the new wheat or barley genotypes make the better use of the environment has been considered as a model to be exported to many other crops. For the first time knowledge of genetics, statistics, biochemistry, pathology, physiology, and more recently molecular biology are synthesized by the breeder. The advances linked to genetics in all countries around the world have been remarkable, and breeding practices (pedigree

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