Plant Biotechnology for Agricultural Sustainability

12

Sandra Pérez Álvarez, Eduardo Fidel Héctor Ardisana, and Ramona Pérez Leal

Contents

S. P. Álvarez (✉)
Facultad de Ciencias Agrícolas y Forestales, Universidad Autónoma de Chihuahua, Delicias, Chihuahua, Mexico

E. F. H. Ardisana
Instituto de Posgrado, Universidad Técnica de Manabí, Portoviejo, Ecuador

R. P. Leal
Facultad de Ciencias Agrotecnológicas, Universidad Autónoma de Chihuahua, Ciudad Universitaria, Chihuahua, Chihuahua, Mexico

S. Kumar et al. (eds.), *Resources Use Efficiency in Agriculture*,
https://doi.org/10.1007/978-981-15-6953-1_12

Abstract

Plant biotechnology is an essential tool that allows agriculture improvement by increasing food production through tissue culture, molecular biology, and crop improvement. At present, agriculture is facing many problems that affect food production seriously; some of these problems are degradation of soils, salinity, contamination with heavy metals and hydrocarbons, drought, desertification, deforestation, and one of the solutions is biotechnology. This chapter will discuss aspects related to sustainable agriculture and food challenge, plant biotechnology, and plant biotechnology and sustainability. First, the incidence of agriculture is analyzed, on the one hand, in the reduction of hunger, and on the other, in the degradation of the environment, which can only be resolved through a sustainable model. Secondly, the most relevant applications of modern biotechnology in the accelerated propagation of plants, germplasm conservation, and genetic improvement are described. Next, both elements are linked, and it is analyzed how biotechnology can contribute to sustainability through modern technologies. The contribution of modern biotechnologies to sustainability in agriculture is illustrated through the presentation of examples of work done with the genus *Lupinus*. This genus comprises species useful for sustainable agriculture, which serve as a source of proteins and secondary metabolites, as well as in crop rotation. This chapter shows some of the results achieved in the multiplication and in vitro conservation of species from *Lupinus*, as examples of the application of biotechnology with an environment friendly approach.

Keywords

Agriculture · Environment · Food security · Sustainable · Tissue culture

Abbreviations

2,4-D	2,4-dichloro phenoxy acetic acid
AFLP	Amplified fragment length polymorphism
BA	Benzyladenine
Bt	*Bacillus thuringiensis*
CH	Casein hydrolysate
CRISPR	Clustered regulatory interspaced short palindromic repeats
DCR	Douglas-fir cotyledon revised
g l^{-1}	Grams per liter
GM	Genetic modified
GMCs	Genetically modified crops
GMOs	Genetically modified organisms
H_2SO_4	Sulfuric acid
IAA	Indoleacetic acid
IBA	Indol-3-butyric acid
ITS2	Internal transcribed spacer 2

kg ha^{-1}	Kilograms per hectare
Kin	Kinetin
mg l^{-1}	Milligrams per liter
MS	Murashige and Skoog
NAA	Naphthaleneacetic acid
PCR	Polymerase chain reaction
PPT	Glufosinate ammonium
RAPD	Randomly amplified polymorphic DNA
RFLP	Restriction fragment length polymorphism
RNA	Ribonucleic acid
SI	Sustainable intensification
SSN	Sequence-specific nucleases
SSRs	Simple sequence repeats
TAL	Transcription-activator-like
TALEN	Transcription-activator-like effector nucleases
TDZ	Thidiazuron
ZFN	Zinc-finger nucleases
μg l^{-1}	Micrograms per liter

12.1 Introduction

The world population on this planet is expected to a continuous increase from 6.7 billion to 9 billion by 2050. To fulfil the food demand, that will increase, the agricultural production needs to rise by 50% by 2030 (Royal Society 2009). It is also vital to notice that arable lands are limited because part of them are used for urbanization, or lost by abiotic stresses such as salinization, desertification, drought. The water needed for drink has also decreased in the past 60 years (United Nations Environment Programme 2002). The majority of the loses mentioned together with the loses caused by biotic factors (pathogens) occurs after the plants are entirely grown because at this point most or all of the land and water required to grow a crop has been invested (Dhlamini et al. 2005).

One solution to solve those problems is genetic improvement of crops, where new crops can be created with resistant to increasing temperatures, less water, flooding, salinity, pathogen, and insect (Gregory et al. 2009; Royal Society 2009). Biotechnology is an important technology that supports the protection and preservation of the environment by, for example, reducing the application of chemical pesticides and herbicides. Some plants have been genetically engineered to clean up heavy metal pollution from contaminated soil (Bagwan et al. 2010). The ecological point of view of biotechnology includes the application of several technologies including farming, agroindustry, forestry, fishing and aquaculture, and different objectives such as conservation of genetic resources, the diagnosis of several diseases of plants, and the production of fermented foods (Bagwan et al. 2010; Dash et al. 2016). This chapter aims to describe the importance and challenge of biotechnology as a sustainable agricultural resource.

12.2 Sustainable Agriculture and Food Challenges

12.2.1 Sustainable Agriculture

Sustainability in agricultural systems as a definition may include terms as agroecology, biodynamic, ecology, organic supply, sensitivity to the environment, low input and some others (McNeely and Scherr 2003). Some of the main principles for sustainability are (Pretty 2008):

a. The food production process is mainly taking account the nutrient cycle in plants, nitrogen fixation, regeneration and conservation of the soil, pathogens, predation, and parasitism;
b. To preserve the environment through the minimal use of non-renewable resources;
c. To use wisely what farmers know and the skills of them, and;
d. To use the knowledge and capacities of the people to solve the main problems of agriculture and natural resources, for example, plant pathogens, water, soil, and others.

According to Dobbs and Pretty (2004) and MEA (2005), sustainability mainly implies the use of technology to increase crop productivity without damage to the environment for agricultural systems. The principal objective of agriculture must be the maintenance of sustainable development to guarantee food safety for the population of the world not only today but also in future too. It is crucial to stand out sustainable agricultural development activities for the preservation and maintenance of natural resources; but at the same time, these resources must increase for future generations taking an account the increase in food demand and also the world population that in 2050, according to predictions, will reach nine billion peoples. Also, abiotic stress events such as drought, floods, scarce rain, salinity are growing, and they will decrease food production (Hans and Colaco 2019).

In sustainable agriculture, the systems include social and human resources at high levels (Olsson and Folke 2001; Pretty and Ward 2001). It does not imply the decrease or reduction in the use of resources (more land is needed to produce the same quantities of food). Some shreds of evidence indicate that sustainable agriculture initiatives and projects arise from modifications in some factors like use of fertilizers in several crops, pesticides and biological control, and so on (Buttel 2003; Tegtmeier and Duffy 2004). Agriculture has great importance in sustainable development, and hunger and poverty eradication. Sustainable agriculture must avoid soil degradation, guarantee biodiversity protection and conservation and achieve social and economic welfare (Hans and Colaco 2019).

12.2.1.1 Challenges and Proposals of the Food Security

The actual world crisis in food is caused mainly for the inequality in the access and distribution of food. It means that regardless of the overproduction of food in all countries, the hunger situation is still critical, with many people in this condition

(FAO 2011; CINU 2011). According to FAO-FIDA-PMA (2014), several millions of people suffer from hunger in the world, while many billion tons of food is wasted every year (Gustavsson et al. 2011; FAO 2014a). The enormous food waste (54%) happens in the first stages of post-harvest, management, and storing, and the rest (46%) occurs in processing, delivery, and consumption of food (Parfitt et al. 2010; Meena et al. 2018).

The growth of world population is globally slowing down, but in Africa and Asia, the population continues to increase. Many communities depend on agriculture for employment and income generation, and they cannot further develop by pressure to which the lands and water resources are already subjected (FAO 2017). Another challenge for the present and future agriculture is the deforestation caused mainly by the expansion of the agricultural lands. Almost half of the forests that once covered the planet have disappeared, and the underground waters run out quickly. The biodiversity has been severely eroded every year; one of the principal causes is the emission into the atmosphere of billions of tons of greenhouse gases, whose consequences are global warming and climate change (FAO 2017).

Agricultural systems or agroecosystems have a variety of properties that characterize them as modified ecosystems (Dalgaar et al. 2003; Swift et al. 2004). Some of these properties are (Gliessman 2005):

a. Productivity that is medium in healthy ecosystems, high in modern ecosystems, medium (possibly high) in sustainable agroecosystems;
b. Species diversity that is high in healthy ecosystems, low in modern ecosystems, medium in sustainable agroecosystems;
c. Functional diversity that is high in healthy ecosystems, low in modern ecosystems, medium-high in sustainable agroecosystems;
d. Output stability that is medium in healthy ecosystems, low-medium in modern ecosystems, high in sustainable agroecosystems;
e. Biomass accumulation that is high in healthy ecosystems, low in modern ecosystems, medium-high in sustainable agroecosystems;
f. Nutrient recycling that is closed in healthy ecosystems, open in modern ecosystems, semi-closed in sustainable agroecosystems;
g. Trophic relationships that are complex in healthy ecosystems, simple in modern ecosystems, intermediate in sustainable agroecosystems;
h. Natural population regulation that is high in healthy ecosystems, low in modern ecosystems, medium-high in sustainable agroecosystems;
i. Resilience that is high in healthy ecosystems, low in modern ecosystems, medium in sustainable agroecosystems;
j. Human displacement of ecological processes that is low in natural ecosystems, high in modern agroecosystems, low-medium in sustainable agroecosystems;
k. Sustainability that is high in natural ecosystems, low in modern agroecosystems, and high in sustainable agroecosystems.

According to Haberl et al. (2004) and Firbank et al. (2006, 2008), systems of modern agriculture have modified some of the above characteristics to increase

production. Sustainable agroecosystems, on the contrary, need to change some of those properties to the natural systems without sacrificing productivity. It is necessary to maximize the renewable sources of energy and some energy flows that are directed to feed trophic essentials interactions to reach the goal of sustainability and maintain other ecosystem functions.

12.2.1.2 Agricultural Productivity in a Sustainable Way

Since 2005, several farmers are practicing integrated farming that is a step to sustainability, because they found that this system is safer in buying and supplying, while many moderns farming systems are inefficient (wasteful) (EA 2005). By adopting integrated farming practices, waste is less and the benefit to the environment is higher; so, farmers can save inputs by replacing regenerative technologies with external contributions, such as legumes or organic fertilizers for inorganic or biological control for pesticides (Pretty and Ward 2001).

Ostrom (1990) and Pretty (2003) declare that sustainable agroecosystems, as some relevant characteristics, have progressive effects in assisting to construct natural capital, strengthen populations (social capital), and improve human abilities. Examples of this include (according to Pretty 2008):

- Enhancements to usual investment that include increased water maintenance in soils, drinking water availability in the waterless period, and reduced soil erosion by the combination of organic matter;
- Improvements to social investment that include more public groups that are stronger, several new procedures to work with communal natural resources, and connections to some outside strategy organizations that are better;
- Improvements to human capital, increasing local capacity to face problems, the status of women, respect for marginalized groups, improving child health and nutrition, more employment and reversed migration.

Agricultural sustainability, in a conventional way, may involve a reduction of some inputs (fertilizers, water, pesticides) but the requirement of land is higher to produce the same amount of food that other systems—such as organic ones—where they may have lower yields but an increase of positive impact on natural capital. Some pieces of evidence show that active agricultural projects in agricultural sustainability arise from changes in factors of agricultural production (Tilman et al. 2011; Meena et al. 2019). In this sense compatibility between definitions of "sustainable" and "intensification" was suggested in the 1980s (Raintree and Warner 1986), and "intensification" became synonymous of harm in agriculture to produce food (Conway and Barbier 1990). Similarly, "sustainable" implies to the people good agriculture (Royal Society 2009). According to the Royal Society (2009), sustainable intensification (SI) is defined as a process or system where productivity (yields) increases without damaging the environment and using less land for cultivation. The definition is not a close concept, so any favoritism is made to any interpretation or vision of agriculture (Smith 2013), and both definitions (SI and "agricultural intensification") can be differentiated by priorities and goals than only

to determine productivity improvement. Sustainable intensification based on Smith (2013) includes several options like the application of new technologies and improving the efficiency of current crop production, so for SI, the following aspects are to be considered:

- The mechanism in agriculture that increases the productivity of crops are: (a) better nutrient supply according to plant needs; (b) to improve recycling of nutrients; (c) to improve the use of the soil by reducing erosion, increase fertility, nutrients improvement; (d) to improve the use of crops according to bioclimatic regions.
- It is expanding the limits of crop production by using molecular techniques that will allow obtaining new crops more quickly compared to the past, making this possible without the increase of water use and intensity in fertilizing.

The SI has several advantages, going from climate change mitigation (reduced soil erosion and emissions from processes like nitrification), environmental improvement through the reduction in the use of fertilizers and pesticides (innovation, application of new technologies, transfer of knowledge), and social sector (Pretty et al. 2011).

12.2.2 Sustainable Agriculture in Latin America

Agriculture is one of the main productive activities in Latin America, where it constitutes a primary source of food and raw materials for various industries. To a greater or lesser extent, all the original peoples that populated the American continent before the arrival of the first Europeans were farmers, and there was an outstanding development of the forms of agricultural production in the territories that today occupy countries such as Mexico, Peru, Ecuador, and Bolivia. However, agrarian production techniques were transformed to the extent that European practices were introduced in Latin American agriculture, although traditional production practices were maintained in all countries of the area.

In the second half of the twentieth century, the growing need for food led to the implementation of the "Green Revolution" practices, among which are the new varieties of plants arising from genetic improvement, mineral fertilizers, synthetics pesticides, agricultural machinery of all kinds, irrigation systems, and other technologies (Gliessman 2013; Meena et al. 2020a, b). In the last 30 years, new products of science and technology have been incorporated; these include genetically modified organisms (GMOs) that in 2016 already occupied 185 million hectares (ISAAA 2016). The application of these intensive technologies has undoubtedly led to an increase in product volumes and yields per unit area. However, the criteria for their use have not always been based on scientific recommendations, but on guidelines imposed by the market, which have as a paradigm the sale of their formulations with the recommendation of a supposed excellent result. In South America, for example, the consumption of fertilizers and

Table 12.1 Fertilizer and pesticide consumption in the countries of South America (Adopted, Héctor et al. 2018)

Country	Fertilizer consumption (kg ha^{-1})				Pesticide consumption (kg ha^{-1})
	Nitrogen fertilizers (1)	Phosphoric fertilizers (2)	Potassium fertilizers (3)	Total fertilizers (1 +2+3)	
Argentina	25.65	18.47	0.82	44.94	6.55
Bolivia	5.09	2.30	1.30	8.69	7.96
Brazil	45.23	51.22	59.55	156.00	NA
Chile	243.77	68.77	39.93	352.47	11.36
Colombia	150.55	72.64	68.62	291.81	13.46
Ecuador	70.11	13.11	40.81	124.03	6.85
Guyana	14.26	11.31	0.60	26.17	0.90
Paraguay	25.21	44.67	35.47	105.35	NA
Peru	55.82	20.79	14.05	90.66	3.09
Surinam	142.18	21.23	20.66	184.07	14.40
Uruguay	28.98	39.30	31.56	99.84	9.44
Venezuela	87.69	22.46	31.38	141.53	NA
AVERAGE	74.54	32.18	28.72	135.46	

NA not available

pesticides is excessive (Table 12.1). Countries such as Brazil, Chile, Colombia, Suriname, and Venezuela contribute to raising the average consumption of fertilizers in the subcontinent. In particular, Chile triples the total volume of fertilizers applied in South America, and the amount of nitrogen fertilizers used in its agriculture (243.77 kg ha^{-1}—kilograms per hectare) is comparable to that of China, which reaches 296.8 kg ha^{-1} (FAO 2014b). Even the figures of seemingly small consumption of countries such as Bolivia and Guyana do not reflect the reality since the amount of chemical inputs applied is not proportional to the amount of agricultural land in the countries of the region (Héctor et al. 2018; Meena et al. 2020a). Chile, Colombia, and Ecuador are also the countries in the area that most pesticides apply, with more than 10 kg ha^{-1} of these dangerous synthetic products.

Intensive practices in agriculture, such as mechanization and the use of excessive synthetic chemicals, lead to physical and chemical degradation of soils. Among the effects that occur are: the decrease in organic matter content, which is very degraded lands can be reduced to levels four times lower than usual (Mor-Mussery et al. 2015); the increase in the sandy fraction of the soil, with loss of cation exchange capacity and increase in saturation by aluminum (Reichert et al. 2016); the loss of nutrients and the immobilization of others (Casierra and Aguilar 2007); the reduction of the arable layer and the water retention capacity (Bestelmeyer et al. 2015), and other effects.

Undoubtedly, the growing population must be fed, and for this, a proportional supply of food is needed whose primary source is agriculture. However, the indiscriminate exploitation of soils, water sources, and other natural resources can only lead to their depletion, and consequently to the loss of the productive capacity of the

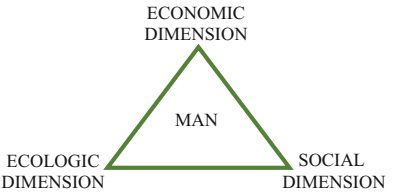

Fig. 12.1 The three dimensions of the sustainability triangle (Modified, Dyllick and Hockerts 2002)

planet, with the gradual extinction of life. Amid this concern, the concepts of sustainability and its application to agriculture emerge. Concerns for the preservation of the environment date from the mid-twentieth century, but approaches to development in terms of sustainability are attributed to the "Brundtland Report" (Brundtland 1987) in which the relationship between development and environmentalism is first raised. From this postulate, two trends developed: the so-called *weak sustainability*, which advocates economic growth over ecological protection, and *strong sustainability*, which reverses the equation giving preponderance to environmental conservation over advances in the economy (Norton 1995). Subsequently, Dyllick and Hockerts (2002) proposed that sustainability should be developed in three equivalent dimensions (economic, ecological, and social). A triangle with three dimensions, whose center is man, as the managing agent of the three aspects of sustainability, and also as a beneficiary of them, could be see in Fig. 12.1.

Apparently, from what is presented in Fig. 12.1, a definition of sustainable agriculture could be reached with relative ease, considering it as an agricultural production system in which economic and social benefits are obtained without affecting the environment. However, as noted by Velten et al. (2015), the picture is much more complicated. From a bibliographic analysis of journals dedicated to the topic of sustainable agriculture, these authors found that:

a. Although—in general—the three dimensions proposed by Dyllick and Hockerts (2002) are present in the sources consulted, these tend to focus more specifically on any of them.
b. Organizations that work for sustainable agriculture have diverse strategies.
c. Sustainable agriculture is present in several fields of action.

Table 12.2 shows the elements detected by Velten et al. (2015).

The concept of sustainability in agriculture, based on these trends, has evolved into a multifunctional agricultural production system. This should not be only a supplier of food and raw materials, but also a generator of multiple benefits in the area of ecosystem services, with resulting collateral activities such as biodiversity recovery, landscaping, and tourism (Huang et al. 2015). In Latin America, a stream of thought has been developed that defends the sustainability of agroecosystems based on a powerful ecological component. Authors such as Altieri and Nicholls (2017) consider Latin America as the area where agroecology emerged in the late 1970s and 1980s, strengthened by intellectual currents of a sociocultural nature. This

Table 12.2 Goals, strategies, and areas of action of sustainable agriculture (Adapted, Velten et al. 2015)

Goals	Strategies	Fields of action
• Environmental (production- and non-production specific)	• Adaptive management	• Agrifood system
• Social	• Cooperation	• Management and technological solutions
• Economic	• Ecology-based	• Social and environmental challenges
	• Economics-based	• Social and human capital
	• Holistic and complex systems thinking	• The social, political, and economic environment
	• Knowledge and science	
	• Subsidiary	

trend predominates in the area and has been strengthened through the influence of intellectuals, universities, non-governmental organizations, peasant movements, and other social organizations. However, much depends on government policies, while these are decisive when implementing massive strategies that can be accessible to small producers and guarantee a space where they can compete with the great companies that support the mode of production for conventional agriculture (Altieri et al. 2012; Yadav et al. 2020). Latin American political instability allows us to see a particularly complex scenario, in which the predominance of ancestral agricultural practices or new technologies, or of the complementation between the two, will depend more on power struggles between political groups and business interests than on the benefits that both trends can bring to the economy, the preservation of the environment, and social benefits.

12.3 Plant Biotechnology

12.3.1 Plant Tissue Culture

Tissue culture is the cultivation in the artificial nutrient medium of explants (any part of the plant, namely roots, stem, leaves, seeds, or protoplasts) under aseptic conditions (Touchell et al. 2008; Levitus et al. 2010). The first idea of growing an individual plant in the artificial medium was of Gottlieb Haberlandt in 1902. Haberlandt never realized the relevance of his approach, but more than 100 years after, this definition is still an essential tool for plant sciences (Touchell et al. 2008). Tissue culture is used for an increasing number of purposes such as crop improvement programs, embryo rescue, haploid and dihaploid production within a short time (Abraham 2009), species conservation, and rescue of species in danger of extinction. Plant propagation through tissue culture has several advantages compared to conventional propagation; according to Dominguez et al. (2008) these advantages are:

a. It is a propagation system based in cloning, which means that all the genotypic characteristics of the original material are maintained.
b. The entire process is carried out in a laboratory under controlled environments, totally independent of external conditions; so, the material is not affected by the seasonal changes during the year, drought, frost, high temperatures, or other environmental factors.
c. Around 10,000 plants can be obtained in a little time from a single donor.
d. The space required is minimal, and the time in which the process can take place is relatively short.
e. The plants obtained are free of phytopathogenic bacteria, fungi, and nematodes, and with more specific techniques (like meristems culture) plants can be free even from viruses and viroids.

"Totipotency" is the physiological base of the tissue culture and is defined as the capacity of any part of the plant to regenerate a whole plant in a basal medium. Tissue culture develops protocols for plant regeneration (thousands of plants from a piece of root, leaves, buds, and seeds) free of pathogens and with good yield (Yildiz 2012).

12.3.1.1 Micropropagation

The plant and the selected explant are significant for micropropagation because it is a cloning technique. The genotype of the plant is determinant since not all the plants have the same regeneration capacity. Some dicotyledon plants have an excellent regeneration capacity; meanwhile, woody plants such as fruit trees, pines, and some others are hard to regenerate (Pierik 1987). Species from *Lupinus* genus, such as *Lupinus campestris* L. and *Lupinus montanus* L. from the family Fabaceae, are known for having seeds with sturdy seminal covers, so several scarification treatments are used. The same procedure is used with *Acacia farnesiana* (L.) Wild, which belongs to the same family (Fig. 12.2) (unpublished results).

Explants should be isolated from healthy plants. Also, it is essential to notice that the regeneration capacity of mature tissues is quite low, such as the plant seeds in a resting stage (dormant) (Pierik 1987). There are several types of research in micropropagation of many different plant species. In the Center for Basic Sciences of the Autonomous University of Aguascalientes, projects have been developed aimed to establish methodologies for cultivation and propagation *in vitro* of several species from the genus Agave. The selection of species is based on their possibility of mezcal and pulque production, as is *A. cupreata*, *A. karwinskii*, *A. palmeri*, *A. potatorum*, and *A. salmiana*. Some other were selected for their ornamental value as *A. bracteosa*, *A. chiapensis*, *A. difformis*, *A. nizandensis*, *A. obscura*, *A. ornithobroma*, *A. peacockii*, *A. titanota*, and *A. victoria-reginae*. In vitro propagation technique of all these species was based in basal meristems selection. Basal seedling segments germinated in vitro were cultured in nutrient media supplemented with cytokinins such as benzyladenine (BA), 6-(γ,γ-Dimethylallylamino) (2iP), kinetin (Kin), thidiazuron (TDZ), and metaTopolin (mT). The efficiency of these systems goes from the production of averagely 2.2 shoots for each explant in

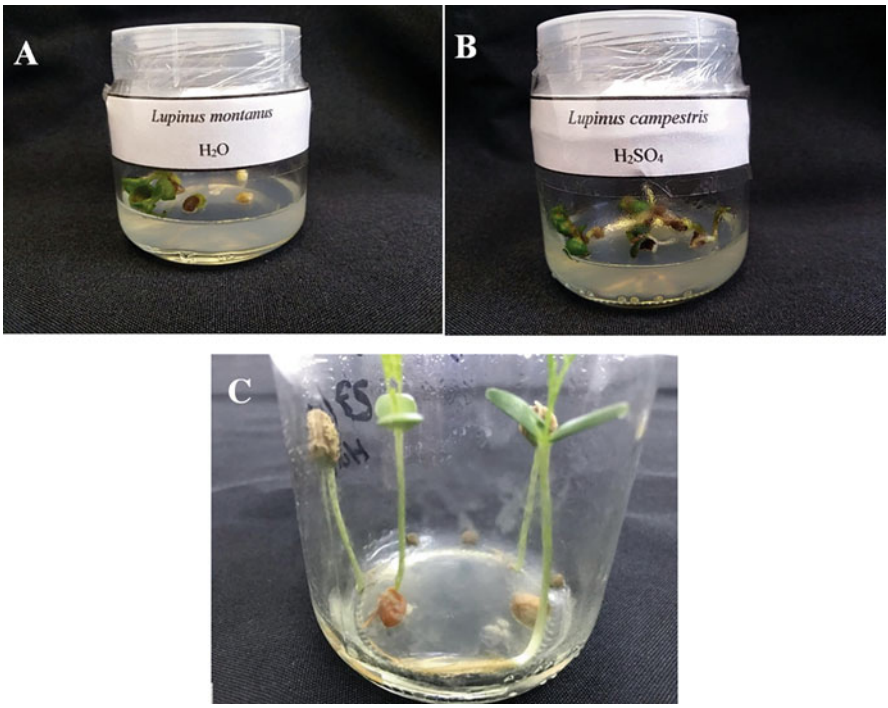

Fig. 12.2 Seed germination in the agar-water medium after scarification treatment, (**a**) *L. montanus*, boiling water for 24 h; (**b**) *L. campestris*, H_2SO_4 (sulfuric acid) for 15 min; (**c**) *A. farnesiana*, H_2SO_4 for 15 min

A. palmeri, up to 30 shoots per explant in *A. victoria-reginae*, in a propagation cycle of 40–60 days (Dominguez et al. 2008).

The morphogenesis of several cultivars of tomato (*Solanum lycopersicum* L.) was tested with the application of different antibiotics (carbenicillin, kanamycin, ampicillin, and cefotaxime). Murashige and Skoog (1962) was used for the experiment and the vegetable material used was cotyledons. As results kanamycin caused damage to explants and carbenicillin and ampicillin (100–400 mg l^{-1}) induced the regeneration of bud and non-toxic effect (Gerszberg and Grzegorczyk-Karolak 2019).

Research in *Cymbopogon schoenanthus* subsp. *proximus* used as renal antispasmodic was done by Abdelsalam et al. (2018). They studied the influence of several phytohormones (naphthaleneacetic acid—NAA, BA), different carbon sources, methyl jasmonate, and vitamins. The higher callus induction (100%) was obtained with 4 mg l^{-1} NAA combined with 0.5 mg l^{-1} BA; when NAA was used at 1.0 and 4.0 mg l^{-1} combined with 0.5 mg l^{-1} of BA the number of shoots increased; also, 6% sucrose induced root induction efficiently and sugar at 3% had a good effect increasing shoot numbers. Different concentrations of methyl jasmonate, biotin, and calcium pantothenate were used for root formation, but shoot induction was reduced.

Ramirez-Mozqueda and Iglesias Andreu (2017) studied friable calluses in *Vanilla planifolia*. Immature seeds were cultured in MS medium supplemented with 0.45 μM TDZ, and friable callus was obtained. The effect of another growth regulator (BA) was evaluated in different concentrations with the same culture medium but without gelling agent (liquid) supplemented with 8.88 μM BA, a 0.5 g of inoculum density was obtained and at 16 days the growth of the cell suspension culture was high, with 80% cell viability.

12.3.1.2 Callus Culture

The main objective of using callus (a mass of undifferentiated cells, Fig. 12.3) is to develop an efficient, fast, and large-scale micropropagation methodology, as well as to induce and generate plant structures that, due to their characteristics of totipotence, undifferentiation, and regeneration capacity, allow the development and implementation of modern biotechnological techniques for the non-traditional genetic improvement.

A research was done with *Vanilla planifolia* Jacks. ex Andrews (*Orchidaceae*) to develop a massive, efficient, and fast propagation methodology. The calluses were formed from an undifferentiated and transient structure generated from the radical

Fig. 12.3 Callus from in vitro root of Lupinus species (Unpublished results)

apices grown in the absence of light, in a liquid MS medium supplemented with 30 g l^{-1} (grams per liter) sucrose, 1 mg l^{-1} BAP, and 1 g l^{-1} of hydrolyzed casein. The highest percentage of calluses (72%, $p < 0.05$) was formed in solid MS medium supplemented with 0.5 mg l^{-1} of 2,4-dichloro phenoxy acetic acid (2,4-D) in the dark (Gätjens-Boniche et al. 2018).

Callus obtained through in vitro culture allow the production of secondary medicinal metabolites and three varieties of *Artemisia annua* L., an aromatic Asteraceae plant, were cultured with this aim. Plant leaves were cultured in MS medium supplemented with (a) 0.5 mg l^{-1} BA, 0.5 mg l^{-1} NAA, 0.5 g l^{-1} casein hydrolysate (CH), (b) picloram (0, 0.5, 1.0, 1.5, and 2.0 mg l^{-1}), and (c) 2,4-D (0, 0.5, 1.0, 1.5, and 2.0 mg l^{-1}). The highest callus formation was accomplished in MS medium with 0.5 mg l^{-1} BA, 0.5 mg l^{-1} NAA, and 0.5 g l^{-1} of CH. Calluses observed on 0.5 mg l^{-1} picloram were more easily dispersed than calluses from other media (Keong et al. 2018).

In mango (*Mangifera indica* L.) var. Ratnagiri, nucellar tissue was used to induce somatic embryogenesis. The MS medium was supplemented with five TDZ concentrations (0.45, 2.27, 4.54, 9.08, and 11.35 μM), alone or combined with 4.52 μM 2,4-D, without any other plant growth regulators. After 4–9 weeks, a medium with 4.52 μM 2,4-D and 2.27 μM TDZ (induction medium) was used for somatic embryos. A total of 35 somatic embryos per gram of fresh weight can be obtained after several weeks (Malabadi et al. 2011a).

Somatic embryogenesis is the formation of an embryo from a somatic cell, without the need of gamete fusion (Tisserat et al. 1979). According to Yeung et al. (1996) this method, theoretically, is the most efficient for the mass production of plants in vitro due to the bipolar nature of the embryo, the possibility of the entire automation of production process, and the high multiplication coefficients in short periods. Its disadvantages lie in the lack of knowledge about the parameters that regulate this process; thus, the number of species in which efficient somatic embryogenesis allows productive use of the method is still limited. Malabadi et al. (2011b) used immature zygotic embryos of several commercial varieties of papaya (*Carica papaya* L.) for the obtaining somatic embryos in an MS medium supplemented as described by Malabadi et al. (2011a) with similar results. Concerning the varieties used, the authors found the best results of somatic embryogenesis in Taiwan-786 (87.0 ± 4.2), followed by Taiwan-785 (85.0 ± 3.0) and Coorg Honey Dew (81.0 ± 3.2).

Malabadi et al. (2004) worked with apical dome section of *Pinus kesiya* Royle ex Gordon. The goal of the research was the initiation, maintenance, and maturation of somatic embryos. The apical dome section was cultivated in half and full strength DCR (Douglas-fir cotyledon revised) (Gupta and Durzan 1985) basal medium supplemented with 0.2 g l^{-1} polyvinyl pyrrolidine (PVP), 7 g l^{-1} agar (Difco-bacto), 30 g l^{-1} maltose, and 0.2, 0.3, or 0.4% activated charcoal without growth regulators. Explants were incubated in the dark at 4 °C for 1–10 days. Another culture condition was the application to the basal medium for three days of 0.3% activated charcoal at different temperatures (10, 15, and 20 °C). For the initiation stage of embryogenic callus several concentrations of indoleacetic acid (IAA),

indol-3-butyric acid (IBA), NAA, and 2,4-D with Kin and BAP were used in half and full strength (inorganic salts) DCR basal media. The maintenance phase was done with callus showing pro-embryonal masses in half of basal DCR medium containing 40 g l^{-1} maltose, 4 g l^{-1} gellan gum supplemented with 2.26 μM 2,4-D, 2.68 μM NAA, and 0.88 μM BA. A desiccation treatment was used after maturation stage where a half-strength DCR basal medium with 60 g l^{-1} maltose, 37.84 μM abscisic acid (ABA), and 5 g l^{-1} gellan gum was used. The use of NAA in the medium for callus induction produced light white embryogenic callus, whereas the mixture of NAA, 2,4-D, and BA produced white friable embryogenic callus when apical dome sections were cultured on half DCR basal medium. In the maintenance medium, 79.2% of the shoot produced somatic embryos on 2 g l^{-1} gellan gum, while 1, 3, 4, and 5 g l^{-1} of gellan gum formed less than 7% of somatic embryos.

12.3.1.3 Plant Regeneration

Propagation of plants through plant tissue culture is very useful (Hammschlag et al. 1995). Callus production with *in vitro* techniques and plant regeneration are the first stages for plant manipulation (Islam et al. 2005). A research was carried out with *Sorghum bicolor* (L.) Moench variety Róna 1. As plant material, seeds were germinated for the obtaining of shoot tips and a basal medium used was MS supplemented with 2,4-D, Kin, proline, vitamin C, sucrose, and Bacto™ Agar. For the determination of the induction and regeneration of potential of calluses, the control medium was supplemented with CH, polyvinylpyrrolidone, honey, and sucrose; the explants were incubated in the dark. The best callus induction (80.0%) was obtained in the medium supplemented with honey and sucrose. For plant regeneration MS medium was also used with two treatments: (1) BAP and sucrose at 2.0 mg l^{-1} and 30 g l^{-1}, respectively, and (2) BAP and sucrose at 2.0 mg l^{-1} and 15 g l^{-1}, respectively, with honey (15 g l^{-1}). The medium with sucrose and honey led to better shoot regeneration from the calluses (Dreger et al. 2019).

Iriawati and Rodiansyah (2017) used basal shoot explants from 10-day old seedlings for the in vitro regeneration of foxtail millet (*Setaria italica* (L.) Beauv.). Basal MS medium was supplemented with two different concentrations of 2,4-D, Kin, 6 BAP, and 1.5 mg l^{-1} nickel sulfate ($NiSO_4$). The best shoot induction was achieved in MS basal medium supplemented with 0.5 mg l^{-1} Kin, 2 mg l^{-1} 6 BAP, and 0.1 mg l^{-1} 2,4-D with 60% of explants developing direct shoot organogenesis. Several light treatments (provided by blue, green, yellow, red, and clear cellophane film covers) were used by Mohamed et al. (2017) for the in vitro regeneration, growth, and proliferation of strawberry (*Fragaria* sp.) plants. They used leaf discs for shoot regeneration. Leaf discs were cultured in MS medium supplemented with 3% sucrose, 0.7% agar plus 6.9 μM TDZ; for shoot proliferation, shoot tip explants from the cultivars FES, SW, TD, Camarosa (CAM), and Gaviota (GA) were collected from 6-week old plantlets after removal of all leaves and roots, and they were placed on a similarly supplemented MS medium with 1.32 μM BA. For the rooting phase, explants and cultivars as in shoot proliferation were placed on supplemented MS with 4.9 μM IBA. Red and green light led induced the

best shoot regeneration (10 shoots explant^{-1}), and green light induced the highest frequency for shoot proliferation (15.3 shoots explant^{-1}). In the stage of root formation, the best results were obtained with white light followed by yellow or blue light. Blue and yellow light rendered high total chlorophyll content.

Balwinder et al. (2011) worked in an efficient protocol for *Citrus jambhiri* Lush. (rough lemon) using cotyledons as explants. They obtained a 91.66% of callus induction in MS medium supplemented with 2,4-D at 2 mg l^{-1} in combination with malt extract (ME) at 50 mg l^{-1}. For plant regeneration, calli were divided into small pieces and cultured in MS basal medium supplemented with BA at 3 mg l^{-1} where 87.50% of shoot regeneration was obtained. The regeneration and control of explants necrosis for an endemic tree of India named *Soymida febrifuga* (Roxb.) A. Juss., (Meliaceae) was investigated by Chiruvella et al. (2011). Nodal segments were cultured in MS basal medium supplemented with BA (2.0, 3.0, and 5.0 mg l^{-1}), Kin (1.0 2.0, and 3.0 mg l^{-1}), NAA (0.2 mg l^{-1}), and IAA (0.2 mg l^{-1}) with different combinations. The best result was observed with the combination in MS medium of BA (2 mg l^{-1}), and NAA (0.2 mg l^{-1}) where a frequency of 80.4% was obtained. The explant necrosis was controlled at 98% in MS medium supplemented with calcium nitrate (556 mg l^{-1}), calcium pantothenate (1.0 mg l^{-1}), activated charcoal (20 mg l^{-1}), and fructose (100 mg l^{-1}).

The species *P. kesiya* is a conifer of the family of the Pinaceae, specifically to the genus Pinus. Malabadi et al. (2005) worked with embryogenic cultures of this species using mature zygotic embryos with half of the MS germination basal medium with maltose, gellan gum, 2, 4-D and several concentrations of triacontanol (1, 2, 3 4, 5, 7, 10, 15, 20, 25, and 30 μg l^{-1}) where 10 μg l^{-1} (micrograms per liter) produced white-mucilaginous embryogenic callus. The white-mucilaginous embryogenic calli were subcultured in a medium with 2.0 μM 2,4-D and 2.0 μg l^{-1} triacontanol. Somatic embryos were cultured for germination in half-strength MS germination medium without growth regulators.

12.3.2 Plant Breeding

12.3.2.1 Marker-Assisted Selection

Genetic markers were used for the first time to determine the order of genes along chromosomes when Sturtevant (1913) made the first genetic map in *Drosophila melanogaster* (fruit fly). After that, Sax (1923) worked with *Phaseolus vulgaris* L. in the generation of gene linkage between seed color and size. Since those studies, genetic markers have changed from morphological traits to isozymes and finally to DNA markers; today they are used in many research areas such as plant breeding, characterization of plant germplasm, and others (Henry 2001). According to Jiang (2013) genetic markers can be classified into two categories: (1) classical markers where it is possible to find morphological markers, cytological markers, and biochemical markers and (2) DNA/molecular markers where some representative examples are: RFLP (restriction fragment length polymorphism), AFLP (amplified fragment length polymorphism), SSRs (simple sequence repeats), SNP

(single-nucleotide polymorphism), and DArT (diversity arrays technology). Morphological markers, as the name said, are used to differentiated qualities that can be seen, like the color of the flower, the structure of different seeds, and so on, and they do not need biochemical and molecular techniques or instruments for their study. Their principal disadvantage is that they are few, and can be influenced by several environmental factors and growth stages of the plant (Eagles et al. 2001). For the research of plant variation, these markers have been used for plant breeding (Weeden et al. 1994).

In cytology, the structural characteristics of chromosomes can be shown by the chromosomal karyotype and bands. Band patterns, which are shown in color, width, order, and position, reveal the difference in the euchromatin and heterochromatin distributions. For example, the Q bands are produced by quinacrine hydrochloride, the G bands are produced by Giemsa staining, and the R bands are the inverted G bands. These chromosomal referents points are used not only for the characterization of normal chromosomes and the detection of chromosomal mutation but also for physical mapping and identification of linkage groups. Physical maps based on morphological and cytological markers laid the groundwork for mapping genetic links with the help of molecular techniques. However, the direct use of cytological markers has been very limited in genetic mapping and plant breeding (Jiang 2013).

Biochemical markers (isozymes) are enzymes codified by several genes but with the same functions. They were used effectively in genetic diversity detection within the structure of the population. The disadvantages of these markers are that they are few; also, the polymorphism they detect is weak, and they can be affected by extraction methods, tissues, plant growth stages, biotic and abiotic stress (Paterson 1996; Baird et al. 1997; Henry 1997).

Molecular markers are based in the polymorphism present among the nucleotide sequences of any individual. That is, they can indicate the genetic differences between species and organisms (Henry 1997). These markers are handy because of their abundance, their neutrality (they are frequently located in non-coding regions of deoxyribonucleic acid-DNA), and because they are not affected by environmental factors and/or the plant growing phase (Winter and Kahl 1995). Some molecular markers useful in plant breeding are:

a. *Restriction Fragment Length Polymorphism (RFLP)*: This was the first marker used, and it is the only one based on hybridization. This marker was created by Botstein et al. (1980), and the polymorphism is due to insertions/deletions, point mutations, translocations, duplications, and inversions. For this technique, the DNA is extracted, purified, and mixed with restriction enzymes to excise DNA at recognition sites. For this technique, the DNA is extracted, purified, and mixed with restriction enzymes to excise DNA at recognition sites and the results are visualized in agarose or polyacrylamide gel electrophoresis (PAGE) were several bands (fragments with different length) are separated (Ni et al. 2002).
b. *Polymerase Chain Reaction (PCR)-based markers*: Kary Mullis in 1983 developed a new technique that made possible the synthesis of large amounts of DNA from a fragment without cloning: polymerase chain reaction. With this procedure, it is possible to synthesize millions of copies in a couple of hours, having then a

sufficient amount to study a sequence of interest representing only a ten-millionth part within a mixture of DNA as complex as the human genome itself (Mullis 1990).

c. *Randomly Amplified Polymorphic DNA (RAPD)*: This analysis was described by Williams et al. (1990), and it is based in DNA amplification by PCR using a single, short (10 nucleotides), and random primers. The amplified fragment depends on the length and size of both the primer and the target genome. The absence or presence of the band is corroborated in gel electrophoresis, and this is the confirmation of the polymorphism (Winter and Kahl 1995).
d. *Amplified Fragment Length Polymorphism (AFLP)*: These markers are a combination of RFLP and PCR markers; DNA is digested, and then the PCR is implemented (Farooq et al. 1996). The AFLP has the advantage that sequence information is not needed, turning it into a cost-effective technique. Two restriction enzymes are used to excise the DNA, and the fragments are then joined at each end by complementary adapters and subsequently amplified by PCR; sizes finally separate the products by electrophoresis (Ni et al. 2002).
e. *Simple Sequence Repeats (SSRs) or microsatellites*: SSRs are tandem repeat motifs of 1–6 nucleotides that abound in the genome of various taxa from prokaryotes and eukaryotes (Hancock 1999). The microsatellites are distributed in coding and non-coding regions and are characterized by being highly polymorphic in terms of their length; therefore, they are suitable regions to be used as molecular markers at the population level (Zane et al. 2002). This high polymorphism level is due to a high mutation rate because events of deletion and insertion during DNA replication and this polymorphism can be easily detected by PCR (Schlötterer 2000; Mohler and Schwarz 2005).

Molecular markers are handy for the study of genetic diversity in several crops. Kumar et al. (2008) studied genetic diversity in several accessions of beans (*P. vulgaris*) using RAPD, and they found that 95% of the amplified products were polymorphic, demonstrating a right quantity of variation at the DNA level among these accessions. AFLP markers were used by Dehmer and Hammer (2004) for characterization of the genetic diversity between 44 accessions of the *Solanum nigrum* L. complex, and through this research, they were able to classify taxonomically unknown material and to correlate the clustering of the examined accessions with their geographic origin.

Several tomato determinate and indeterminate inbred lines were collected from different countries (China, Japan, South Korea, and the USA) and for the diversity analysis, 35 SSR markers were used. Gene distances between 0.72 and 1 rs showed diversity at a moderate level and a significant number of alleles that are unique (Benor et al. 2008). Microsatellites SSR were used for the identification of genetic variation and characterization of 46 parthenocarpic genotypes of round zucchini shrub type squash (*Cucurbita pepo* L.), and the polymorphic loci were 100% with five groups identified (Méndez-López et al. 2019).

12.3.2.2 Genetic Engineering

According to Shetty et al. (2018), genetically modified crops (GMCs) are plants to which the DNA was modified using genetic engineering techniques (addition, deletion, or manipulation of nucleotides or genes) to obtain a change or a desired characteristic that cannot occur in nature. The process of generating a genetically modified crop can be divided into six stages: (1) identification and characterization of the desired gene, (2) incorporation of the gene of interest in a suitable genetic construction, (3) introduction of the development in plant cells, (4) selection of transformed plants, (5) regeneration of the whole plant from transformed cells, and (6) incorporation of the new GMC as a commercial variety (Gutiérrez et al. 2015). Several efficient protocols and methods are available to create genetically modified crops. Plant transformation techniques offer the possibility of accessing an unlimited number of genes that were previously not accessible to plant breeders. Specifically, for gene transfer from non-sexually compatible species, significantly increasing genetic improvement options are open (Basu et al. 2010). Some of the methods used are:

a. *Agrobacterium tumefaciens* mediated transfer: This method is well established, and it uses the bacteria *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes*. These bacteria contain a genetic element outside their chromosome, called the tumor-inducing (Ti) plasmid. The genes of biotechnological interest are introduced into the Ti plasmid so that the bacteria can transfer it into the plant. A segment is stably assigned to the chromosomes of the plant with which it is co-cultivated. This system can move large and intact parts of DNA with low copy numbers and stable integration. This method has been well tested in dicotyledonous plants like potatoes (*Solanum tuberosum* L.), tomatoes, and tobacco (*Nicotiana tabacum*) (Gutiérrez et al. 2015; Shetty et al. 2018).
b. Gene guns (Biolistic): This is a great method that uses gold or tungsten microparticles covered with DNA of interest, which is accelerated at high speed to the target tissues. The introduced DNA can reach the nucleus and insert stably. It is also possible to add sequences in chloroplasts or mitochondria for the expression of the proteins of interest in these organelles. This ability to transform organelles is very desirable in the generation of organisms expressing recombinant proteins or enzyme overproduction. Some disadvantages include low transformation efficiency and reported transgene-silencing due to multi-copy insertions (Shetty et al. 2018).
c. Clustered regulatory interspaced short palindromic repeats (CRISPR): This method uses short, repetitive base sequences present in segments of prokaryotic DNA, and the repetition is followed by a spacer DNA that is exposed to a foreign DNA (virus or plasmid) (Shetty et al. 2018).

The decision about the release of genetically modified plants is not directly related to commercialization because some of these plants have passed the regulatory procedures positively but never were released to the market (Baranski et al. 2019). Bt maize is a crop that expresses *Cry* protein, naturally produced by *Bacillus*

thuringiensis (Bt; a bacterium used as an insecticide since 1938); it is toxic to stem borer insects that die when eating Bt corn leaves or stalks (Bates et al. 2005; Kumar et al. 2016a). Qaim (2009) summarized studies of impacts related to Bt cotton (*Gossypium* spp.) (resistant to insects) in three regions of India, finding an increase in productivity of 37, 33, and 24% due to the improvement of that character. A research was done by Du et al. (2019) to eliminate a marker (gene *egfp*) in transgenic maize using a heat-inducible auto-excision vector that combines a site-specific recombinase. Consequently, transgenic maize plants free of the marker were obtained. Waltz (2015) from J R Simplot company obtained a potato (Innate potato) resistant to blackspot bruising browning and with less content of asparagine (an amino acid that is converted into acrylamide when the potato is fried). Weeds that are not desired in agriculture are a problem; herbicides like Roundup™ and Liberty Link™ are used to eliminate those unwanted plants (Bahadur et al. 2015). Roundup ready™ soybeans (*Glycine max*) contain genes conferring tolerance to glyphosate (an herbicide that kills weeds). In this way, this herbicide can be used without damage the crop (Padgette et al. 1996).

12.3.2.3 Genome Editing

The technology of recombinant DNA is a method used for genetic engineering where the exact place of a changed fragment of DNA in a host organism is difficult to locate. In genetic transformation, expertise is necessary because for every sequence to cut a new specific molecule must be created. The new genome editing technology (CRISPR-Cas9) solves this problem: CRISPR-Cas9 cuts DNA in different places, and the cell itself repairs DNA, turning this technology into a faster, more comfortable, less expensive, and more accurate procedure (Habets et al. 2019).

The discovery of sequence-specific nucleases (SSN) allowed the creation of modification at the genetic level and the regulation of DNA sequences in several organisms (Doudna and Charpentier 2014; Barrangou and Horvath 2017). The SSN can be reprogrammed to produce DSBs (DNA double-strand breaks) at a desired genomic location. Until now, four main classes of protein have been used for an accurate edition of the genome: mega nucleases (Smith et al. 2006; Paques and Duchateau 2007); zinc-finger nucleases (ZFN) (Maeder et al. 2008); TALEN (Transcription-Activator-Like Effector Nucleases) (Bogdanove and Voytas 2011), and several endonucleases derivate from CRISPR-Cas (Abudayyeh et al. 2016, 2017). The technologies from the first generation (ZFN, TALEN, and mega nucleases) are excision DNA systems, and the CRISPR-Cas endonucleases are excision systems of DNA and RNA (Ribonucleic acid) guided by programmable RNA (Langner et al. 2018).

a. Meganucleases: Mobile introns encode these enzymes, and they happen naturally (Smith et al. 2006; Paques and Duchateau 2007). They can identify new DNA target sites. An example of these enzymes is the I-SceI meganucleases from yeast (best-characterized) used in genome editing (Voytas 2013).

b. ZFNs: According to Voytas (2013) and Puchta and Fauser (2014) they were the first endonuclease created for the recognition and cleave of chromosomal DNA, and they are artificial bipartite enzymes with a length of ~310 amino acids.
c. TALENs: They are derived from TAL (transcription-activator-like) effectors of the bacterial plant pathogen. The construction of a new TALEN is hard and expensive; also, it is not appropriate for multiple gene editing because of the large size and the requirement of two proteins needed to identify antiparallel DNA strands (Voytas 2013).
d. CRISPR-Cas9 Nucleases: This technology only needs two components: (1) An endonuclease (Cas9) from a monomeric DNA, and (2) a single RNA sequence that is a guide which binds to the DNA target (Steinert et al. 2015).

New CRISPR Nucleases: This system has five target nucleases from DNA and two target nucleases from RNA (Mitsunobu et al. 2017; Koonin et al. 2017). The function of some of the target nucleases from DNA was proved in vivo and in vitro (Burstein et al. 2017; Stella et al. 2017). The activity of Cpf1 (Cas12a) was authenticated in plants using Cpf1 orthologs from a bacterium *Francisella novicida* that belongs to the Francisellaceae family, which consists of gram-negative pathogenic bacteria (FnCas12a), *Lachnospiraceae bacterium* ND2006 from an anaerobic family (Lachnospiraceae) (Nogue et al. 2014) (LbCas12a), and *Acidaminococcus* sp. bacterium belongs to the phylum Firmicutes (AsCas12a). An evaluation between these nucleases revealed that LbCas12a is more efficient than AsCas12a and FnCas12a (Wang et al. 2017a).

Examples of the use of CRISPR-Cas9 technology are the works in maize (*Zea mays* L.) and potato to obtain modified crops with homogenous starch composition rather than a mixture (amylose or amylopectin, not both). Granule-bound starch synthase I (GBSSI), the key enzyme required for amylose synthesis, has been targeted in tetraploid potato plants by transfecting protoplasts with preassembled Cas9/gRNA RNPs (Shure et al. 1983; Andersson et al. 2018).

The obtainment of pathogens-resistant crops is an essential aim in plant improvement. Wheat (*Triticum* sp.) plants resistant to *Blumeria graminis* f. sp. *tritici*, the fungal pathogen responsible for powdery mildew disease is one example (Wang et al. 2014). Also, cucumber (*Cucumis sativus* L.) plants resistant to Potyvirus were obtained using CRISPR/Cas9 technology (Chandrasekaran et al. 2016). Finally, disease resistance has also been achieved by using CRISPR/Cas9 in Wanjincheng orange (*Citrus sinensis* Osbeck) plants to target the promoter of the susceptibility gene CsLOB1, resulting in plants with enhanced resistance to citrus canker (Peng et al. 2017). Agrobacterium, in the genome editing topic, is more used for the creation of transgenic crops, and several efficient protocols of transformation and regeneration of plant species exist. In this context, agrobacterium can be used in tobacco leaves to express the CRISPR/Cas9; this allowed the recovering of edited plants with the non-transgenic genome, so 17% of the edited plants with improved genome using this method were non-transgenic (Chen et al. 2018).

12.4 Plant Biotechnology and Sustainability

12.4.1 Biotechnology and Food Production

It is estimated that by 2050 the world population will increase by a third, and for this reason, agricultural production enhances up to 70% will be necessary. Also, the demand for food and forage crops will double over the next 50 years. In 2008, the World Bank estimated that around 10 million people die each year from hunger and food diseases (Dixon and Tilson 2010). An immediate and efficient solution to those problems is plant biotechnology. Plant biotechnology is responsible for generating sufficient and healthy food, in addition to the fact that it has managed to transform the agricultural techniques to enhance plant production by increasing crop resistance to weather changes, pests and diseases, and other (Espinoza 2018; Kumar et al. 2016a, b, 2019). More than 50 years after the start of the application of biotechnology in agriculture, it remains a discussion about its benefits and social costs. The main target of the accusations has been transgenic crops because of their possible impacts on the environment and society (Altieri 2003). Other "softer" technologies have suffered minor criticism.

For more than three decades, discussion about if biotechnology is compatible and can support or not sustainable agriculture has gone on. Believers in plant biotechnology as a tool to transform agriculture in a more sustainable process underline that biotechnology increases crop production increase, while environmental impacts related to agriculture are reduced (Brookes and Barfoot 2018). The largest companies that produce genetically modified seeds (Monsanto, Dow Agrosciences, DuPont, Syngenta, Bayer) explain that biotechnology supports sustainability in agriculture because production can be increased through it as well as farmer incomes, also biotechnology can reduce some environmental impact in agriculture (pesticides). Similarly, the use of plant biotechnology will allow obtaining new varieties that can survive and produce under stress situation such as salinity and drought (Scientific American 2009). Several professors and researchers at universities and government executives defend the fact that using genetic modification methods can be friendly with sustainable agriculture and, under right situations, could be consistent with organic farming (Ronald 2008; Ronald and Adamchak 2008).

Plant genetic resources are essential for humanity, as they constitute the source for genetic improvement and obtaining new varieties of plant species. However, anthropic activity has caused a negative impact that led to a significant decrease in wild germ plasma. In the period from 1996 to 2004, the alarming amount of 8321 plant species entered the Red List of threatened species of the International Union for the Conservation of Nature and Natural Resources. It is estimated that this number increases every year in proportions not always calculable (Sarasan et al. 2006). Although authors such as Rao (2004) point out the annual loss of more than 15 million hectares of tropical forest, it is difficult to appreciate how many species (even still unknown to humanity) disappear with them. Biotechnology can help with

the protection of these endangered plant species by using tissue culture and the creation of in vitro germplasm banks.

One example of how useful plant biotechnology is for agriculture sustainability is the tissue culture of species from the genus. At present, 200–300 species form this genus have been described, and most of these live in American territory. Plants from this genus are associated with nitrogen-fixing bacteria. Lupinus synthesizes quinolizidine alkaloids (AQ) as part of a defense strategy against herbivores. Currently, Lupinus species find numerous applications, as a source of protein in food (Tapia and Fries 2007) and secondary metabolites with various biological activities (Fornasini et al. 2012), also improving the soil in crop rotation (Stepkowski et al. 2011). Another potential use of the species of the genus Lupinus is as green manure or in ecological restoration and reforestation programs (Ramírez-Contreras and Rodríguez-Trejo 2009). During the monitoring and collection of biological material, it was observed that the farmers of the Amecameca region in Mexico allow the establishment of Lupinus plants in their plots, as they "improve the crops." To validate this practice, the effect of the incorporation of the biomass of a native species, *L. bilineatus*, into a nut orchard was evaluated; results evidence that it provides the same amount of nitrogen (N_2) as the chickpea, natural manure used by nut producers (Figueroa-Rodríguez 2016). At the same time, the diversity of beneficial bacteria associated with the rhizosphere of *L. montanus* was evaluated, finding the presence of the genera *Pseudomonas*, *Serratia*, *Rahnella*, *Plantibacter*, *Microbacterium*, *Pantoea*, *Staphylococcus*, *Arthrobacter*, *Paenibacillus*, and *Chryseobacterium*, which have a close correlation with the phenology of the plant (López-Jaimes 2014). However, Mexican species are not cultivated, so their use as a source of secondary metabolites is limited. The working group from the Instituto Politécnico Nacional, Universidad Autonoma de Chihuahua (Facultad de Ciencias Agrícolas y Forestales), and some other Mexican institutions initiated studies to achieve in vitro propagation of these species and the creation of a germplasm bank; recently, a Science Basic CONACyT project was approved to work on these topics. Some preliminary and unpublished results with *L. montanus* and *L. campestris* have been obtained in multiplication (6 BAP and Kin) and conservation (mannitol) (Fig. 12.4).

The seeds of the Lupinus genus species undergo physical dormancy. According to Rodríguez and Rojo (1997), the germination of the seed of *L. montanus* improves after the application of pre-germinative treatments to soften the seed coat. Some species of this genus such as *L. campestris*, *L. bilineatus*, and also *L. montanus* have been tested with scarification treatments such as boiling water, H_2SO_4, and cut off the seed. The best results (unpublished) achieved in a multidisciplinary project from the Universidad Autónoma de Chihuahua, Facultad de Agronomía (Dra. Sandra Perez Álvarez and Lic. Edgar Omar Carrasco Rivera), and the Instituto Politécnico Nacional (CEPROBI) (Dr. Kalina Bermudez Torres) were with H_2SO_4 by 12 min in *L. montanus* and by 15 min in *L campestris*, *L. bilineatus*, and cut off the seed in the laminar flow chamber (Fig. 12.5).

Biofortification is another application of biotechnology in agriculture that influences in sustainability. This technology is based on the application of

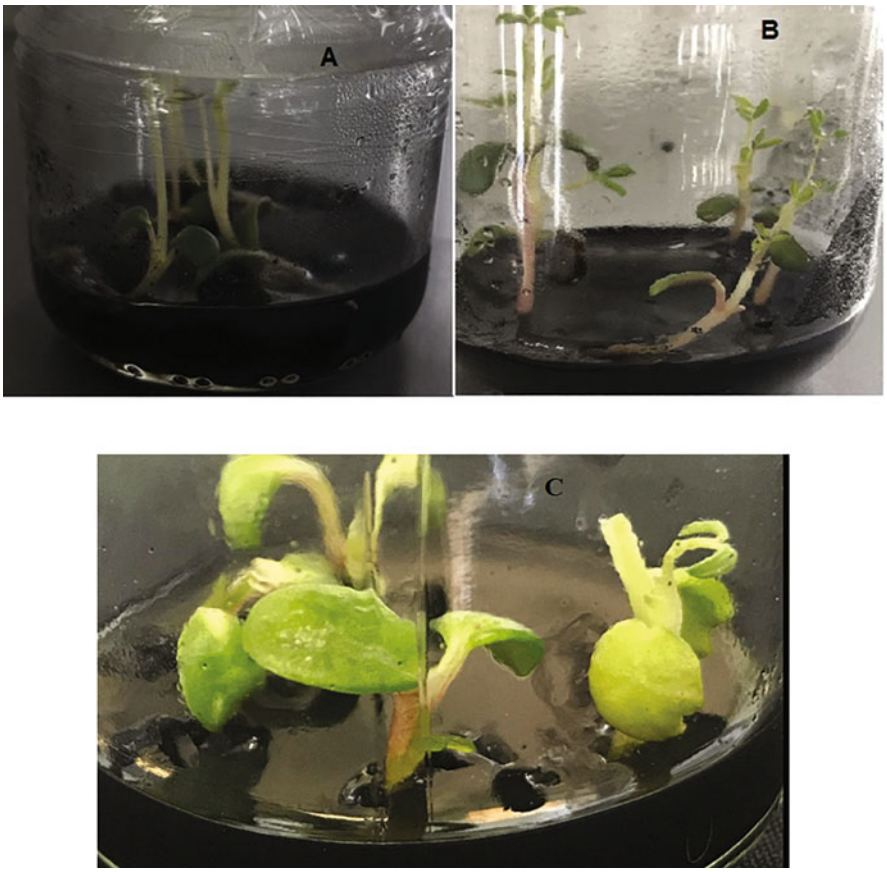

Fig. 12.4 Multiplication in MS medium supplemented with BAP 1 mg l^{-1} and kinetin (Kin) 0.5 mg l^{-1} and with activated carbon (**a**) *L. montanus*, (**b**) *L. campestris*, (**c**) Conservation medium with mannitol

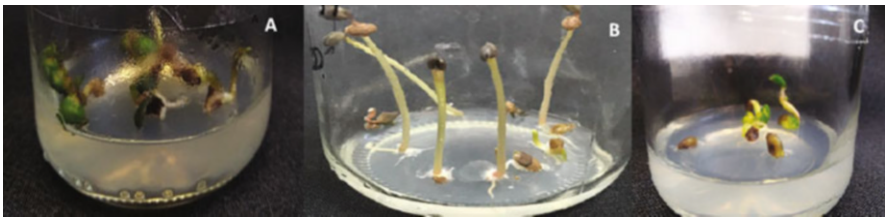

Fig. 12.5 Scarification methods of Lupinus seed with H_2SO_4, (**a**) *L. campestris*; (**b**) *L. bilineatus*; (**c**) *L. montanus*

micronutrients to crops like beans, rice (*Oryza sativa* L.), and wheat by using conventional plant breeding and biotechnology, making the basic food more nutritious, specifically in developing countries (Khush 2008). Children and women (because menstruation and childbirth) are the most vulnerable part of the population to the micronutrient deficiencies (Singh 2009). About half of the planet population suffers deficiencies of micronutrients such as Zn, Fe, and vitamins like vitamin A inducing to several symptoms related to impaired immune function, iron deficiency anemia, and xerophthalmia. The solution to this nutrition problem is to diversify the diet, but the poverty of these affected people makes it impossible; thus, biofortification of crops may take part in the solution (Jena et al. 2018). According to Bouis et al. (2017), some fortified foods include iodized salt, cooking oil, and sugar with added vitamin A, and iron biofortified flour, dairy foods, condiments, sugar, and salt.

12.4.2 Genetically Modified Organisms and Sustainability

The role of GMOs technology in the sustainable development of agriculture is yet debatable in many countries, mainly in topics like pests and diseases, drought, malnutrition, and food insecurity in developing countries (James 2014). According to Adenle et al. (2013), the GMOs technology, at that time, had not impacted notoriously on food security because of the debate about regulation of GMO products and also because of the disagreement surrounding the adoption of GMOs. The first genetically modified food authorized for human consumption was the Flavr Savr tomato in 1994. This tomato spoils more slowly than the conventional one, which allows farmers to collect the fruits when they are ripe, instead of before reaching maturity, unlike traditional tomatoes. However, it turned out to be a commercial failure (Weasel 2008). A tomato that can fight cancer because it contains three more times lycopene than conventional varieties was developed by Purdue University and the United States Department of Agriculture's Agricultural Research Service (Awais et al. 2010). Lycopene is a carotenoid pigment with antioxidant properties, and it can trap molecules that damage tissues in the human body, lowering the possibility of breast and prostate cancers.

Regardless of all the excellent results of biotechnology in agriculture, transgenic foods are still connected with a deficiency of information about their effects on the environment and human health (Boccia and Sarnacchiaro 2015).Altieri (2003) points out that although the declared end of plant biotechnology is the reduction of hunger, the planet generates enough food for it, and that the problem lies not in production, but the unequal distribution and the conversion of food production in agribusiness. One of the risk elements that most concern about GMOs is their effects on health, which have been widely speculated. Favorable or unfavorable positions can be assumed towards biotechnology, according to the optimistic or pessimistic views of those who do the analysis (Wilches 2010). However, there is already some research that points out the effects of transgenic products on health. Carman et al. (2013) studied the influence of transgenic foods in pigs that were fed with transgenic

soybean and corn; the autopsy of the pigs showed stomach inflammations 2.6 times higher than those of pigs that ate conventional soybean and corn. Also, female pigs fed with the mixture of genetic modified (GM) soybean and corn had significantly higher uteri than those who ate non-GM soybean and corn. According to the authors, this could be associated with different types of pathologies, several of them malignant, a situation that deserves more detailed studies. A very alarming element is that humans and pigs have very similar anatomical characteristics, particularly in the digestive system, so that more in-depth studies in humans should be carried out before further enhancing the consumption of transgenic foods (Héctor et al. 2016).

Bøhn et al. (2014) showed that high concentrations of glyphosate and aminomethylphosphonic acid (AMPA) accumulate in Roundup Ready soybeans from Monsanto Corporation. This is the result of glyphosate herbicide applications, which are not present in conventional soybeans or organic soybeans, as this species is susceptible to that herbicide. Besides, significantly lower levels of protein were found in transgenic soybeans and considerably higher concentrations of fatty acids that can lead to obesity compared to conventional and organic soybeans. Other herbicide-tolerant crops are those carrying the gene *pat* derived from the common soil bacterium *Streptomyces viridochromogenes* for the herbicide glufosinate ammonium (PPT). This herbicide inhibits the enzyme glutamine synthetase, which causes abnormal accumulation of ammonia in plants, and the acetylated form of PPT is inactive (Oberdoerfer et al. 2005). There are several crops displaying resistance such as sugar beet (*Beta vulgaris*), canola (*Brassica* spp.), soy, rice, and corn (CERA 2010).

While the results of modern biotechnology are unquestionable in terms of solving the scientific problem that they intend to, it is also true that behind these advances, a powerful profit motive move. In 2008 (the landscape had not changed) ten large companies controlled more than 30% of world seed trade, and five companies (AstraZeneca, DuPont, Monsanto, Novartis, and Aventis) had the control of 60% of the world pesticide trade, plus 20% of the seed market and practically 100% of the transgenic seed trade (De la Torre 2008). Under these conditions, it is impossible to coincide with García-Gonzáles et al. (2010) when they state that access to technology is no longer exclusive to developed countries and that everyone needs to recognize its potential and exploit it in all its dimensions. It is not enough to want to do it; we need to be able to do it (Héctor et al. 2016). Farmers that start to reduce the application of chemicals try to use other ecological practices even when they do not practice yet all sustainable technologies (Hubbell and Welsh 1998).

12.4.3 Biotechnology and Sustainable Agriculture

Biotechnology works with living organisms, so many public opinion and debates have been developed in this matter. According to Singh (2000), biotechnology, biodiversity, and sustainable agriculture are complementary to each other, independent and free of contradictions even when topics like food security and biosafety can be in illogicality. Genetic engineering through horizontal gene transfer is part of this

contradiction because it can be a threat to biodiversity and sustainability. The similarity between organisms gets to the dilution of smooth gene transfer risk (Singh 2000). The contribution of biotechnology to sustainable agriculture has been indifferent topics such as (Singh 2000):

- Resistance to biotic stresses has been increased (pest and diseases);
- Resistance to abiotic stresses has been improved (salinity, drought, cold);
- Solutions to soils contaminated with heavy metals (bioremediation, phytoremediation);
- Crops productivity and quality have grown;
- Fermentation technology has been improved;
- Nutrient uptake and efficient use have been increased, and nitrogen fixation has been enhanced; and some others.

In other words, biotechnology contributes to sustainable agriculture with the obtainment of crop resistance to pesticides which trends to reduce the dependence on agrochemicals; the production of crops would also achieve a reduction of chemicals fertilizers with better uptake and efficient use of nutrients; improvement of productivity and quality of crops increases market offers (Persley 2000). Biotechnology can include traditional and local knowledge, organic practices, tissue culture and genomic techniques; marker-assisted technology, transgenics, and others (Heinemann 2009). Causes of environmental degradation that biotechnology elude are poverty and socioeconomic differences. These causes also lead to political insecurity and social conflicts, resulting in more unsustainability. In the other hand, the actual tendency of biotechnology—development generally has been pro-rich and must of the results are applying in the private sector of developed countries—is not sustainable. The responsibility of promoting modern biotechnology in favor of poor people is in the hands of developing countries. Some of these contradictions respond to the fact that biotechnology stores high amounts of national resources for research and technology development at the cost of some of the conventional but vital programs (Singh 2000). Godfray et al. (2010a, b) mention the arguments that support using technology to boost yields are typically predicated on global models that project rising demand for food due to population growth and increasing affluence.

To transform countries that are not industrialized into industrialized ones, biotechnology techniques can offer support to guarantee sustainability and to decrease adverse environmental impacts that can occur. In agricultural business progress, for example, mediations might start from contributions and agricultural modernization, actual processing technologies, packing of delicate foods, the promotion of food safety in the processing and regulatory environment; and interventions to improve competitiveness and productivity (Lokko et al. 2018). Besides, in agriculture, the identification of species will require the sequence of the whole genome of each new species. Specifically, in entomology, the use of biotechnology is recommended to overcome the disadvantages of morphological identification, molecular techniques for insect identification have been adapted. Nowadays, most of the DNA extraction

protocols of insect tissues are based on the manual methods of CTAB, phenol-chloroform, SDS/proteinase K, or commercial kits available (Calderón-Cortés et al. 2010; Shams et al. 2011).

For PCR, use should be made of RFLP (Qin et al. 2008), DNA barcode (Yang et al. 2012), species-specific primer (SS-PCR) (Zhao et al. 2016), multiplex endpoint PCR, and qPCR TaqMan multiplex (Arif et al. 2015), among others. Currently, some molecular markers such as the cytochrome c oxidase subunit I (IOC), the 16S ribosomal RNA gene (16S rDNA), the internal transcribed spacer 2 (ITS2) and microsatellites have proven effective in identifying insect species (Li et al. 2011).

In recent years, the microarray technique has begun to be used for the identification of insects. The sequencing of two mitochondrial genes (IOC and ND2) and the ribosomal gene (ITS2) have been used to design species-specific probes. In addition to the gene chip method developed in that same way, it has allowed the identification of species of genera important ones such as Aedes, Anopheles, Armigeres, and Culex (Wang et al. 2017b). The developing countries should widely apply the results of the biotechnology. In this matter plants free of diseases obtained by tissue culture are used by small farmers in these countries. Another example is papaya plants resistant to the virus that was developed in Hawaii, which are used in developing countries (Serageldin 1999). In the Democratic Republic of the Congo, tissue culture is used in food security, and cassava clones free of diseases are propagated in Nigeria (FAO 2001). In Kenya, free-disease banana plants, obtained through tissue culture, help in the increase of yield and to protect incomes of farmers threatened because of the loss of commercial coffee crops. In Uganda, as a result of cooperation between the International Potato Center in Peru and Ugandan National Agricultural Research Organization, potato plants free of diseases are introduced and growth. All these are examples that imply biotechnology helping the poor and the hungry (Wambugu 2001). Rural populations poverty is mainly because the water resources are not enough; the crops yield is low, which leads to the deficient food supply, food insecurity, damaged environment, and hunger (Serageldin 1999). Biotechnology in many countries means economic development and social progress (DaSilva 1998) giving access to technology by credits, especially to poor rural farmers (Holaday 1999).

12.5 Conclusions

Since before the first products appeared on the market, high expectations had been created on the potential of the new biotechnology as a vital tool in the supply of food to a continuously growing human population. Agricultural genetic engineering has been considered as the spearhead of a new revolution capable of improving productivity by reducing costs, helping in the adoption of more environmentally friendly agricultural practices, and serving as a development engine for developing countries. At present, both the use of traditional techniques and innovative techniques to achieve sustainable agriculture are considered. An efficient agroecological approach requires the effective implementation of new technologies, which can be adjusted in

sustainable development programs, drawing various alternatives; the products obtained from biotechnology must serve to overcome different problems: diseases, pests, and environmental limitations of plant production.

12.6 Future Perspectives

Biotechnology is a tool that will continue to be used in agriculture due to its wide applications, many of which contribute to sustainability. Obtaining transgenic crops with all the necessary tests before releasing them to the market, can contribute to agricultural sustainability. These crops can resist high temperatures, frost, salinity, drought, pathogens (which implies the reduction in the application of pesticides), crops that require less chemical fertilizers, like pesticides that pollute the environment (soil, water, air). Tissue culture also opens paths in this matter because it is a technology that allows cloning plants with characteristics of resistance to the factors mentioned above. Also, biotechnology offers several gains for agricultural productivity that represent contributions to sustainability such as decreasing poverty and increasing food security in developing countries. Another technology that is already used, but is part of the future of agricultural sustainability is nanotechnology, which also helps to reduce contamination by applying chemicals for pest control and fertilizers at the nano level.

References

Abdelsalam A, Chowdhury K, El-Bakry A (2018) Efficient adventitious morphogenesis from *in vitro* cultures of the medicinal plant *Cymbopogon schoenanthus*. Plant Tissue Cult Biotechnol 28(2):147–160

Abraham A (2009) Agricultural biotechnology research and development in Ethiopia. Afr J Biotechnol 825(29):7196–7204

Abudayyeh OO, Gootenberg JS, Konermann S, Joung J, Slaymaker IM et al (2016) C2c2 is a single component programmable RNA-guided RNA-targeting CRISPR effector. Science 353:5573–5673

Abudayyeh OO, Gootenberg JS, Essletzbichler P, Han S, Joung J et al (2017) RNA targeting with CRISPR-Cas13. Nature 550:280–244

Adenle AA, Morris EJ, Govindan P (2013) Status of development, regulation and adoption of GM agriculture in Africa: Views and positions of stakeholder groups. Food Policy 42:159–166

Altieri MA (2003) Dimensiones éticas de la crítica agroecológica a la biotecnología agrícola. Acta Bioethica Año 9(1):47–61

Altieri MA, Nicholls CI (2017) Agroecology: a brief account of its origins and currents of thought in Latin America. Agroecol Sustain Food Syst 41(3-4):231–237

Altieri MA, Funes-Monzote FR, Petersen P (2012) Agroecologically efficient agricultural systems for smallholder farmers: contributions to food sovereignty. Agron Sustain Dev 32:1–13

Andersson M, Turesson H, Olsson N, Fält AS, Ohlsson P, Gonzalez-Matías N, Samuelsson M, Hofvander P (2018) Genome editing in potato via CRISPR-Cas9 ribonucleoprotein delivery. Physiol Plant 164:378–384

Arif M, Opit G, Mendoza-Yerbafria A, Dobhal S, Li Z, Kučerová Z, Ochoa-Corona FM (2015) Array of synthetic oligonucleotides to generate unique multi-target artificial positive controls and molecular probe-based discrimination of Liposcelis species. PLoS One 10(6):e0129810

Awais MA, Pervez A, Yaqub R, Sarwar F, Siraj S (2010) Current status of biotechnology in health. Am Eurasian J Agric Environ Sci 7(2):210–220
Bagwan JD, Patil SJ, Mane AS, Kadam VV, Vichare S (2010) Genetically modified crops: food of the future. Int J Adv Biotechnol Res 1(1):21–30
Bahadur S, Verma SK, Prasad SK, Madane AJ, Maurya SP, Gaurav VVK, Sihag SK (2015) Eco-friendly weed management for sustainable crop production-a review. J Crop Weed 11 (1):181–189
Baird V, Abbott A, Ballard R, Sosinski B, Rajapakse S (1997) DNA diagnostics in horticulture. In: Current topics in plant molecular biology: technology transfer of plant biotechnology. CRC Press, Boca Raton, pp 111–130
Balwinder SS, Singh GV, Kaur AN (2011) An efficient plant regeneration protocol from callus cultures of *Citrus jambhiri* Lush. Physiol Mol Biol Plants 17(2):161–169
Baranski R, Klimek-Chodacka M, Lukasiewicz A (2019) Approved genetically modified (GM) horticultural plants: A 25-year perspective. Folia Hortic 31(1):3–49
Barrangou R, Horvath P (2017) A decade of discovery: CRISPR functions and applications. Nat Microbiol 2:17092
Basu SK, Dutta M, Goyal A, Bhowmik PK, Kumar J, Nandy S, Scagliusi SM, Prasad R (2010) Is genetically modified crop the answer for the next green revolution? GM Crops 1(2):68–79
Bates SL, Zhao JZ, Roush RT, Shelton AM (2005) Insect resistance management in GM crops: past, present and future. Nat Biotechnol 23(1):57–62
Benor S, Zhang M, Wang Z, Zhang H (2008) Assessment of genetic variation in tomato (*Solanum lycopersicum* L.) inbred lines using SSR molecular markers. J Genet Genomics 35(6):373–379
Bestelmeyer BT, Okin GS, Duniway MC, Archer SR, Sayre NF, Williamson JC, Herrick JE (2015) Desertification, land use, and the transformation of global drylands. Front Ecol Environ 13 (1):28–36
Boccia F, Sarnacchiaro P (2015) Genetically modified foods and consumer perspective. Recent Pat Food Nutr Agric 7:28–34
Bogdanove AJ, Voytas DF (2011) TAL effectors: customizable proteins for DNA targeting. Science 333:1843–1846
Bøhn T, Cuhra M, Traavik T, Sanden M, Fagan J, Primicerio R (2014) Compositional differences in soybeans on the market: glyphosate accumulates in Roundup Ready GM soybeans. Food Chem 153:207–215
Botstein D, White RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage mapping man using restriction fragment length polymorphisms. Am J Hum Genet 32:314–331
Bouis H, Saltzman A, Low J, Ball AM, Covic MN (2017) An overview of the landscape and approach for biofortification in Africa. Afr J Food Agric Nutr Dev 17:11848–11864
Brookes G, Barfoot P (2018) GM crops: global socio-economic and environmental impacts 1996–2006. PG Economics Ltd, Dorchester, p 204
Brundtland GH (1987) Our common future. Oxford University Press, Oxford, p 300
Burstein D, Harrington LB, Strutt SC, Probst AJ, Anantharaman K, Thomas BC, Doudna JA, Banfield JF (2017) New CRISPR-Cas systems from uncultivated microbes. Nature 542:237–241
Buttel FH (2003) Internalising the societal costs of agricultural production. Plant Physiol 133:1656–1665
Calderón-Cortés N, Quesada M, Cano-Camacho H, Zavala-Páramo G (2010) A simple and rapid method for DNA isolation from xylophagous insects. Int J Mol Sci 11(12):5056–5064
Carman JA, Vlieger HR, Ver Steeg LJ, Sneller VE, Robinson GW, Clinch-Jones CA, Haynes JI, Edwards JW (2013) A long-term toxicology study on pigs fed a combined genetically modified (GM) soy and GM maize diet. J Org Syst 8(1):38–54
Casierra F, Aguilar OE (2007) Estrés por aluminio en plantas: Reacciones en el suelo, síntomas en vegetales y posibilidades de corrección. Una revisión. Rev Colomb Cienc Hortic 1(2):246–257
CERA (2010) GM crop database. ILSI Research Foundation, Washington DC

Chandrasekaran J, Brumin M, Wolf D, Leibman D, Klap C, Pearlsman M, Sherman A, Arazi T, Gal-On A (2016) Development of broad virus resistance in non-transgenic cucumber using CRISPR/Cas9 technology. Mol Plant Pathol 17:1140–1153

Chen L, Li W, Katin-Grazzini L, Ding J, Gu X, Li Y, Gu T, Wang R, Lin X, Deng Z et al (2018) A method for the production and expedient screening of CRISPR/Cas9-mediated non-transgenic mutant plants. Hortic Res 5:13

Chiruvella KK, Mohammed A, Dampuri G, Gopal RG (2011) *In vitro* shoot regeneration and control of shoot tip necrosis in tissue cultures of *Soymida febrifuga* (Roxb.) A. Juss. Plant Tissue Cult Biotechnol 21(1):11–25

CINU (2011) Alimentación. CINU. http://www.cinu.org.mx/temas.htm

Conway GR, Barbier EB (1990) After the green revolution. Sustainable agriculture for development. Earthscan, London, p 210

Dalgaar T, Hutchings NJ, Porter JR (2003) Agroecology, scaling and interdisciplinarity. Agric Ecosyst Environ 100:39–51

Dash A, Kundu D, Das M, Bose D, Adak S, Banerjee R (2016) Food biotechnology: a step towards improving nutritional quality of food for Asian countries. Recent Pat Biotechnol 10:43–57

DaSilva EJ (1998) Biotechnology, developing countries and globalization. World J Microbiol Biotechnol 14:463–486

De la Torre J (2008) Aspectos éticos del uso de los organismos modificados genéticamente (OMG) en la agricultura y alimentación. ICADE 73:141–169

Dehmer KJ, Hammer K (2004) Taxonomic status and geographic provenance of germplasm accessions in the *Solanum nigrum* L. complex: AFLP Data. Genet Resour Crop Evol 51:551–558

Dhlamini Z, Spillane C, Moss JP, Ruane J, Urquia N, Sonnino A (2005) Status of research and application of crop biotechnologies in developing countries. Food and Agriculture Organization of the United Nations Natural Resources Management and Environment Department, Rome, p 65

Dixon GR, Tilson EL (2010) Soil microbiology and sustainable crop production. Springer, Dordrecht, p 435

Dobbs T, Pretty JN (2004) Agri-environmental stewardship schemes and multifunctionality. Rev Agric Econ 26:220–237

Dominguez MS, Jiménez LG, Rosales CG, Quiñones CV, Delgadillo SD, Mireles SJ, Pérez EM (2008) El cultivo in vitro como herramienta para el aprovechamiento, mejoramiento y conservación de especies del género Agave. Investig Cienc 41:53–62

Doudna JA, Charpentier E (2014) Genome editing. The new frontier of genome engineering with CRISPR-Cas9. Science 346:1258096

Dreger M, Mól R, Deja A, Raj E, Mańkowska G, Wielgus K (2019) Improved plant regeneration in callus cultures of *Sorghum bicolor* (L.) Moench. In Vitro Cell Dev Biol Plant 55:190–198

Du D, Jin R, Guo J, Zhang F (2019) Construction of marker-free genetically modified maize using a heat-inducible auto-excision vector. Gene 10(374):1–17

Dyllick T, Hockerts K (2002) Beyond the business case for corporate sustainability. Business Strat Environ 11(2):130–141

EA (2005) Assessment of win–win case studies of resource management in agriculture. Bristol, EA and English Nature, p 132

Eagles HA, Bariana HS, Ogbonnaya FC, Rebetzke GJ, Hollamby GJ, Henry RJ, Henschke PH, Mcneil MD (2001) Implementation of markers in Australian wheat breeding. Crop Pasture Sci 52(12):1349–1356

Espinoza EFM (2018) Biotecnología para la agricultura moderna. Ciencia 69(4):1–6

FAO (2001) Action against undernutrition and poverty. Redirecting food assistance to those who need it most. In: The state of food insecurity in the world. http://www.fao.org/docrep/003/y1500e/y1500e05.htm#P0_0

FAO (2011) Biotechnologies for agricultural development. FAO, Rome, p 569

FAO (2014a) The water-energy-food nexus: a new approach in support of food security and sustainable agriculture. FAO, Rome, p 28
FAO (2014b) Anuario Estadístico: La alimentación y la agricultura en América Latina y el Caribe. https://www.fao.org/3/a-i3592s.pdf
FAO (2017) El futuro de la alimentación y la agricultura: Tendencias y desafíos. p 52, www.fao.org/publications
FAO-FIDA-PMA (2014) El estado de la inseguridad alimentaria en el mundo 2014: fortalecimiento de un entorno favorable para la seguridad alimentaria y la nutrición. FAO, Roma, p 62
Farooq S, Iqbal N, Arif M (1996) Detection of genetic variability in basmati and non-basmati rice varieties and their radiation induced mutants through random amplified polymorphic DNA (RAPD). International Atomic Energy Agency, Vienna (Austria); Food and Agriculture Organization of the United Nations, Rome (Italy); Proceedings series, p 748
Figueroa-Rodríguez MI (2016) Efecto de especies nativas y silvestres del género Lupinus sobre la fertilidad del suelo agrícola. Maestría en Ciencias en Desarrollo de Productos Bióticos, CeProBi-IPN, México, p 107
Firbank LG, Rothery P, May MJ, Clark SJ, Scott RJ, Stuart RC, Boffey CWH, Brooks DR, Champion GT, Haughton AJ, Hawes C, Heard MS, Dewar AM, Perry JN, Squire GR (2006) Effects of genetically modified herbicide-tolerant cropping systems on weed seedbanks in two years of following crops. Biol Lett 2:140–143
Firbank LG, Petit S, Smart S, Blain A, Fuller RJ (2008) Assessing the impacts of agricultural intensification on biodiversity: a British perspective. Philos Trans R Soc B 363:777–787
Fornasini M, Castro J, Villacres E, Narvaez L, Villamar M, Baldeon ME (2012) Hypoglycemic effect of *Lupinus mutabilis* in healthy volunteers and subjects with dysglycemia. Nutr Hosp 27 (2):425–433
García-Gonzáles R, Quiroz K, Carrasco B, Caligari P (2010) Plant tissue culture: current status, opportunities and challenges. Cien Investig Agrar 37(3):5–30
Gätjens-Boniche O, Acuña-Matamoros CL, Montero-Carmona W, Díaz C, Torres S (2018) Vanilla mass propagation and protocormic callus formation from root tips. PoliBotánica 45:157–180
Gerszberg A, Grzegorczyk-Karolak I (2019) Influence of selected antibiotics on the tomato regeneration in *in vitro* cultures. Notulae Bot Hortic Agrobot Cluj-Napoca 47(3):558–564
Gliessman SR (2005) Agroecology and agroecosystems. In: Pretty J (ed) The Earthscan reader in sustainable agriculture. Earthscan, London
Gliessman SR (2013) Agroecología: plantando las raíces de la resistencia. Agroecología 8(2):19–26
Godfray H, Charles J, Beddington JR, Crute IR, Haddad L, Lawrence D et al (2010a) Food security: the challenge of feeding 9 billion people. Science 327(5967):812–818
Godfray H, Charles J, Crute IR, Haddad L, Lawrence D, Muir JF et al (2010b) The future of the global food system. Philos Trans R Soc B Biol Sci 365(1554):2769–2777
Gregory PJ, Johnson SN, Newton AC, Ingram JSI (2009) Integrating pests and pathogens into the climate change/ food security debate. J Exp Bot 60:2827–2838
Gupta PK, Durzan DJ (1985) Shoot multiplication from mature trees of Douglas-fir and sugar pine. Plant Cell Rep 4:177–179
Gustavsson J, Cederberg C, Sonesson U (2011) Global food losses and food waste. Food and Agriculture Organization of the United Nations, Rome
Gutiérrez DF, Ruiz RM, Xoconostle BC (2015) Estado actual de los cultivos genéticamente modificados en México y su contexto internacional. Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, México, p 194
Haberl H, Schulz NB, Plutzar C, Erb HK, Krausmann F, Loib W, Moser D, Sauberer N, Weisz H, Zechmeister G, Zulka P (2004) Human appropriation of net primary production and species diversity in agricultural landscapes. Agric Ecosyst Environ 102:213–218
Habets M, van Hove L, van Est R (2019) Genome editing in plants and crops – Towards a modern biotechnology policy focused on differences in risks and broader considerations. Rathenau Institute, The Hague, p 48

Hammschlag F, Ritchie D, Werner D, Hashmil G, Krusberg L, Meyer R, Huettel R (1995) *In vitro* selection of disease resistance in fruit trees. Acta Hortic 392:19–26
Hancock JM (1999) Microsatellites and other simple sequences: genomic context and mutational mechanisms. In: Goldstein DB, Schlötterer C (eds) Microsatellites, evolution and applications. Oxford University Press, Oxford, pp 1–10
Hans BV, Colaco VM (2019) Sustainable agriculture and economic growth. JETIR 6(1):483–512
Héctor E, Pérez-Álvarez S, Moreira R, Millet B (2016) Perspectivas futuras e impacto social de las biotecnologías vegetales. Alternativas 17(2):44–51
Héctor E, Millet B, Torres A, Fosado O (2018) Agricultura en Sudamérica: La huella ecológica y el futuro de la producción agrícola. Rev Chakiñan 5:90–101
Heinemann J (2009) Hope not hype: The future of agriculture guided by the international assessment of agricultural knowledge, science and technology for development. Third World Network, Penang
Henry RJ (1997) Practical applications of plant molecular biology. Chapman & Hall, London, p 280
Henry RJ (2001) Plant genotyping: the DNA fingerprinting of plants. CABI Publishing, Oxford, pp 239–249
Holaday JW (1999) Competing in the new millennium: challenges facing small biotechnology firms. Congressional Testimony -Subcommittee on Technology, 27 October, 1999. http:www.house.gov/science/holaday-102799.htm
Huang J, Tichit M, Poulot M, Darly S, Li S, Petit C, Aubry C (2015) Comparative review of multifunctionality and ecosystem services in sustainable agriculture. J Environ Manag 149:138–147
Hubbell B, Welsh R (1998) Transgenic crops: engineering a more sustainable agriculture? Agric Hum Values 15:43–56
Iriawati PMR, Rodiansyah A (2017) *In vitro* regeneration of foxtail millet (*Setaria italica* (L.) Beauv.) cv. Buru Hotong. J Math Fund Sci 49(2):171–180
ISAAA (2016) Global status of commercialized biotech/ GM crops: ISAAA brief 52. ISAAA, Ithaca
Islam MM, Ahmed M, Mahaldar D (2005) *In vitro* callus induction and plant regeneration in seed explants of rice (*Oryza sativa* L.). Res J Agric Biol Sci 1:72–75
James C (2014) Global status of commercialized biotech/GM crops: 2014, executive summary. Dedicated to the late Nobel Peace Laureate, Norman Borlaug, (ISAAA Brief 49), p. 32 http://www.isaaa.org/resources/publications/briefs/49/executivesummary/default.asp and top ten facts: http://www.isaaa.org/resources/publications/briefs/49/toptenfacts/default.asp and http://www.askforce
Jena J, Sethy P, Jena T, Ranjan SM, Kumar SS, Kumar GD, Palai JB (2018) Rice biofortification: a brief review. J Pharmacogn Phytochem 7(1):2644–2647
Jiang GL (2013) Molecular markers and marker-assisted breeding in plants. In: Andersen SB (ed) Plant breeding from laboratories to fields. InTech, Rijeka, pp 45–83
Keong C, Nordin FA, Bhatt A, Keng C (2018) Culture medium and varietal selection for establishment of callus culture of *Artemisia annua*. Educ JSMT 5(2):67–76
Khush GS (2008) Biofortification of crops for reducing malnutrition. Proc Indian Natl Sci Acad 74 (1):21–25
Koonin EV, Makarova KS, Zhang F (2017) Diversity, classification and evolution of CRISPR-Cas systems. Curr Opin Microbiol 37:67–78
Kumar V, Sharma S, Kero S, Sharma S, Sharma AK, Kumar M, Bhat KV (2008) Assessment of genetic diversity in common bean (*Phaseolus vulgaris* L) germplasm using amplified fragment length polymorphism (AFLP). Sci Hortic 116:138–143
Kumar S, Kakraliya SK, Lakhran H, Sheoran S, Ram M, Meena RS (2016a) Use of *Bacillus thuringiensis* (Bt) Gene: a biotechnological tool for crop improvement. Prog Res 11:6247–6251
Kumar S, Sheoran S, Kakraliya SK, Kumar P, Meena RS (2016b) Drought: A challenge for Indian farmers in context to climate change and variability. Prog Res 11:6243–6246

Kumar S, Meena RS, Jakhar SR, Jangir CK, Gupta A, Meena BL (2019) Adaptation strategies for enhancing agricultural and environmental sustainability under current climate. In: Meena RS (ed) Sustainable agriculture. Scientific Publisher, Jaipur, pp 226–274

Langner T, Kamoun S, Belhaj K (2018) CRISPR crops: Plant genome editing toward disease resistance. Annu Rev Phytopathol 56:22.1–22.34

Levitus G, Echenique V, Rubinstein C, Hoop E, Mroginski L (2010) Biotecnología y mejoramiento vegetal II. ArgenBio. Consejo Argentino para la Información y el Desarrollo de la Biotecnología, Argentina, p 650

Li ZH, Kučerová Z, Zhao S, Stejskal V, Opit G, Qin M (2011) Morphological and molecular identification of three geographical populations of the storage pest *Liposcelis bostrychophila* (Psocoptera). J Stored Prod Res 47(3):168–172

Lokko Y, Heijde M, Schebesta K, Scholtés P, Van Montagu M, Giacca M (2018) Biotechnology and the bioeconomy-Towards inclusive and sustainable industrial development. New Biotechnol 40:5–10

López-Jaimes EL (2014) Diversidad de bacterias asociadas a la rizosfera de *Lupinus montanus* Kunth. Tesís de Maestría en Ciencias. Instituto Politécnico Nacional, Morelos

Maeder ML, Thibodeau-Beganny S, Osiak A, Wright DA, Anthony RM (2008) Rapid "opensource" engineering of customized zinc-finger nucleases for highly efficient gene modification. Mol Cell 31:294–301

Malabadi RB, Choudhury H, Tandon P (2004) Initiation, maintenance and maturation of somatic embryos from thin apical dome sections in *Pinus kesiya* (Royle ex. Gord) promoted by partial desiccation and Gellan gum. Sci Hortic 102:449–459

Malabadi RB, Mulgund GS, Nataraja K (2005) Plant re generation via somatic embryogenesis in *Pinus kesiya* (Royle ex. Gord.) influenced by triacontanol. Acta Physiol Plant 27(4A):531–537

Malabadi RB, Teixeira da Silva JA, Nataraja K, Vijaykumar S, Mulgund SG (2011a) Induction of somatic embryogenesis in mango (*Mangifera indica* L.). Int J Biol Technol 2(2):12–18

Malabadi RB, Vijaya SK, Mulgund GS, Nataraja K (2011b) Induction of somatic embryogenesis in Papaya (*Carica papaya*). Res Biotechnol 2(5):40–55

McNeely JA, Scherr SJ (2003) Ecoagriculture. Island Press, Washington, DC, p 319

MEA (2005) Ecosystems and human well-being. Island Press, Washington, DC, p 155

Meena RS, Kumar V, Yadav GS, Mitran T (2018) Response and interaction of Bradyrhizobium japonicum and Arbuscular mycorrhizal fungi in the soybean rhizosphere: a review. Plant Growth Regul 84:207–223

Meena RS, Kumar S, Bohra JS, Lal R, Yadav GS, Pandey A (2019) Response of alley cropping-grown sesame to lime and sulphur on yield and available nutrient status in an acidic soil of Eastern India. Energy Ecol Environ 4:65–74

Meena RS, Kumar S, Datta R, Lal R, Vijayakumar V, Brtnicky M, Sharma MP, Yadav GS, Jhariya MK, Jangir CK, Pathan SI, Dokulilova T, Pecina V, Marfo TD (2020a) Impact of agrochemicals on soil microbiota and management: a review. Land 9(34):1–22. https://doi.org/10.3390/land9020034

Meena RS, Lal R, Yadav GS (2020b) Long term impacts of topsoil depth and amendments on soil physical and hydrological properties of an Alfisol in Central Ohio, USA. Geoderma 363:1141164

Méndez-López A, Villanueva-Verduzco C, Rojas-Martínez RI, Sahagún-Castellanos J, Colinas-León MT, Sánchez-Vega M (2019) Genetic diversity of parthenocarpic genotypes shrubby squash using molecular genetic markers. Rev Mex Cienc Agríc 10(1):1–9

Mitsunobu H, Teramoto J, Nishida K, Kondo A (2017) Beyond native Cas9: manipulating genomic information and function. Trends Biotechnol 35:983–996

Mohamed FH, Omar GF, Ismail MA (2017) *In vitro* regeneration, proliferation and growth of strawberry under different light treatments. Acta Hortic 1155:361–368

Mohler V, Schwarz G (2005) Genotyping tools in plant breeding: From restriction fragment length polymorphisms to single nucleotide polymorphisms. In: Molecular marker systems in plant breeding and crop improvement. Springer, Cham, pp 23–38

Mor-Mussery A, Leu S, Budovsky A, Lensky I (2015) Plant-soil interactions and desertification: a case study in the Northern Negev, Israel. Arid Land Res Manag 29(1):85–97
Mullis KB (1990) The unusual origin of the polymerase chain reaction. Sci Am 262(4):56–61
Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497
Ni J, Colowit PM, Mackill DJ (2002) Evaluation of genetic diversity in rice subspecies using microsatellite markers. Crop Sci 42(2):601–607
Nogue PY, Alberti A, Weissenbach J, Salanoubat M, Tolonen AC (2014) Functional diversity of carbohydrate-active enzymes enabling a bacterium to ferment plant biomass. PLoS Genet 10 (11):1–12
Norton BG (1995) Evaluating ecosystem states: two competing paradigms. Ecol Econ 14:113–127
Oberdoerfer RB, Shillito RD, De Beuckeleer M, Mitten DH (2005) Rice (*Oryza sativa* L.) containing the bar gene is compositionally equivalent to the nontransgenic counterpart. J Agric Food Chem 53:1457–1465
Olsson P, Folke P (2001) Local ecological knowledge and institutional dynamics for ecosystem management: a study of Lake Racken watershed, Sweden. Ecosystems 4:85–104
Ostrom E (1990) Governing the commons: the evolution of institutions for collective action. Cambridge University Press, New York, p 298
Padgette SR, Re DB, Barry GF, Eichholtz DE, Delannay X, Fuchs RL et al (1996) New weed control opportunities: development of soybeans with a Roundup Ready™ gene. Herbicide Resist Crops 2009:53–84
Paques F, Duchateau P (2007) Meganucleases and DNA double-strand break-induced recombination: perspectives for gene therapy. Curr Gene Ther 7:49–66
Parfitt J, Barthel M, Macnaughton S (2010) Food waste within food supply chains: quantification and potential for change to 2050. Philos Trans R Soc B 365:3065–3081
Paterson AH (1996) Making genetic maps. In: Paterson AH (ed) Genome mapping in plants. R G Landes Company, Austin, pp 23–39
Peng A, Chen S, Lei T, Xu L, He Y, Wu L, Yao L, Zou X (2017) Engineering canker-resistant plants through CRISPR/Cas9-targeted editing of the susceptibility gene CsLOB1 promoter in citrus. Plant Biotechnol J 15:1509–1519
Persley GJ (2000) Agricultural biotechnology and the poor: promethean science. In: Persley GJ, Lantin MM (eds) Agricultural biotechnology and the poor: Proceedings of an international conference Washington DC, 21-22 October 1999. Washington, Consultative Group on International Agricultural Research, pp 1–12
Pierik RLM (1987) *In vitro* culture of higher plants. Springer, Dordrecht, p 348
Pretty J (2003) Social capital and the collective management of resources. Science 302:1912–1915
Pretty J (2008) Agricultural sustainability: concepts, principles and evidence. Philos Trans R Soc B 363:447–465
Pretty J, Ward H (2001) Social capital and the environment. World Dev 29:209–227
Pretty JN, Toulmin C, Williams S (2011) Sustainable intensification in African agriculture. Int J Agric Sustain 9(1):5–24
Puchta H, Fauser F (2014) Synthetic nucleases for genome engineering in plants: prospects for a bright future. Plant J 78:727–741
Qaim M (2009) The economics of genetically modified crops. Ann Rev Resour Econ 1:665–694
Qin M, Li ZH, Kucerova Z, Cao Y, Stejskal V (2008) Rapid discrimination of the common species of the stored product pest Liposcelis (Psocoptera: Liposcelididae) from China and the Czech Republic, based on PCR-RFLP analysis. Eur J Entomol 105(4):713
Raintree JB, Warner K (1986) Agroforestry pathways for the intensification of shifting cultivation. Agrofor Syst 4:39–54
Ramírez-Contreras A, Rodríguez-Trejo DA (2009) Plantas nodriza en la reforestación con *Pinus hartwegii* Lindl. Revista Chapingo. Ser Cienc Forest Ambiente 15(1):43–48

Ramirez-Mozqueda MA, Iglesias Andreu LG (2017) Vanilla (*Vanilla planifolia* Jacks.) cell suspension cultures: establishment, characterization, and applications. Biotechnology 7 (4):242–248
Rao NK (2004) Plant genetic resources: Advancing conservation and use through biotechnology. Afr J Biotechnol 3(2):136–145
Reichert JM, Carneiro TJ, Reinert DJ, Rodrigues MF, Sanchez LEA (2016) Land use effects on subtropical, sandy soil under sandyzation/desertification processes. Agric Ecosyst Environ 233:370–380
Rodríguez DA, Rojo C (1997) Estudio de la semilla del arbusto *Lupinus montanus* H.B.K. (Leguminosae). Rev Chapingo Ser Cienc Forest 3:39–45
Ronald P (2008) The new organic. The Boston Globe, March 16. Available at http://www.boston.com/bostonglobe
Ronald P, Adamchak R (2008) Tomorrow's table: Organic farming, genetics, and the future of food. Oxford University Press, New York, p 228
Royal Society (2009) Reaping the benefits: science and the sustainable intensification of global agriculture. The Royal Society, London, p 86
Sarasan V, Cripps R, Ramsay MM, Atherton C, McMichen M, Prendergast G, Rowntree JK (2006) Conservation in vitro of threatened plants-progress in the past decade. In Vitro Cell Dev Biol Plant 42:206–214
Sax K (1923) The association of size differences with seed-coat pattern and pigmentation in *Phaseolus vulgaris*. Genetics 8:552–560
Schlötterer C (2000) Evolutionary dynamics of microsatellite DNA. Chromosoma 109:365–371
Scientific American (2009) Biotech's plans to sustain agriculture. Sci Am 301(4):86–94
Serageldin I (1999) Biotechnology and food security in the 21st century. Science 285:387–389
Shams SS, Vahed SZ, Soltanzad F, Kafil V, Barzegari A, Atashpaz S, Barar J (2011) Highly effective DNA extraction method from fresh, frozen, dried and clotted blood samples. Bioimpacts 1(3):183
Shetty MJ, Chandan K, Krishna HC, Aparna GS (2018) Genetically modified crops: an overview. J Pharmacogn Phytochem 7(1):2405–2410
Shure M, Wessler S, Fedoro N (1983) Molecular identification and isolation of the Waxy locus in maize. Cell 35:225–233
Singh RB (2000) Biotechnology, biodiversity, and sustainable agriculture: a contradiction. http://www.bic.searca.org/seminar_proceedings/Bangkok/2000/Hplenary_papers/singh.pdf
Singh MV (2009) Effect of trace element deficiencies in soil on human and animal health. Bull Indian Soc Soil Sci 27:75–101
Smith P (2013) Delivering food security without increasing pressure on land. Glob Food Secur 2:18–23
Smith J, Grizot S, Arnould S, Duclert A, Epinat JC, Chames P, Prieto J, Redondo P, Blanco FJ, Bravo J, Montoya G, Pâques F, Duchateau P (2006) A combinatorial approach to create artificial homing endonucleases cleaving chosen sequences. Nucleic Acids Res 34:e149
Steinert J, Schiml S, Fauser F, Puchta H (2015) Highly efficient heritable plant genome engineering using Cas9 orthologues from *Streptococcus thermophilus* and *Staphylococcus aureus*. Plant J 84:1295–1305
Stella S, Alcon P, Montoya G (2017) Class 2 CRISPR-Cas RNA-guided endonucleases: Swiss Army knives of genome editing. Nat Struct Mol Biol 24:882–892
Stepkowski T, Zak M, Moulin L, Króliczak J, Golińska B, Narożna D, Safronova V, Mądrzak CJ (2011) *Bradyrhizobium canariense* and *Bradyrhizobium japonicum* are the two dominant Rhizobium species in root nodules of lupin and serradella plants growing in Europe. Syst Appl Microbiol 34(5):368–375
Sturtevant AH (1913) The linear arrangement of six sex-linked factors in Drosophila, as shown by their mode of association. J Exp Zool 14:43–59
Swift MJ, Izac AMN, van Noordwijk M (2004) Biodiversity and ecosystem services in agricultural landscapes-are we asking the right questions? Agric Ecosyst Environ 104:113–134

Tapia ME, Fries AM (2007) Guía de Campo de los cultivos andinos. FAO-ANPE, Lima
Tegtmeier EM, Duffy MD (2004) External costs of agricultural production in the United States. Int J Agric Sustain 2:1–20
Tilman D, Balzer C, Hill J, Befort BL (2011) Global food demand and the sustainable intensification of agriculture. Proc Natl Acad Sci U S A 108:20260–20264
Tisserat B, Esan E, Murashige T (1979) Somatic embryogenesis in angiosperms. Hortic Rev 1:1–78
Touchell D, Smith J, Ranney TG (2008) Novel applications of plant tissue culture. Comb Proc Int Plant Propagat Soc 58:196–199
United Nations Environment Programme (2002) State of the environment and policy retrospective: 1972–2002. In: United Nations Environment Programme (ed) Global environment outlook 3. United Nations, New York, pp 1–30
Velten S, Leventon J, Jager N, Newig J (2015) What is sustainable agriculture? A systematic review. Sustainability 7:7833–7865
Voytas DF (2013) Plant genome engineering with sequence-specific nucleases. Annu Rev Plant Biol 64:327–350
Waltz E (2015) USDA approves next-generation GM potato. Nat Biotechnol 33:12–13
Wambugu F (2001) Modifying Africa: how biotechnology can benefit the poor and hungry, a case study from Kenya. In: Action against undernutrition and poverty, Nairobi, Kenya, p 76
Wang Y, Cheng X, Shan Q, Zhang Y, Liu J, Gao C, Qiu J-L (2014) Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. Nat Biotechnol 32:947–951
Wang M, Mao Y, Lu Y, Tao X, Zhu JK (2017a) Multiplex gene editing in rice using the CRISPR-Cpf1 system. Mol Plant 10:1011–1013
Wang X, Tu WC, Huang EJ, Chen YH, Chen JH, Yeh WB (2017b) Identification of disease-transmitting mosquitoes: Development of species-specific probes for DNA Chip Assay using mitochondrial COI and ND2 genes and ribosomal internal transcribed spacer 2. J Med Entomol 54(2):396–402
Weasel LH (2008) Food fray: inside the controversy over genetically modified food. AMACOM, New York, p 258
Weeden N, Timmerman G, Lu J (1994) Identifying and mapping genes of economic significance. Expanding the production and use of cool season food legumes. Curr Plant Sci Biotechnol Agric 19:726–737
Wilches AM (2010) La biotecnología en un mundo globalizado. Rev Colomb Bioet 5(2):164–169
Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535
Winter P, Kahl G (1995) Molecular marker technologies for plant improvement. World J Microbiol Biotechnol 11(4):438–448
Yadav GS, Lal, R, Meena RS (2020) Vehicular traffic effects on hydraulic properties of a Crosby silt loam under a long-term no-till farming in Central Ohio, USA. Soil Till Res. https://doi.org/10.1016/j.still.2020.104654
Yang Q, Kučerová Z, Li Z, Kalinović I, Stejskal V, Opit G, Cao Y (2012) Diagnosis of Liposcelis entomophila (Insecta: Psocodea: Liposcelididae) based on morphological characteristics and DNA barcodes. J Stored Prod Res 48:120–125
Yeung E, Rahman H, Thorpe T (1996) Comparative development of zygotic and microspore-derived embryos in *Brassica napus* L. cv Topas. I. Histodifferentiation. Int J Plant Sci 157 (1):27–39
Yildiz M (2012) The prerequisite of the success in plant tissue culture: High frequency shoot regeneration. In: Recent advances in plant *in vitro* culture. IntechOpen, Budapest, pp 63–90
Zane L, Bargelloni L, Patarnello T (2002) Strategies for microsatellite isolation: a review. Mol Ecol 11:1–16
Zhao ZH, Cui BY, Li ZH, Jiang F, Yang QQ, Kučerová Z, Li FJ (2016) The establishment of species-specific primers for the molecular identification of ten stored-product psocids based on ITS2 rDNA. Sci Rep 6:21022