Microbial Genes, Enzymes, and Metabolites: To Improve *Rhizosphere* and Plant Health Management

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Bahman Fazeli-Nasab and Ahmad Farid Rahmani

Abstract

In today's world, there are other important problems, such as climate change and environmental problems and the loss of genetic resources, in addition to the issue of human societies supplying food and creating more food. One of the reasons being the improper use of chemical products in agriculture (such as pesticides and chemical fertilizers, etc.). In recent decades, the growing population of the world and the growing market for food have led to a serious and imminent change from conventional agriculture to advanced agriculture in the agricultural and food sciences and to the use of modern genetic technologies in the production of crops and livestock. The application of the techniques of genetic evolution and molecular genetics in the use of microorganisms and microbial genes to improve the amount and efficiency of goods, on the one hand, and, on the other hand, to minimize costs and processing time, has made the use of these techniques very useful in the different branches of agriculture. So far, microorganisms have been used in different sectors of agriculture as follows; reducing the toxicity of antibiotics and herbicides (beta-lactam gene), production of fungicides and biocides (chitinase gene), resistance to pathogenic bacteria (WRKY gene family), resistance to citrus bacterial canker (pthA gene), dissolution of soil phosphorus (gabY, Mps, pKKY, pKG3791 and OlpA-Cm genes), tolerance to abiotic stresses (Flavdex gene (Fld), PR5 gene family), coexistence with plants in water and mineral production (mycorrhizal fungus), and editing the plant genome (CRISPR/

B. Fazeli-Nasab (✉)
Research Department of Agronomy and Plant Breeding, Agricultural Research Institute, University of Zabol, Zabol, Iran
e-mail: bfazeli@uoz.ac.ir

A. F. Rahmani
Department of Horticulture, Faculty of Agriculture, Herat University, Herat, Afghanistan

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Cas9 gene). Various genes have also been used in the removal of soils contaminated with heavy metals and herbicides (*atzA*, *atzB*, *atzC*, *atzD*, *atzE* and *atzF*, *BPDO*, *CotA,* and *merA* genes). Production of sugar biopolymers (Asr gene), production of biofilms, production of dietary supplements for oil enrichment (fatty acyl-ACP thioesterase gene), development of immunity against gas spoilage (*alfa-Toxin* gene), bioethanol synthesis (*Cel6B* gene), Baker's yeast engineered to promote the bakery industry, and engineered yeasts for the production of engineering and industrial alcohol that have also been controlled by other genes. We also attempted to review in this chapter the form and manner in which microbial genes are used directly and indirectly to improve the quantity and efficiency of agricultural products.

Keywords

Beta-lactam · Chitinase · CRISPER · Flavodoxin Flv · Laccase · WRKY gene family

Abbreviations

ABC	ATP-binding cassette
AMF	Arbuscular mycorrhizal fungi
ASR	Alternate sucrase
BPDO	Biphenyl dioxygenase
CRISPR	Clustered regularly interspaced short palindromic repeats
crRNA	CRISPR RNA
Cry1Ac protoxin	Is a crystal protein produced by the gram-positive bacterium, Bacillus thuringiensis (*Bt*) during sporulation
DDD	Domain-driven design
DDT	Dichlorodiphenyltrichloroethane
GDH	Glucose dehydrogenase holoenzyme
GMOs	Genetically modified organisms
HPLC	High-performance liquid chromatography
LMOs	Living modified organisms
PAL	Phenylalanine ammonialyase
PPP1	Phosphoprotein phosphatase 1
PQQ	Pyrroloquinoline quinine
PthA	*Pathogenicity gene*
T-DNA	Transfer DNA
TracrRNA	Small trans-encoded RNA
WRKY gene family	The length of the WRKY domain is approximately 60 amino acids long and also they have one or two DNA binding domains that contain the conserved heptapeptide WRKYGQK and also they are responsible for the recognition with W-box sequence "(C/T)TGAC(T/C)"

19.1 Introduction

Food availability will be one of the most significant obstacles ahead as the population rises. Although the green revolution could help people get the food they need, but with the increasing population and the need for more food, the need for another green revolution is felt, with greater focus on environmental values and resource management and preservation, so that food will rise by 50% over the next 20 years (Khan et al. 2009). The Green Revolution, which was produced with the advent and delivery of chemical fertilizers, posed threats to the atmosphere and human beings along with increasing production. Therefore, by creating organic agriculture with a greater focus on soil capacity and biological capacities, human beings have chosen to use approaches that are more consistent with nature and uphold the ecological equilibrium of soil and climate to maintain productivity and conserve their basic resources (Sarikhani et al. 2014).

In recent decades, the growing population of the world and the growing food market have led to a serious and imminent change from conventional agriculture to advanced agriculture in the agricultural and food sciences and the use of modern genetic technologies in the production of crops and livestock. As we know, plants are the world's primary and most essential renewable resources that also fulfill non-nutritional, chemical, and industrial requirements, such as photosynthesis, in addition to supplying food for humans and animals. For this reason, the application of genetic engineering and molecular genetics methods in the use of microorganisms and microbial genes to increase the quantity and quality of products, on the one hand, and reduce costs and production time, on the other hand, the use of these methods in various branches of agriculture is very valuable.

All food cycles are related by bacteria to higher nutritional levels, so it would be a significant factor in deciding the role of an ecosystem because of the range of reactions that bacteria cause, role and likely bacterial diversity (Khodashenas et al. 2010).

In soil composition, bacteria play a significant role. Bacteria-produced polysaccharides bind soil particles together and help form the foundation of the soil. Bacterial humus also forms complexes of clay-organic matter that assist in the granulation of the soil. Actinomycete community bacteria create hyphae that bind soil particles together, thereby playing a role in the granulation of soil. Soil granulation lowers soil erosion, increases the infiltration of water and adequate aeration of the soil (Khodashenas et al. 2010).

Endophytic bacteria, by maintaining their survival in the host plant, in addition to not harming their host, but with the help of various mechanisms, directly and indirectly increase plant growth. Indirect stimulation of plant growth occurs when bacteria counteract the harmful effects of one or more plant pathogens, which can be achieved in two ways. In one method, bacteria stop the activity of the pathogen by secreting siderophore, producing hydrogen cyanide, and secreting extracellular enzymes such as chitinase, beta-one, and three gluconases, protease, and lipase. In another method, the bacterium activates the induced systemic resistance mechanism in the plant (Etminani and Etminani 2018). These include siderophores,

lipopolysaccharides, and salicylic acid. Siderophore can specifically help improve the growth of the host plant in addition to its indirect influence.

In fact, iron absorption by microorganisms and plants in iron deficiency conditions usually depends on chelating agents for the breakdown and transport of inorganic (mineral) iron. The most diverse biosynthetic chelates are microbial siderophores and to a lesser extent phytosiderophores produced by Geramineaes. Siderophores are low molecular weight compounds (less than 1000 Da) with a high affinity for trivalent iron that are secreted by various bacteria to dissolve trivalent iron in the extracellular medium. *Pseudomonas* was introduced as the predominant endophytic genus with the ability to produce growth-promoting compounds in this study. Bacteria of *Pseudomonas* have spread extensively in nature and are isolated from most environments (Alexander and Zuberer 1993; Fazeli-Nasab and Sayyed 2019). These bacteria are important in terms of a broad range of metabolites supporting plant growth, such as production of hydrogen cyanide, production of siderophores, solubilization of phosphates, and production of auxins (Costa-Gutierrez et al. 2020; Schippers et al. 1987). This genus has been introduced as a growth-promoting endophytic bacterium in crops (Maheswari et al. 2013) and it has been shown that *Pseudomonas fluorescens* and *Pseudomonas putida* bacteria in pine and *Pseudomonas aureofaciens* bacteria in Fir plant can increase plant height and biomass (Ahmadzadeh 2013). *Pseudomonas* has also been identified in *Arabidopsis* and soybeans (Chaudhry and Patil 2020; Panchal and Ingle 2011).

19.2 Importance of Different Microbial Populations Associated with the Plant

The rhizosphere is a microecological area near the plant root, where rapid and numerous chemical interactions take place and its environment is more competitive than the soil mass. This environment is divided into three regions: internal, middle, and external, based on proximity to the root and the extent of its impact. Compounds added to the soil by the roots are classified into four categories: exudates (passively removed from the roots), secretions (actively removed from the roots), dead cells, and gaseous compounds. The compounds in the substances left by the roots, by acidifying or changing the redox conditions in the rhizosphere or directly chelating the elements, help to provide nutrients such as nitrogen, phosphorus, iron, etc. As the soil dries, the hydraulic potential decreases, after which the root seepage begins to return water to the soil, increasing the degree of stability of the rhizosphere. Conventional nutrient management strategies are highly dependent on the use of chemical fertilizers, and the potential biological potential of soil and plants has in many cases received less attention. Of course, the growing demand of the world for food and, as a result, the need to achieve high yields of agricultural products, has been a pressure lever for this lack of attention. In many cases, the expediency of producing more and obtaining food has even violated other environmental considerations. The chemical and biological processes that take place in the rhizosphere not only determine the mobility and uptake of soil nutrients but also control

the efficiency of nutrient consumption. Establishing an integrated nutrient management strategy in the root zone is an effective way to solve the problem between high crop yield, nutrient efficiency, and environmental protection (Dadivar 2015).

Plants are an important source of organic matter in the soil and organic matter is a major source of energy for microbial activity. In most cases, it defines the basis of the tendency of microorganisms to the roots and the formation of the interaction of microorganisms and plants in the form of cooperation and coexistence. The formation of root–microorganism interactions in the rhizosphere has caused many physical, chemical, and biological properties of soil in this environment to be different from soil mass. This difference is very favorable for both the plant and the rhizosphere microorganisms (Dadivar 2015).

The uniqueness of rhizosphere conditions in terms of plant nutrition and also the difference of rhizosphere between different plants can provide effective management strategies for farmers and producers (Dadivar 2015; Ryan et al. 2009). Provision of nutrients locally and in the environment adjacent to the roots, instead of consumption as a spread in the soil mass, uses the capabilities and benefits of efficient plants in improving the nutrition and growth of inefficient or inefficient plants in the form of mixed cultivation of different plant species or different cultivars of a species can increase production and reduce the consumption of agricultural inputs, including chemical fertilizers (Dadivar 2015; Gqozo et al. 2020; Rehman et al. 2020). Also, adopting appropriate crop rotations by considering the rhizosphere characteristics of each plant, so that the appropriate conditions created in the soil by one crop can be used in cultivation and subsequent crop production, including cultivation management strategies. Emphasis is placed on the rhizosphere, which can increase productivity and reduce chemical fertilizers (Ayub et al. 2020; Dadivar 2015).

The reaction of calcium carbonate in calcareous soils leads to an increase in soil pH, especially in areas with low rainfall. These reactions in the surface horizon of calcareous soils limit the solubility and uptake of many elements such as Fe, Cu, Mn, Zn, P. It also impairs plant and root growth and ultimately reduces yields unless large amounts of chemical fertilizers are applied. Therefore, the solubility and low uptake of nutrients in calcareous soils have attracted the attention of many nutritionists due to the high cost of chemical fertilizers, the environment, and public health. Many studies have shown that in calcareous soils, organic acids from plant root secretions can act as an effective factor in extracting a significant portion of plant nutrients and improve the efficiency of fertilizer and water consumption in these soils (Khademi et al. 2009).

Different bacterial species protected by a polymer substrate are known as biofilms. In adverse environmental conditions, this polysaccharide coating plays a part in preserving the cells in the biofilm and giving them certain capabilities to maintain and withstand bacteria in adverse environmental conditions. The biofilm produced by them may have positive or negative effects on human life due to the presence of bacteria in different ecological environments. The production of biofilms in plant pathogens causes many problems in killing bacteria because biofilms prevent the effects of disinfectants, antibiotics, and chemical toxins on harmful bacteria. On the other hand, the prominent effect of heavy metal decomposing

bacteria in contaminated soils and waters, wastewater, and air purifying bacteria, as well as beneficial bacteria effective in improving plant growth cannot be ignored. Considering the issues raised, by increasing the level of knowledge and examining various aspects of bacterial life, biofilm-producing bacteria can be used to improve the quality of human life (Khezri 2019).

The use of antagonistic bacteria in the biological regulation of plant diseases and the quantitative and qualitative enhancement of crop growth has recently been found to be of considerable importance by researchers. Increasing use of chemical compounds in the form of fertilizers and pesticides to control pests and plant diseases has caused serious pollution in the environment, human health and other organisms. For this reason, scientists are looking for alternative methods to these harmful compounds in controlling plant pests and diseases and improving plant growth. The use of biologically inhibitory agents that have high capabilities in the production of secondary metabolites effective in reducing or inhibiting plant diseases is one of the strategies that has been welcomed by researchers (Khezri 2019).

Most of the bacteria that inhibit plant diseases are located in the soil around the roots, called the *rhizosphere*. In the form of compounds rich in sugars and organic acids, the root of the plant secretes a large part of the stabilized compounds into the soil atmosphere through the roots. For this cause, the rhizosphere is an ideal location for various types of microorganisms to expand and multiply (Khezri 2019).

The findings of studies conducted by different researchers suggest that biofilms formed by beneficial bacteria may be useful for disease control (Younessi et al. 2017).

Bacillus subtilis, *B. cereus*, and *Pseudomonas fluorescent* bacteria can be mentioned as effective bacteria in biologically controlling plant diseases (Younessi et al. 2017). Different strains of these bacteria have high potential in producing a variety of secondary metabolites such as biofilms, biosurfactants, extracellular fluid secretions, antifungal volatile compounds, antibiotics, and various enzymes and reduce disease in different ways (Younessi et al. 2017). In one study, the probability of managing tomato blight bacterial disease was assessed using *B. subtilis* 6051 strain on the model plant of Arabidopsis and the results showed that the reduction of the disease relied directly on the development of biofilm by the antagonist bacterium (Younessi et al. 2017).

19.3 The Role of Microorganisms in Soil Protection

Soil conservation as a living organism is considered to be the main goal in bio-agriculture, so that other factors and institutions should be such that in the long run, they cause more soil fertility and revitalization of its living ecosystem. Production of high quality food, sufficient quantity, preservation and increase of soil fertility in the long run, preservation of genetic diversity, and consideration of the wider social and ecological effects of the crop system are the goals of bio-agriculture. Soil microorganisms, especially bacteria, cause many reactions that are necessary for the production of agricultural products. These reactions

include the following; Food cycle, soil structure preservation, organic matter decomposition and food release, decomposition of agricultural chemicals, decomposition of other contaminants, production of plant humus, control of plant and animal pests. Soil bacteria are dynamic reservoirs of nutrients in all ecosystems and are directly or indirectly important in improving plant health (Forouzandeh et al. 2019; Jahantigh-Haghighi et al. 2020; Mehrban and Fazeli-Nasab 2017; Naghavi et al. 2004).

Many reactions that are essential for the production of agricultural products are caused by soil microorganisms, especially bacteria. These include the food cycle, soil structure preservation, decomposition of organic matter and food release, decomposition of agricultural chemicals, decomposition of other contaminants, humus production, and control of pests and plants and animals. Soil bacteria are dynamic sources and reservoirs of nutrients in all ecosystems and are critical for improving plant health directly or indirectly (Khodashenas et al. 2008).

Microbial decomposition has been introduced as the most important natural mechanism for removing non-volatile hydrocarbon pollutants from the environment. Although biodegradation occurs slowly, the use of microbial species that decompose pollutants more effectively or through improving environmental conditions such as food addition and aeration can increase biodegradation. Creating different conditions related to native microbial communities such as energy sources, pH, electron receivers and donors, food, temperature, etc., in contaminated sites is also required in the bioremediation method (Gerhardt et al. 2009; Megharaj et al. 2011).

Owing to its wide distribution in the biosphere compared with other living species, bacteria play a prominent role in Atrazine degradation. Biodegradation of the herbicide atrazine in microorganisms is mostly carried out by bacteria due to the presence of *atzA*, *atzB*, *atzC*, *atzD*, *atzE,* and *atzF* genes, which encode the degrading enzymes of this organic pollutant. Since the mid-1990s, there have been reports of atrazine degradation by a large number of degrading bacteria, including the genera *Pseudomonas*, *Rhizobium*, *Acinetobacter*, *Agrobacterium*, and *Pseudoaminobacter*. Atrazine is often used as a source of nitrogen and carbon by bacteria (Fernandes et al. 2014; Ma et al. 2017; Qingyan et al. 2008).

Biological oxidation of organic compounds is the main advantage of microbial decomposition. Mineralization is said to take place as organic compounds are converted to H_2O and CO_2. It should be remembered that mineralization never happens entirely because the cell is converted to part of the organic matter and an essential part of the cell mass is in some way non-degradable. With hazardous substances combined with H_2O, CO_2 and new bacterial cells will solve a number of problems, but this itself needs to be corrected. In biodegradation, mineralization does not always occur. Changes in the molecular structure of a contaminant during bioremediation may result in the production of different materials from raw materials that are still toxic or hazardous (Abdollahi and Abdollahi 2008).

In general, the three metabolic pathways by which contaminants are modified and degraded by microorganisms are: aerobic, anaerobic, fermentation or fermentation methods (King et al. 1997). The aerobic process can cause many of these contaminants to degrade, but when reacted, heavy halogen compounds are not readily affected by aerobic microorganisms and become more toxic. It is important

to note that the rate of reactions for the aerobic metabolism process is often faster and typically easier to control (Gibson 1988; Heitkamp et al. 1988; Tabak et al. 1964).

Almost all methods of bioremediation are aerobic, but since they can catalyze most reactions and degrade certain compounds that are immune to aerobic decomposition, anaerobic bacteria should not be ignored. A wide variety of compounds can be used by anaerobic bacteria to generate energy. In fuel products, these compounds include carbohydrates, amino acids, fats, pesticides, and aromatic compounds. Anaerobic microbes can reduce (reduce) chlorogenic molecules that are resistant and less attacked by aerobic bacteria. This toxin, like other chlorine toxins, is very resistant and its decomposition in the soil contributes to DDD, which is also resistant and both compounds may be contained in fats and are detrimental to the nutrition of animal products. DDT is one of the toxins that is highly regarded in terms of environmental contamination. Some researches have shown that the breakdown of DDT and ddd in anaerobic conditions is much faster than in aerobic ones. Normally, only one to two percent of it remains after a few months. About 26 microbial species have the ability to degrade ddt to ddd. These microbes include *Aerobacter*, *Aerogenase*, *Enterobacter*, and *E. coli*. (Abdollahi and Abdollahi 2008; Morrison et al. 2000; Tang et al. 1999).

In the climate, hydrocarbons are degraded primarily by *filamentous fungi*, yeast, actinomycetes, and bacteria. Biodegradation efficiencies have been recorded for soil fungi at 6–82%, soil bacteria at 13–50%, and marine bacteria at 0.003–10% (Das and Chandran 2011). While many microorganisms are capable of degrading crude oil in the soil, bacteria are increasingly recognized for their biodegradability (Sebiomo et al. 2011), and are commercially available as lyophilized hydrocarbon decomposition bacteria. The way bacteria do the biological process is well known; and the bacteria that can break down petroleum products are *Pseudomonas*, *Aeromonas*, *Moraxella*, *Bijrinkia*, *Flavobacter*, *Korobacter*, *Nocardia*, *Corynebacteria*, *Acinetobacter*, *Mycobactena*, *Monococcus*, *Streptococcus*, *Streptococcus aureus*. Microbial deformation and mineralization are the most important methods of decomposing pesticides in soil. The size and activity of soil microbial biomass affect the rate of decomposition (Burken and Schnoor 1996; Burken et al. 2011).

Microorganisms have been used in different agricultural industries so far. Examples include the role of microorganisms in biofilms and the development of dietary supplements to enrich oil (Franklin et al. 2019, 2020), the reduction of antibiotic toxicity (Allen et al. 2009), the promotion of engineered bread yeast for the bakery industry (Prieto et al. 2005), and the development and industrialization of engineered alcohol yeast.

19.4 The Role of Microorganisms (Biofertilizers) in Sustainable Agriculture

The use of bio-fertilizers to increase soil fertility in agricultural production has been proposed to achieve sustainable agriculture, expand the production of products without toxins and chemical fertilizers as an alternative to chemical fertilizers.

Types of biofertilizers include molecular nitrogen-fixing bacteria (diazotrophs), root fungi (mycorrhiza), microorganisms (soluble microorganisms), and insoluble phosphates, rhizosphere bacteria that stimulate plant growth, and plant-farming microorganisms, microorganisms, plant-producing microorganisms. It is used to expand the biological regulation of plant diseases (Sabbagh and Valizadeh 2019).

One of the forced coexistences of plants that are useful in water absorption and nutrients, as well as in soil formation and soil stabilization, is arbuscular mycorrhizal fungi. The coexistence of arbuscular mycorrhizal fungi also increases plant production capacity. In this regard, the mycorrhizal relationship can be mentioned as a living structure in which there is coexistence between the fungus and the root and increases the potency of both (Xu et al. 2019). Since most mycorrhizal fungi do not have a specific host, large populations of plants coexist with these fungi. Approximately 240 species of arbuscular mycorrhizal fungi (AMF) have been identified using the morphology of their spores, and in the absence of spores, the presence of fungal organs within roots such as arbuscules and vesicles as well as their structural features is the best means of identification. In addition to decreasing the adverse effects of nutrient shortages and drought and salinity stresses, coexistence with mycorrhizal fungi often improves reversibility after plant stress. And their vegetation has declined and the biosynthesis of secondary metabolites in plants has also increased (Hatami et al. 2020).

In order to stimulate rooting and root production, in rosemary (*Rosmarinus officinalis*) a two-factor factorial experiment was performed in three replications (first factor (phosphorus fertilizer (ammonium phosphate) equivalent to zero, 25, 50, 75 and 100 kg / ha). And the second factor (two levels of *mycorrhizal fungus* treatment including *Glomus intraradices* and *Glomus mosseae*)). Some traits such as plant height, root length, stem diameter, shoot fresh weight, shoot dry weight, root wet weight, stem dry weight and number of leaves per plant were also investigated. The results showed that in addition to both types of fungi were effective on all morphological indices of the plant (Table 19.1), even fungi in the presence of phosphorus fertilizer could affect the morphological indices more so that the highest plant height (1/1) 195 mm) was obtained from the treatment of *glomus muse* A and the use of 100 kg/ha of ammonium phosphate and the lowest amount (110.1 mm) was obtained from the treatment of *glomus muse* A and no application of ammonium phosphate (control) (Table 19.2) (Bagheri et al. 2018).

The use of nitroxin, vermicompost fertilizers in sesame plants has had a positive and important impact on most of the characteristics evaluated, so that in some characteristics, including the number of capsules per plant, the yield of grain and the yield of oil, the use of these fertilizers have an impact equal to half the effect (Sajadi et al. 2011). Nitroxin biofertilizer contains a set of the most effective strains of nitrogen-fixing bacteria of *Azospirillum*, *Azotobacter* and phosphate solvent of *Pseudomonas*. *Azotobacter* and *Azospirillum* are the most important growth-promoting bacteria in the plant, which in addition to bio-stabilizing nitrogen and helping the plant easily access soil nutrients, produce significant amounts of growth-promoting hormones, especially auxin, gibberellin, and cytokine (Sabbagh and Valizadeh 2019).

Table 19.1 Comparison of the average simple effects of rosemary under the influence of *arbuscular mycorrhiza*

Sources of changes	Bush height (mm)	Root length (mm)	Stem diameter (g)	Wet weight of aerial parts (g)	Dry weight of aerial parts (g)	Root wet weight (g)	Dry weight of the stem (g)	Number of leaves per plant
Glomus Musa A	130.1^{b}	144.1^{a}	1.83^{a}	7.83^{a}	6.90^{a}	0.73^{a}	1.02^{b}	29.80^{b}
Glomus intraradices	143.4^{a}	126^{b}	1.92^{a}	8.00^{a}	7.20^{a}	0.76^{a}	1.19^{a}	34.86^{a}
Control without fungi	119.85^{c}	88.95^{c}	1.78^{b}	7.20^{b}	6.27^{b}	0.34^{b}	0.71^{c}	19.16^{c}

Similar letters in each column indicate no significant difference at the 5% level based on Duncan's multiple range test

Table 19.2 Mean interactions on growth characteristics of rosemary under the influence of *mycorrhiza* and ammonium phosphate fertilizer

Arbuscular mycorrhizae	Ammonium phosphate	Number of leaves per plant	Dry weight of the stem (g)	Root wet weight (g)	Bush height (mm)
Glomus intraradices	100 kg/hectare	31.00^{b}	1.37^{b}	1.21^{b}	147.02^{bc}
	75 kg/hectare	36.00^{b}	0.92^{dc}	0.71^{c}	124.96^{dc}
	50 kg/hectare	25.33^{c}	1.29^{b}	0.59^{dc}	121.66^{d}
	25 kg/hectare	37.00^{b}	0.71^{e}	0.65^{c}	127.79^{dc}
	No fertilizer application (control)	13.66^{d}	0.83^{de}	0.48^{d}	129.54^{dc}
Glomus musa A.	100 kg/hectare	45.00^{a}	1.65^{a}	1.59^{a}	195.10^{a}
	75 kg/hectare	35.66^{b}	1.81^{a}	0.69^{c}	159.21^{b}
	50 kg/hectare	40.00^{b}	0.76^{de}	0.62^{dc}	130.08^{dc}
	25 kg/hectare	29.00^{c}	0.72^{e}	0.68^{c}	122.89^{dc}
	No fertilizer application (control)	24.66^{c}	1.01^{c}	0.21^{e}	110.17^{d}

Similar letters in each column indicate no significant difference at the 5% level based on Duncan's multiple range test

19.5 The Role of Genetic Engineering in the Use of Microbial Genes in Agriculture

By engineering the fatty acyl-ACP thioesterase gene, they have reduced the carbon chain, increased the degree of saturation, quality and efficiency of the oil. Asparagus converts to aspartic acid and consequently reduces the formation of acrylamide (a carcinogen during cooking). Genes involved in the biosynthesis pathway of isobutanol and ethanol in *S. Cerevisiae* yeast have been engineered to increase production (FDA 2013). Later, recombinant microorganisms (such as recombinant probiotics) will appear in dairy products such as yogurt and cheese (Aguilera et al. 2013).

Numerous studies have been performed to increase the efficiency of pest control agents such as *Bacillus thuringiensis* (*Bt*) through genetic engineering, during which genes encoding crystalline proteins or other toxins are transferred to Bt bacteria and their expression is increased or for the first time, a specific gene has been transferred to the target strain because each strain of this bacterium has a specific number of such cry genes (Driss et al. 2011; McDade 2019). In 2011, with the transfer of the chitinase gene to the Bt bacterium, the insecticide capacity of this bacterium increased by 50% (Driss et al. 2011). Insect-specific neurotoxin gene along with *cry1Ac* gene was transferred to a Bt strain and it was found that its insecticidal rate was increased (Li et al. 2012).

Another approach is to transfer to other microbial agents the genes of a biological agent, such as *Bt*. The *vip3A* and *cry1I* genes, for example, have been isolated from the *Bt* bacterium and transferred to the *Pseudomonas fluorescens* bacterium, which is a biological agent to control plant diseases, so that it may also have insecticidal properties (Hernández-Rodríguez et al. 2013). The *cry9Aa* gene has also been transferred to *E. coli*, leading to insecticidal activity against *Spodoptera exigua* (Naimov et al. 2014).

Concerning the genetic engineering of plant disease antagonists, several studies have been performed so far to improve the production and controllability of plant pathogens, including *Trichoderma* sp. (Kowsari et al. 2013; Malmierca et al. 2012) and *Streptomyces* (Clermont et al. 2011), *Bacillus subtilis* (Leclere et al. 2005) and *Psuedomonas* sp. (Hernández-Rodríguez et al. 2013), respectively, observed. Genetic engineering has increased the efficiency of microbial growth stimulants (biofertilizers) through the production of recombinant nitrogen-fixing bacteria (Rhizobium) with the ability to bioremediate soil heavy metals (Ike et al. 2007), *Anabaena* sp. With higher growth stimulant and nitrogen fixation (Chaurasia and Apte 2011) and *Azospirillum* with high auxin production and higher growth stimulus (Baudoin et al. 2010; Malhotra and Srivastava 2006).

The *BADH* gene was transfected into the walnut (Juglans regia L.) cultivar "Chandler" by *Agrobacterium* LBA4404, plasmid pBI121, CaMV 35S promoter, and *NPTII* gene as a selectable marker (in turn driven by a NOS promoter) to confirm that *BADH* transgenes were successfully incorporated into the plant genome using PCR and Southern blot analysis. Transgenic and wild plants grown from embryos exposed to four levels of osmotic stress (i.e., zero, 2, 4, and 8% PEG) and four levels of salinity (i.e., zero, 50, 100, and 200 mM NaCl) and after 21 days, they found that the transgenic plants grew under almost extreme salinity and drought stress, but the wild-type plants showed a lagging growth rate and did not survive the cradle stage (Fig. 19.1) (Rezaei Qusheh Bolagh et al. 2020).

Results of research on *Rhizobium leguminosarum* showed that the introduction of a high catalase activity *VKT* gene into this bacterium resulted in a 1.7 to 2.3-fold increase in the nitrogen fixation activity of its nodes relative to control bacteria (Orikasa et al. 2010). The gcd gene was also cloned from *E. coli* in *Azotobacter vinelandii*, which resulted in increased phosphorus solubility and growth of sorghum (Sashidhar and Podile 2009). However, it should be noted that despite extensive studies and research on the creation of recombinant microorganisms for the biological control of pests and diseases, as well as biofertilizers, a significant number of such products have not yet been commercialized. The engineered strain of *Sinorhizobium meliloti*, called RMBPC-2, in which the *nifA* gene was inserted and nitrogen fixation increased, was one of the first commercialized strains (Bosworth et al. 1994). However, engineered nitrogen stabilizing strains have become increasingly commercialized in both the United States and Australia (Kunjapur and Prather 2015; Mindt et al. 2020).

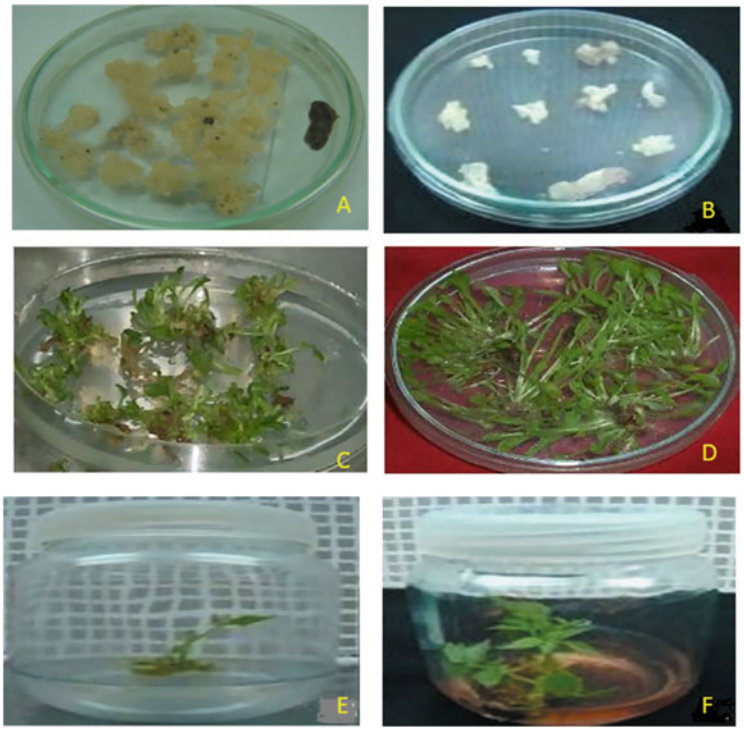

Fig 19.1 The stages of transformation of *BADH* gene for salt and drought tolerance to Persian walnut using *Agrobacterium*-mediated (section A: Difference in growth of transgenic walnut (left) and non-transgenic (right) somatic embryos on DKW (Driver and Kuniyuki Walnut) containing 100 mM kanamycin); Shoot formation directly from transgenic somatic embryos of walnut 5 weeks after culture on selective medium in dark, 5 weeks on germination medium, and weekly on regeneration medium ((b) Selective medium, (e) Germination medium, (f) Proliferation medium)

19.5.1 The Role of WRKY Gene Family in Bacterial Resistance

The *WRKY* gene family is the most significant group of regulatory transcriptional factors (Xue et al. 2019). For the activation of *PR* genes, binding of transcription factors to DNA is necessary (van Loon et al. 2006). In rice, one hundred forms of WRKY genes were identified (Ross et al. 2007). Xoo's expression of the *WRKY12* gene in rice increases *NPR1*, *PR1b*, phenylalanine ammonialyase (PAL), and peroxidase (pox) expression. Increased expression of *WRKY70* in Arabidopsis increases resistance to *Pseudomonas syringae* and *Pectobacterium carotovorum* (Li et al. 2006). It was suggested that *WRKY* is a transcriptional regulator in JA and SA-dependent signal cascades (Song and Goodman 2001). The result of *WRKY* gene expression in tobacco increases the level of programmed cell death response and *HR* (Oh et al. 2008). It has been suggested that the *WRKY12* transcription activation factor in tobacco causes overexpression of the *PR1* gene by binding to a

protected sequence in the *PR1* gene promoter called *WK-boxes* (TTTTCCAC) (van Verk et al. 2011).

19.5.2 The Role of *pth*A Gene in Developing Resistance to Chancre

One of the most significant genes for the pathogenesis of chancre-causing bacteria is the *pthA* gene (Shiotani et al. 2007). The homologs of this gene, including pthB and pthC, are present in the citrus chancroid bacterial strains that cause forms B and C (Mokhtari et al. 2015). This gene is part of the *avrBs3*/*pthA* (Transcriptional Activator-like (*TAL*) effectors) (*TAL* family) family of genes. This family is widely found in Xanthomonas species (Shiotani et al. 2007). The *pthA* gene in all citrus chancre bacteria is about three to four kilograms long. The gene has 17.5 consecutive repeating regions, each of which is 102 bp and is located in the central part of the gene. This repetitive region is necessary to determine host specificity as well as infectivity (Al-Saadi et al. 2007). *PthA* protein is composed of 1163 amino acids. The middle portion of the *PthA* protein contains 17.5 identical replicates of 34 amino acids, with the exact number and arrangement of replicate units differing in different bacteria. This difference results in function and specificity when resistance or infectivity develops in the respective host species in the absence of resistance genes (Al-Saadi et al. 2007).

The most effective way to control citrus chancre is producing disease-resistant plants. The lack of access to resistant plants and the restriction of the presence of natural genes for disease resistance have resulted in the use of methods based on the genetic modification for other sources of resistance. The use of the resistance mechanism of plant antibody expression (Plantibody-mediated resistance) is one of the new solutions for creating resistant plants. The use of this mechanism has so far led to resistance against a large number of viral, bacterial, and fungal diseases (Mokhtari et al. 2015; Yajima et al. 2010). In the latter mechanism, disruption of the pathogenic process is achieved by targeting essential pathogen proteins by specific antibody–antigen binding. Therefore, *PthA* protein, with its essential functions in causing disease, can be considered as one of the best candidates in creating resistance.

19.5.3 The Role of Beta-Lactam Gene in Reducing the Toxicity of Antibiotics

An unpleasant occurrence and a health alert is the presence of antibiotic resistance genes in bacteria. Studies have shown that human activity contributes to an increase in bacterial genes for antibiotic resistance. Antibiotic resistance genes spread to other species beyond particular bacterial species, and these genes are also known as bio-pollutants. In environments where pollutants put a lot of stress on bacteria, antibiotic resistance is more widespread (Abou-Shanab et al. 2007).

Genes for antibiotic resistance are very complex. To date, 95 distinct antibiotic resistance genes have been isolated from humans, of which only 69.5% are similar to known resistance genes, and other sequences are unknown. Genealogy of *beta-lactamase* genes derived from Alaskan soils, many similarities with known *beta-lactamase* genes (Allen et al. 2009; Huff et al. 2020), and in the phenotypic analysis, bacteria with these genes also had different responses. Because there is no restriction on the transport of plasmids and other genetic elements between bacterial species, the increase in gene contamination leads to the spread and spread of resistant bacteria. Numerous reports indicate that antibiotic resistance genes are highly similar in bacteria isolated from natural habitats to genes of human pathogenic bacteria, and therefore, infected natural habitats can be the origin of resistance genes (Aminov 2009; Sabri et al. 2020).

In most cases, genes associated with bacterial resistance to heavy metals are associated with antibiotic resistance genes. These genes control mechanisms such as detoxification by increasing the flow of substances out of the cell. Because these genes are nonspecific, they reduce the harmful effects of both metals and antibiotics on the cell, and the presence of one of these two genes is needed to motivate these genes and make this group of bacteria more abundant, although the concentration of metals still increases. The abundance of bacteria with antibiotic resistance genes in the soil is not known (Knapp et al. 2011).

Increased resistance to antibiotics, especially against *beta-lactams* (*beta-lactamases*, which break down beta-lactams in bacteria) has become more common in the last two decades. (Boyd et al. 2020; Bush and Jacoby 2010). Antibiotic resistance is primarily due to the transport and maturation of large plasmids, such as several *beta-lactamase* genes, capable of acquiring different resistance genes. Other mechanisms of resistance to *beta-lactams* can be harmful to bacteria (Allen et al. 2009); For example, reducing the efficiency of purines and increasing the flow of substances are mechanisms to reduce the toxicity of antibiotics that reduce the essential nutrients in the bacterium and cause problems for it.

Beta-lactamases are inactivating enzymes of penicillin and related compounds (Hemmati et al. 2015); the genes encoding these enzymes are found chromosomally in bacteria. The study of the frequency of genes that make this enzyme is one of the special methods in estimating the frequency of genes resistant to antibiotics such as penicillin, amoxicillin, and ampicillin (Younessi et al. 2017).

As the only source of carbon and electricity, *beta-lactamase*-producing bacteria are said to often use antibiotics. A variety of species of *Pseudomonas* can use benzyl penicillin, a process involving the synthesis and release of large quantities of *beta-lactamase*, as their sole source of carbon. Since breaking the *beta-lactam* ring in antibiotics is the first step in bacterial detoxification and thus antibiotic resistance, bacteria capable of producing the *beta-lactamase* enzyme show resistance to a wide variety of antibiotics (Younessi et al. 2017).

19.5.4 The Role of PR5 Gene Family in Responding to Stressful Situations

Due to their sequence similarity with the plant protein thaumatin (TL) (Liu et al. 2020), proteins belonging to the *PR5* family are known as Thaumatin-like proteins. So far, the PR5 protein group has been isolated from tobacco, Arabidopsis, rice, wheat, and many other plants (Baek et al. 2019). In response to stressful conditions, such as high salt concentrations, wounds, or pathogen attacks, the accumulation of these proteins in plants has been observed. The permeability of the pathogenic cell membrane is altered by these proteins (Boccardo et al. 2019; Kitajima and Sato 1999). Osmotins are similar to PR5 protein types of play. Osmotin is an inducible protein that has been found in tobacco by salinity tension. Game types, therefore, are referred to as Osmotins. In healthy plants, neutral *PR5* proteins are not present but are caused by ethylene (Liu et al. 2020).

According to studies on the involvement of phenylpropanoid biochemical pathway derivatives in different biological and abiotic stresses (Campbell and Ellis 1992; Chen et al. 2019), changes in enzymes like phenylalanine ammonialyase (*PAL*) in this pathway may be studied. By interacting with the synthesis pathways of phenylpropanoids and isoflavones that have phytoalexin activity, the *PAL* gene plays a very significant role in plant resistance. This gene is involved in the biosynthesis of salicylic acid and other defense-related compounds and is a key signaling compound for the activation of defense-dependent genes, catalysts, and transcription factors (Stotz et al. 2009).

19.5.5 *Bt* gene and Concern Management in Transgenic Crops

Growth in resistance management strategies requires that the biochemical and genetic mechanisms of resistance production are properly understood (Tohidfar and Khosravi 2015). The *Cry1Ac* protein receptor (Fabrick and Tabashnik 2007) is referred to as Cadherin. The formation of resistance in some studies has been correlated with Cadherin Locus. Due to mutations in cadherin protein (Gahan et al. 2010), *Cry1Ac* resistance has been observed in some essential cotton pests. This resistance has been attributed to the degradation by retrotransposons of a gene belonging to the broad cadherin gene family, producing several proteins necessary for larval development. However, there was no association between *Cry1Ac* resistance and cadherin gene orthologs in the two *P. xylostella* breeds isolated from the field, suggesting a separate genetic basis for resistance in farm-bred breeds (Tohidfar and Khosravi 2015).

Loss of adhesion and irreversible binding to the pest's precursor membrane have been due to resistance to *Cry1Ac*. But reducing bond strength is not the only resistance creation mechanism. Mutations in the second 12 cadherin proteins also induced resistance to *Cry1Ac*, but in laboratory assays, they did not fully inhibit toxin binding (Gahan et al. 2010). Another way to build resistance is to mutate ABC protein vectors. ABC proteins are inner membrane proteins that are involved in

many activities, including the transfer of toxic molecules from the cell. In one study, an inactive mutation was made in one of the ABC proteins called ABCC2, which reduced the binding of *Bt* toxin to membrane vesicles. Decreased binding of Bt toxin to cell membranes following mutations in this protein led to the introduction of ABCC2 as one of the proteins involved in the integration of *Bt* protein with membranes (Gahan et al. 2010). In general, the problems associated with the use of *Bt* pesticides have been reduced by producing transgenic *Bt* products containing the *cry1Ac* gene (Tohidfar et al. 2013).

19.5.6 The Role of *rol* Gene Family in Increasing the Sensitivity of Plants to Certain Hormones

The family of rol-genes located on the T-DNA of bacterium *A. Rhizogenesis* is the main cause of capillary root syndrome. They have *rolA*, *rolB*, *rolC*, and *rolD* in these genes. Increasing plant exposure to hormones such as auxin, which has been confirmed by studies on plants such as *L. corniculatus* and *N. tabacum* are the most significant impact of position genes in plants. The *rolC* gene has been studied more than other genes by researchers in terms of its importance in improving decorative and horticultural traits on the plant. In terms of pathogenicity, rolC gene expression is associated with capillary root syndrome and changes such as the production of new secondary compounds, changes in plant hormonal balance, and chlorosis. Increased lateral branches, the formation of needle-shaped leaves, early flowering, and reduction of flower size, and creation of male sterility by reducing pollen grain production are other morphological changes caused by expression of *rolC* gene in plants. One of the most important regulators of *rolC* gene expression is sucrose. According to studies, the sucrose response region in the promoter of this gene is in the range of −94 to −135. The presence of high sources of sucrose in phloem tissues has led to high expression of rolC gene in these tissues (Gardoonpar et al. 2016).

Regarding the effect of rolC gene on plant hormones, it should be noted that this gene affects the number of cytokinins, auxins, and gibberellins (GA). Changes such as decreased vertebral dominance and increased branching indicate changes in cytokinin hormone levels (Boutigny et al. 2020; Zuker et al. 2001). Examination of *rolC* tobacco protoplasts has shown an increase in membrane hyperpolarization of these cells in the presence of auxin, or in other words, increased membrane excitability of protoplasts in the presence of this hormone (Maurel et al. 1991).

A decrease in plant size with a higher number of buds was observed in potato transgenic *rolC* (Bettini et al. 2016; Fladung et al. 1993). It has also been found that less photosynthesis occurs in transgenic *rolC* plants due to the decrease in chlorophyll content and the leaves appear yellowish-green (chlorosis) compared to natural plant organisms.

The 35S-*rolC* (*roleC* gene under the influence of the strong 35S promoter of CaMV virus) potato transgene plants also have a high expression of this gene in the leaves and, as a consequence, these leaves display more chlorosis (Bahramnejad

et al. 2019; Schmülling et al. 1988). In addition to the above, other role models of the rolC gene in plants are stimulation of the development of secondary metabolites and defense proteins in transgenic plants and its relationship with oxygen-free radicals and cyclin-dependent protein kinases (*CDPK*) (Mark et al. 2019; Shkryl et al. 2008).

19.5.7 *PA* Gene Expression of *Bacillus anthracis* in Plants

The use of injectable vaccines in the mucosal tissues receiving the vaccine typically does not induce a sufficient immune response. The principal inputs of pathogenic species are mucosal surfaces, including the mouth and genitals. As a result, oral vaccines were produced which were shown to enhance the immune response of these tissues. There are several production advantages of oral vaccines, such as the fact that there is no need for cleaning anymore, so production costs are significantly reduced and a person receives the appropriate amount of daily fruit or vegetables (Jalali javaran et al. 2011).

Anthrax is a deadly disease common between livestock and humans, and the Protective Antigen (*PA*) gene from *Bacillus anthracis* has the highest potential for a vaccine against anthrax, which is transferred to the lettuce genome and *PA* gene expression is confirmed by ELISA (Honari 2008).

19.5.8 The Role of Gene Encoding the Enzyme *Asr* in Production of Glucose Biopolymers

In addition to plants, bacteria also synthesize different high molecular weight polysaccharides as follows; Alginates, Gellan, Xanthan, Alternan and Mutan (Venkatachalam et al. 2020). Lactic acid bacteria can also generate useful biopolymers such as alternan, which are used in the extracellular environment in industry, agriculture, and medicine, using sucrose, and expressing enzymes of glycosyltransferase. In general, extracellular polysaccharides given by lactic acid bacteria are divided into two groups: hemopolysaccharides and heteropolysaccharides. These enzymes use the energy from the hydrolysis of sucrose chains to transfer fructose or glucose to an acceptor molecule. Glycosyltransferase enzymes, after breaking down sucrose molecules, may transfer glucose units to a growing glucan chain or other substrate, such as maltose or isomaltose, to form glycolic oligosaccharides. In addition, these enzymes may play a hydrolyzing role (transfer of glucose units to water). (Nazarian-Firouzabadi et al. 2019).

Glucansucrase is mainly produced by *Leuconostoc mesenteroides*, *Streptococcus* oral flora, and Lactobacillus species (Yan et al. 2018). So far, more than 60 enzymes of this family have been identified, all produced by bacteria of the four genera *Leuconostoc*, *Streptococcus*, *Lactobacillus*, and *Weissella*. However, some genes encoding these enzymes have also been identified in some other lactic acid bacteria such as *Oenococcus*, *Fructobacillus,* and *Enterococcus* (Gangoiti et al. 2018).

From a medical point of view, alpha-glucans and exopolysaccharides are potentially valuable because they are not digested by human gastrointestinal enzymes. Alpha-glucans pass unchanged deoxopolysaccharide from the upper gastrointestinal tract and are fermented in the large intestine by colon bacteria; therefore, glucansucrase products can be used as fiber in human nutrition, because such biopolymers do not cause the rapid release of blood sugar. Besides, exopolysaccharide alpha-glucans are potentially prebiotic; In other words, these polymers are selectively fermented and cause certain changes in the composition or microbial activity of the gastrointestinal tract that are beneficial to the well-being and health of the host (Roberfroid 2007).

Alternan is an important biopolymer that is mentioned as an alternative to gum arabic, especially in low-viscosity diets. Alternan may also be used as a low-calorie or non-calorie food additive as a filler and bulking agent for food products, in the manufacture of inks, adhesives, cosmetics, creams and ointments, and as a coating for drug release (Nazarian-Firouzabadi et al. 2019). The production of new compounds in plants, using genetic engineering methods, is one of the goals of gene transfer methods. Therefore, Alternan can be produced instead of using bacterial bioreactors, using genetic engineering methods in plants.

The gene encoding the enzyme Alternan sucrase (*Asr*) of *Leuconostoc mesenteroides* was transferred to the sugar beet plant and sugar analysis of fresh beet plants showed that the control plant with 19.6% bridge (sucrose) had more sucrose than the wet bridge plants was with medium bridge 14.4. Also, the amount of Brix in wet transgenic plants was lower than control plants and the rate of reduction of sugar (sucrose) in transgenic plants with *Asr* gene was about 36.1% compared to the control. Bacterial alternan sucrase enzyme can produce 36.6 mg/g FW of Alternan biopolymer in sugar beet-roots and convert significant amounts of root sucrose to Alternan biopolymer for industrial and pharmaceutical applications (Nazarian-Firouzabadi et al. 2019).

19.5.9 The Role of Food Coloring Phycocyanin

Phycocyanin has two subunits of protein, alpha (a) and beta (β), of which there is one site in the alpha subunit, and two sites in the beta subunit to bind phycocyanobilin to the apoprotein. Full phycobilin protein synthesis depends on the synthesis of alpha and beta chains simultaneously, and the proper placement of phycobilin in these two chains. Therefore, the recombinant production of this phycobili-protein is more difficult than other proteins (Eriksen 2008). Although the halo protein of the alpha phycocyanin subunit belongs to the cyanobacterium *Synechocystis* sp. PCC6803 has been reported in Escherichia coli (Tooley et al. 2001).

In fruit, medicine, and cosmetics, phycocyanin is a blue pigment that can be used as a natural dye to substitute carcinogenic synthetic dyes. Today, it is used to a lesser degree in immunoassay and cytometry, in addition to being used as a food coloring. Because of its antioxidant, anti-inflammatory, and anti-cancer roles, phycocyanin in Spirulina is also beneficial to human health, which is why it has gained more

attention in recent years. Considering that phycocyanin accounts for 20% of the total cellular protein of Spirulina platensis, it has been selected as a suitable model for commercial production of phycocyanin in phototrophic cultures. Phytocyanin production in phytotrophy is associated with problems. One way to reduce the problems of phycocyanin production is to produce heterotrophy (the production of recombinant protein is one way to produce heterotrophy). (Shoja et al. 2015).

19.5.10 The Role of *Alpha-toxin* Gene in Creating Immunity Against Gas Gangrene

Using the toxic protein *alpha-toxin* with various phospholipase, sphingomyelinase, and biological pathogenic activities, the bacterium *Clostridium perfringens* contributes to a series of cellular reactions and ultimately induces cell death, lethality, and death of the skin. This bacterium has caused diseases such as gas gangrene with symptoms of pain, fever, and swelling, and in this way has caused significant damage to livestock parts, including high casualties of sheep. Injecting alpha-toxin into animals such as rabbits and sheep can cause symptoms of the disease and reveal signs of tissue damage. On the other hand, vaccination of animals such as mice with portions of the *alpha-toxin* protein of the bacterium *Clostridium perfringens* leads to immunity and makes the animal resistant to infection with the pathogenic bacterium *Clostridium perfringens*, so that no symptoms of Gas gangrene have been observed in mice (Rasani et al. 2020).

In a *Clostridium perfringens* immunization analysis of broilers with a recombinant *alpha-toxin* toxin, it was found that birds vaccinated with recombinant *alpha-toxin* were 35.1% damaged by necrotic enteritis. The rate of damage was measured at an average of 37.2% for unvaccinated birds. The concentration of IgG antibody in vaccinated birds was five times higher than in unvaccinated birds. These results showed that in addition to its pathogenic role, alpha-toxin can also be used as an immunogen (Sakurai et al. 2009). In one study, the amplification, expression, and immunization of the alpha and beta combination gene of *Clostridium perfringens* were studied and the result showed that the alpha and beta protein produced could be resistant to the attack of alpha and beta toxins. It has been suggested that an alternative method for using *alpha-toxin* domains as a vaccine can be the natural form of the toxin or engineered various forms of the toxin with reduced toxicity (Bai et al. 2006).

19.5.11 The Role of *BPDO* Genes in Reducing Environmental Pollution

Polychlorinated biphenyls (PCBs) are chlorinated cyclic compounds which, because of their properties such as heat resistance and stability, were commonly used in different industries in the 1930s and 1980s. In the 1980s, this function of stability and resistance to decay, along with the adverse effects on human health, led to their

cessation of development. The contamination of water and soil around the world with PCBs is one of the major environmental problems. Due to the toxicity of these compounds and their accumulation in the adipose tissue of living organisms and due to their adverse effects on humans, such as cancer, genetic abnormalities in infants, and liver and thyroid tumors, it is necessary to remove and degrade *PCBs* from the environment. One way to reduce PCBs contamination is to transfer and express the bacterial genes of biphenyl dioxygenase (*BPDO*) (which have the ability to break down *PCBs*) to plants. This enzyme has three components of oxygenase with two subunits *bphA* and *bphE*, a *bphF* ferredoxin and a *bphG* reductase (Alizadeh Arimii et al. 2015).

Most gene transfer experiments in plants involve the transfer and expression of a single gene, and the simultaneous transfer of several genes to plants for a biochemical pathway remains a difficult task. There are various ways to transfer several foreign genes into plant cells. One method is to create a polyprotein structure in which the coding sequence of several proteins joins together to form a single copy. It is also possible to use sequential or simultaneous transmission of several vectors carrying different genes. Another way to express several genes in a plant is by crossing translocated plants containing different genes or cloning several genes into one vector by assembling the cassettes (Tzfira et al. 2007).

Simultaneous transferring of *bphA*, *bphE*, and *bphG* genes, which are encoding components of *BPDO* enzyme, to Arabidopsis was investigated and based on the obtained results, 3 *bphA*, *bphE*, and *bphG* genes cloned in pGreen vector into *E. coli* and *Agrobacterium* LBA4404 and C58C1 and finally transferred to Arabidopsis. In terms of gene transfer efficiency to plants, there was a difference between the two strains of *Agrobacterium* LBA4404 and C58C1 used. The highest number of transgenic plants (0.85%) was obtained with LBA4404 strain.

The transgenicity of Arabidopsis seedlings was confirmed by selecting completely green plants in an environment containing 50 mg/l kanamycin as well as a PCR test and finally, the transgenic plants were successfully transferred to the soil and continued to grow (Alizadeh Arimii et al. 2015).

19.5.12 The Role of Laccases (Such as *CotA*) in Environmental Detoxification

Laccases (EC: 1.10.3.2) are N-glycosylated multi-copper oxidases belonging to a group of proteins containing copper (Hesampour and Mohandesi 2018). The four copper ions in the laccase enzyme structure are divided into three types: copper type 1 (T1), copper type 2 (T2), and copper type 3 (T3). The fungal lactase molecule typically contains four copper atoms, while some types of the lactase enzyme are also found to contain three copper atoms in their structure. Laccases have a molecular weight of about 50–100 kDa and when ABTS is used as a substrate, the optimal pH of the enzyme is in the range of 3–5 (Parand et al. 2015).

Laccases are one of the major proteins capable of catalyzing the oxidation of phenolic compounds used in biotechnology as biocatalysts to detoxify the

atmosphere and explain food industry fruit juices (Hesampour and Mohandesi 2018). The first prokaryotic lactases identified belong to the *Azospirillum lipoferum* bacteria. The most important bacterial lactase that has been well studied and its physical and biochemical properties have been determined is the *CotA* protein of Bacillus subtilis. *CotA kDa65* protein belongs to the outer covering of spores. This protein participates in the biosynthesis of spore brown pigment, a melanin-like product, and appears to be responsible for protecting against UV light and hydrogen peroxide. This protein shows similarities with multi-copper oxidases and has high temperature stability (Zamani et al. 2014).

Other laccases have been isolated from *Escherichia coli* (*E. coli*), *Bacillus halodurans*, and *B. licheniformis*. Most of the lactases that have been identified so far and have biotechnological applications have been isolated from fungi. However, the efficient expression of recombinant fungal lactases, which are essential for biotechnological applications, is more difficult than the expression of bacterial enzymes. Problems and barriers to the use of these enzymes include information on sequences that are not accessible, the presence of exon and intron structures in eukaryotic genes, post-translational changes, and bridge formation. Disulfide noted long fermentation time and low efficiency. Despite the industrial applications of bacteria, so far little attention has been paid to bacterial laccases. Studies in genome analysis have shown that these enzymes are widely distributed in bacteria. The development of bacterial laccases for biotechnological applications has advantages because they have high temperature stability and are produced in a short time in cheap environments (Zamani et al. 2014), which is hoped to be used more in the future.

19.5.13 The Role of Flavodexin (*Fld*) Gene in Tolerance to Abiotic Stresses

In agriculture, the most important factors in reducing yield are abiotic stresses (high temperature, cold, frost, and dehydration due to drought or salinity, intensity of sunlight, flooding, ultraviolet light, and heavy metals). Most of these environmental stresses directly or indirectly generate reactive oxygen-free radicals and eventually lead to oxidative stress. Such stresses are the main reason for the decline in crops worldwide and lead to a reduction in the yield of the most important crops by more than 50%. One of the genes that play a key role in responding to such environmental stresses is the cyanobacterial fld gene. Cyanobacteria induce the expression of electron transporters such as flavodoxin (*fld*) that act similarly to ferredoxin (*fd*) in the plant to prevent the adverse effects of ferredoxin depletion. Flavodoxin plays a role similar to ferredoxin (*fd*) in the plant and acts as an antioxidant and increases plant tolerance when non-biological stress occurs by preventing disturbances and irregularities in the electron transfer cycle and the formation of reactive oxygen forms (Ghoreyshi et al. 2016).

Although ferrodoxins (*fd*) are found in all organisms, from prokaryotes to animals, flaudoxins (*flds*) are found only in some bacteria and oceanic algae.

Flavodoxin in these organisms can induce the function of ferredoxin under conditions of iron deficiency and environmental stresses that lead to a decrease in ferredoxin; therefore, they play an adaptive key role in photosynthetic microorganisms that allow rescue and reproduction under adverse conditions (Abdolmaleki et al. 2013).

Fld expression in tobacco plant chloroplasts compensates for the reduction of ferredoxin levels caused by adverse environmental conditions and increases the tolerance of transgenic lines to oxidative stress and a wide range of environmental challenges. Expression of the *fld* gene in tobacco also caused transgenic tobacco lines to tolerate iron deficiency. Transfer of *fld* to tomatoes and potatoes also increased tolerance to oxidative stress and drought stress (Tognetti et al. 2006; Zurbriggen et al. 2007).

Considering that such a system in crops has disappeared along the evolutionary pathway of vascular plants from the plant genome and the benefits of its expression and function have been lost, due to the successful results in increasing tolerance to non-biological stresses in plants. By transferring the bacterial *fld* gene with useful biotechnology tools, the mentioned crop can be taken to increase the yield of other crops, especially wheat. However, due to the multiplicity and genetic and physiological complexity of tolerance to abiotic stresses, trying to improve these traits with conventional breeding programs, while accompanied by many limitations, also requires a lot of time (Abdolmaleki et al. 2013).

19.5.14 The Role of Bacterial *merA* Gene in Environmental Purification

Mercury is a toxic heavy metal that is widely distributed in ecosystems through industrial pollutants and their sediments. Fossil fuels are one of the major sources of mercury pollution in the environment and wastewater is a major source of two types of organic and inorganic mercury, such as elemental mercury, methyl mercuric chloride, and dimethyl mercury. Mercury is a major environmental pollutant and is one of the bio-accumulative toxins that stay in the environment for a long time (its estimated duration is between 0.5 and 2 years). Mercury changes its chemical forms in the environment, moving from one place to another, and being buried in soil and sediments. Most marine plants and animals absorb mercury, and organisms in the lower branches of the food cycle (such as plankton) trap mercury in their bodies. When vegetarians or carnivores eat the higher branches of the plankton food chain, mercury is transferred to the body of the fish and eventually consumed by humans. Mercury is a mutagenic, growth-inhibiting agent with toxic effects and the cause of most important human diseases and syndromes. The effects of mercury on ecosystem performance are economically and hygienically significant (Dash and Das 2012; Khoshniyat et al. 2018; Teng et al. 2020).

Mercury resistance has been observed in a variety of gram-positive and gram-negative bacteria. In bacteria, mercury resistance genes are mostly located in the operon on the plasmid or transposons. The narrow-spectrum mer resistance to

inorganic mercury (merRTPADE) is only resistant to inorganic mercury, and the broad-spectrum mer operon is resistant to broad-spectrum mercury. Resistance to organomercurials (merRTPAGBDE) resists both organic and inorganic mercury. In this operon, *merA* encodes the mercury ion reductase enzyme, and this cytoplasmic protein plays a key role in mercury removal. This enzyme converts Hg^{2+} to less toxic Hg^0 by the following mechanism. Hg^0 is highly volatile and passes freely through biological membranes out of the cell and back into the atmosphere. Bacteria with the *merA* gene encoding the mineral mercury-lowering enzyme have the genetic ability to remove mercury by reducing the mineral mercury to a gaseous form, thus clearing the contaminated area. This is the last step in the path of non-toxicity of mercury in mercury-resistant bacteria, and thus, the bacteria remove mercury from their environment (Dash and Das 2012; Khoshniyat et al. 2018; Pietro-Souza et al. 2020) and prepare the environment for the cultivation and cultivation of agricultural products.

19.5.15 The Role of Chitinase Gene in Production of Biological Fungicides and Insecticides

Chitinases (EC: 3.2.1.14) are bonds of carbon 1 and 4 between two back-molecules of N-acetylglucosamine (GlcNAc) in chitin chains ranging in size from 20 kDa to approximately 90 kDa; they hydrolyze. Secretory chitinases can be present in chitin-containing (insects, crustaceans, and fungi) or chitin-deficient (plants and bacteria) species. In certain bacterial species, including *Aeromonas*, *Serratia marcescens*, *Myxobacter*, *Vibrio*, *Streptomyces*, and *Bacillus* species, this enzyme is present in abundance. The chitinase enzyme has attracted a great deal of interest due to its wide variety of commercial, agricultural, and medical applications, and the isolation of these enzymes from microscopic organisms has found wide applications in the biocontrol of fungi and nematodes of agricultural pests (Mortazavi et al. 2017).

In addition to their role in the growth and division of fungal cells, *Trichoderma* filamentous fungi, due to the secretion of different chitinase enzymes, are used as a powerful factor in the biological control of fungal diseases. These fungi, chitinase enzymes, have many advantages over other organisms' chitinases, including plant chitinases. For example, plant chitinases, unlike fungal chitinases, only affect the tip of the pathogenic fungal hyphae and are unable to break down the hard chitin structures. Also, these enzymes alone have weak antifungal effects and are effective only on a limited number of fungal species. Studies have shown, on the other hand, that all chitin-containing pathogens in the wall are susceptible to *trichoderma* fungal chitinases, while high concentrations of these enzymes do not have any toxic effects on plants (Berini et al. 2018; Chen et al. 2017).

The possible risk of crop epidemics still exists today. Thus, the introduction of new genes encoding antimicrobials and fungi is what is required in new genetic engineering methods. Therefore, what is needed in new methods of genetic engineering is the introduction of new genes encoding antimicrobials and fungi. Chitinases are one gene class of this type. In insects, nematodes, and some other

species, these genes encode beta-1-4 hydrolyzing enzymes in cell wall chitin of fungi and exoskeletons (Ahmadian et al. 2012).

Chitinases can be used as a biological fungicide and insecticide agent, as well as in animal SCP processing, aquatic organism feeding, fungal protoplast isolation, bioactive cyto-oligosaccharides preparation, and plant pathogens inhibition. Chitin material and environmental purification and its conversion into raw materials, and with population growth and limited natural resources, enzyme technology can be useful for many industries to overcome economic problems soon (Babashpour et al. 2011). Chitinase is a recombinant and decomposing chitin that due to this antifungal property can be used as an effective substance in the treatment of human fungal infections and a safe substance in the elimination of pests and biological contaminants (Ahmadian et al. 2012).

The native Iranian strain of gram-positive *Paenibacillus* sp. bacterium A01 in southern Iran, shrimp ponds have been used for the development of recombinant protein chitinase. After replication of the gene by specific primers for heterologous expression of the recombinant enzyme, cloning was performed in the expression vector pET26b and transferred to Escherichia coli to produce the desired protein. Protein extraction was performed with a nickel-Sepharz affinity column. Its biological activity has also been studied. The results showed that the degradation of chitin by the enzyme chitinase in the bacterium *Paenibacillus* sp. A01 has been developed and it has been suggested that this bacterium be used for mass production of this enzyme in industrial and agricultural applications such as biological pesticides (mortazavi et al. 2017).

19.6 The Role of Microorganisms in Dissolving Phosphate

Phosphorus is one of the most significant plant nutrients and has a little abundance in the soil. Phosphorus, in both organic and inorganic forms, is present in the soil. The ability of some microstructures to convert insoluble phosphorus into a usable form such as orthophosphate is an important feature of PGPR that enhances plant yield. *Pseudomonas*, *Bacillus*, *Pantoea*, and *Rhizobium* are the most potent phosphate solvents. Although the genetic knowledge of phosphate dissolution is still limited, several genes encoding phosphatase have been identified and cloned, and a number of genes involved in mineral phosphate dissolution have been isolated. Transfer and expression of genes involved in phosphate dissolution (organic or inorganic phosphate) in bacteria or plants is a new way to improve the capacity of microorganisms as a microbial inoculum (Sarikhani et al. 2014).

19.6.1 Dissolution of Mineral Phosphate

The activity of mineral phosphate dissolution is linked to the ability of microstructures to release metabolites such as hydrogen ion or proton (H^+) secretion and organic acid (Liu 2019; Surange et al. 1997). Some believe that organic and

inorganic acids with their carboxyl and hydroxyl groups chelate the cations with phosphate anion (Al^{3+}, Fe^{3+}, Ca^{2+}) and thus help the dissolution of phosphate (Purakayastha et al. 2019; Stevenson and Cole 1999); some also believe that the dissolution of phosphate Anion exchange of PO_4^{3-} with anion is an organic acid (Jiang et al. 2019; Omar 1997).

The study of the production of organic acids is often done in liquid media and has been done by methods such as paper chromatography or thin-layer chromatography or by high-performance liquid chromatography (HPLC) and some specific enzymatic methods (Gupta et al. 2020; Gyaneshwar et al. 1998). Among different organic acids, gluconic acid seems to be the predominant and most important acid produced in gram-negative bacteria (de Oliveira Mendes et al. 2020; Goldstein et al. 1993). Production of this organic acid by bacteria such as *Pseudomonas* sp., *Erwinia herbicola*, *P. cepacia*, *Azospirillum* spp., *R. leguminosarum*, *R. meliloti*, *B. firmus*, and *Burkholderia cepacia* has been reported (Fazeli-Nasab and Sayyed 2019; Rodríguez and Fraga 1999; Rodriguez et al. 2004; Saia et al. 2020). Strains of *B. licheniformis* and *B. amyloliquefaciens* have been found to produce a mixture of acetic, lactic, isovaleric, and isobutyric acids. Other organic acids such as succinic, malonic, oxalic, and glycolic acids have also been identified among phosphate solvents (Rodríguez and Fraga 1999; Saia et al. 2020).

In addition to organic acids, phosphatase and phytase enzymes are also involved in the dissolution of phosphates. In the case of enzyme-destroying agent therapy, if there is no improvement in the release of phosphorus, it is suggested that the mechanism involved in the dissolution of phosphate is non-enzymatic and is connected to the processing of organic or inorganic acids. If the dissolution of phosphate is stopped if it is treated with a base, it indicates that the dissolution of phosphate is organic acid (Fazeli-Nasab and Sayyed 2019; Rodríguez and Fraga 1999; Saia et al. 2020). Based on these findings, the cloning of genes related to mineral phosphate dissolution was followed. Oxidation of glucose to gluconic acid and ketogluconic acid is the metabolic basis of mineral phosphate-solubilizing phenotypes in some gram-negative bacteria (Goldstein 1995; Khoshmanzar et al. 2020).

19.6.1.1 The Most Important Genetic Factors Involved in the Dissolution of Mineral Phosphate

In 1987, for the first time, an *Erwinia herbicola* gene involved in mineral phosphate dissolution was cloned in an environment containing hydroxyapatite as the sole source of phosphorus by screening antibiotic-resistant recombinants from the Genomic library. Expression of this gene has led to the production of gluconic acid and the dissolution activity of the mineral phosphate in *E. coli* HB101. Sequencing of this gene indicates its possible involvement in the synthesis of *Pyrroloquinoline quinone* (PQQ), which is an essential factor in the formation of the glucose dehydrogenase holoenzyme (GDH) (Table 19.3). GDH-PQQ catalyzes the formation of gluconic acid by direct oxidation of glucose. *E. coli* is able to produce GDH but cannot make PQQ, so gluconic acid is not produced (Dai et al. 2020; Rodríguez and Fraga 1999; Rodríguez et al. 2006; Saia et al. 2020).

Table 19.3 Inorganic phosphate solubilizing genes from different bacteria

Microorganism	Gene or plasmid	Characteristics	References
Erwinia herbicola	*Mps*	Produces gluconic acid and dissolves mineral phosphorus in *E. coli* HB101, possibly involved in PQQ synthesis	Rodríguez et al. (2006)
Pseudomonas cepacia	*gab Y*	In *E. coli* JM109 produces gluconic acid and dissolves mineral phosphorus, has no similarity with PQQ genes	Rodríguez et al. (2006)
Microbe-derived enzymes	*olpA, phoD, appA, phnX, and phnJ*	Able to release free orthophosphate from organic P form	Liang et al. (2020)
Enterobacter agglomerans	*pKKY*	Dissolve phosphorus in *E. coli* JM109, without lowering the pH	Rodríguez et al. (2006)
Microbe-derived enzymes	*NSAPs*	Releasing inorganic phosphates from nucleotides and sugar phosphates	Sarikhani et al. (2014)
Serratia marcescens	*pKG3791*	Produces gluconic acid and dissolves mineral phosphorus	Rodríguez et al. (2006)
Synechococcus PCC7942	*Pcc gene*	Produces phosphoenol carboxylase	Rodríguez et al. (2006)
Pseudomonas fluorescens	*pqqA,B,C,D,E, and F*	Produces gluconic acid and dissolves mineral phosphorus, possibly involved in PQQ synthesis	Orikasa et al. (2010)
Burkholderia multivorans WS-FJ9	*AP-2*, *GspE*, *GspF, PhoR*, *HlyB, PhoA*, *AP-1* and *AP-3*	Involved in the decomposition of organic and inorganic phosphates	Liu et al. (2020)
Ralnella aquatillis	*KIM10*	In *E. coli* DH5α coli produces gluconic acid and dissolves mineral phosphorus, possibly involved in PQQ synthesis	Rodríguez et al. (2006)
Prokaryotic genes	*phoX, phoA, and phoD*	Conversion of organic phosphate to mineral and accessibility for plants	Wan et al. (2020)

By taking a similar approach, another gene associated with mineral phosphate dissolution was isolated from *P. cepacia* (Table 19.3). Expression of this *gabY* gene, which led to the formation of a mineral phosphate-solubilizing phenotype through the production of gluconic acid in *E. coli* JM109, bore no apparent resemblance to the cloned gene synthesizing *PQQ*. The *gabY* gene was able to play an alternative role in expressing or regulating the direct oxidation pathway in *P. cepacia* (Dai et al. 2020; Rodríguez and Fraga 1999; Saia et al. 2020). Other isolated genes associated with MPS phenotypes do not appear to be limited to *pqqDNA* and *gab-synthesizing* genes. Genomic DNA fragments from *Enterobacter agglomerans* showed MPS activity in *E. coli* JM109, although the ambient pH did not change. These results indicate that acid production is an important method but not the only mechanism

involved in the dissolution of phosphate by bacteria. Isolation of the phosphoenolpyruvate carboxylase encoding gene pcc in *Synechococcus* PCC7942 indicates that it is involved in MPS (Dai et al. 2020; Rodríguez et al. 2006).

19.6.2 Mineralization of Organic Phosphorus

Organic compounds of phosphorus such as phytic acid can account for 20–80% of soil phosphorus (Chiu and Paszkowski 2019; Raghothama and Karthikeyan 2005), although changes have been reported in the range between 4 and 90%. Approximately half of the microorganisms with their phosphatase activity present in the soil and roots of plants mineralize organic phosphorus (Tarafdar et al. 1988; Zhang et al. 2020). In the form of a substrate, acidic and alkaline phosphatases convert organic phosphate into a mineral (Beech et al. 2001; Xu et al. 2020).

Phosphorus can be released from organic compounds in the soil by three enzyme groups. (1) Nonspecific phosphatases that follow the dephosphorylation of phosphoester or phosphoanhydride bonds in organic matter. (2) Specific phosphatases such as phytases that release phosphorus from phytate. Although this division is not correct in terms of gene and protein sequence, but in practice it can be said that the main activity of organic phosphorus mineralization is the responsibility of the first two groups (Rodríguez et al. 2006).

Phytases (Myoinositol hexaphosphate phosphohydrolase) belong to a special group of phosphomonoesterases that are able to release phosphorus from phytate (Davis 2020; Zhao et al. 2019). Phytic acid was first discovered in 1903, and its salts are known as phytates (Haefner et al. 2005; Nobile et al. 2019).

19.6.2.1 The Most Important Genetic Factors Involved in the Dissolution of Organic Phosphorus

19.6.2.1.1 Phosphatases

Different phosphatase activity patterns have been observed in bacteria, and complex regulatory mechanisms such as specific environmental conditions often control the production of these enzymes (Liang et al. 2020; Wan et al. 2020). Probably the main mechanism for regulating the expression of phosphatases is an induction by the amount of mineral phosphate (Pi) present in the medium. This mechanism has been studied for alkaline phosphatase (*pho A*) *E. coli*. When Pi concentration decreases to 0.16 mM, the expression of this gene is induced. This mechanism includes a Pi transport operon Pi as a regulating element, as well as a sensor-activator operon as a solver and activator. Genes controlled by the amount of Pi, their expression is activated by PhoB, which is the main part of the PHO regulon (Rodríguez and Fraga 1999).

The grouping of bacterial phosphatases was focused on the enzyme's biochemical and biophysical properties, such as optimum pH (acidic, neutral, or alkaline), substrate profile (specific or nonspecific), and molecular weight (high versus low molecular weight) (Dai et al. 2020; Gaiero et al. 2020; Rossolini et al. 1998).

Bacterial nonspecific acid phosphatase (NSAP) belongs to three families called molecular groups A, B, and C (Rossolini et al. 1998). Attention to group A of these enzymes for bioremediation of heavy metals has increased in the last decade. Attention is also paid to NSAPs for the transfer and expression of these genes in PGPR bacteria to achieve improved phosphate-solubilizing strains using recombinant DNA technology (Rodríguez et al. 2006).

The existence of preserved domains is seen in the comparison of amino acid sequences of six recognized group A enzymes, and the existence of the GSYPSGH [TA] motif is characteristic of this family (Felts 2007; Rossolini et al. 1998). The presence of highly conserved fragments in them is shown by a comparison of amino acid sequences in group B. In this category (Gaiero et al. 2018; Rossolini et al. 1998), the FDIDDTVLFSSP sequence is suggested as a signature sequence pattern. Although group C is distinct from the other two groups, it is similar in sequence level to group B phosphatases and some plant acid phosphatases. The first identified member of this group is the OlpA-Cm protein, whose gene encoding was isolated from the bacterium Chryseobacterium meningosepticum (Rossolini et al. 1998; Wang et al. 2019). A comparison of the amino acid sequences of this group with other proteins has allowed the identification of protected and common areas between these sequences. The results show that group B and group C acid phosphatases, together with some plant acid phosphatases, can be included in a protected subfamily called "DDDD phosphohydrolase subfamily" due to the presence of four aspartate (D) amino acids (Rossolini et al. 1998; Wang et al. 2019).

Several acid phosphatase genes have been isolated from Gram-negative bacteria and characterized. For example, the *acpA* gene isolated from Francisella tularensis encodes acid phosphatase with optimal activity at 6 pH: with a wide range of action on specific substrates. Class A (*PhoC*) and class B (*NapA*) acid phosphatase encoding genes were also isolated from Morganella morganii. In addition, these enzymes are rebellious or non-inducible and show high activity at pH: 6 and 30 °C and action on various substrates (Rodríguez et al. 2006; Sarikhani et al. 2014).

Among rhizobacteria, a gene that facilitates phosphatase activity has been isolated from *Burkholderia cepacia*. This gene encodes an outer membrane-bound protein that increases its expression in the absence of solution phosphorus and may be involved in transporting phosphorus (Liu et al. 2019; Rodríguez et al. 2000). In addition, cloning of two nonspecific phosphatase genes of periplasmic acid (nap E and nap D) from *Sinorhizobium meliloti* was carried out. Also, cloning and transfer of *napA* phosphatase gene from Morganella morganii to *Burkholderia cepacia* IS-16 was performed using broad-host-range vector *pRK293* and an increase in the extracellular phosphatase activity of the recombinant strain was reported (Ferroni et al. 2011; Rodríguez et al. 2006). Cloning and expression of the phosphotriester gene (*hocA*) of *Pseudomonas monteilii* C11 have been performed (Horne et al. 2002; Latip et al. 2019). It is named because of the hydrolysis of coroxon as a source of organic phosphate. The gene is 501 bp in length and encodes a 19 Kd protein, enabling the bacterium to use this source of organic phosphate as the only source of phosphorus in the environment.

19.6.2.1.2 Phytases

Phytate (Mayo Inositol Hexaxis Phosphate) is one of the major forms of phosphorus in oilseeds, legumes and oilseeds (Mayo Inositol Hexaxis Phosphate). In many grains and oilseeds, phytic acid makes up 1–3% of the weight, and usually 60–80% of the phosphorus in these plants. Phytase is the enzyme that hydrates phytate to lower myoinositols and, in some cases, free myoinositol and mineral phosphorus (Ariyan Nezhad et al. 2013).

Phytase (myoinositol hexaxis phosphate phosphohydrolase) genetic studies began in 1984, and in the mid-1990s, the first commercial phytase developed by engineered microstructures entered the market under the name of Natuphos (Bavaresco et al. 2020; Corrêa and de Araújo 2020). In order to improve the diet of monogastric animals, most genetic engineering studies have concentrated on phytase science. It is also used to dissolve soil phytate, as inoculants with high phytase production are among the favorites to improve plant nutrition and reduce soil phosphorus contamination. Phytase genes are also cloned from fungi, plants, and bacteria. Heat-stable genes (phy) from *Bacillus* sp. DS11 and cloned from *B. subtilis* VTT E-68013 (Corrêa and de Araújo 2020; Konietzny and Greiner 2004; Rodríguez et al. 2006). So far, four distinct groups have been reported based on the sequence of genes and their conserved regions, three-dimensional structure, reaction mechanisms, and enzymatic properties for phytases abbreviated to *HAP*, *PAP*, *CP*, and *BPP* (Hou et al. 2020; Naghshbandi and Moghimi 2020; Sarikhani 2012).

Phytases are also divided into two general groups based on optimal pH of activity: acidic and alkaline phytases. The first group includes fungal phytases and the group of gram-negative bacteria, and *Bacillus* bacteria belong to the group of alkaline phytases (Oh et al. 2004; Vasudevan et al. 2019). In another classification, phytases are named based on which group of phosphates is first removed from the phytate by the enzyme. For example, 3-phytase or 6-phytase, which indicates that phosphate 3 and phosphate 6 are the first phosphates removed from the phytate molecule, respectively. *E. coli* phytase is of 6-phytase type, while fungal phytases and bacilli are of 3-phytase type (Jatuwong et al. 2020; Oh et al. 2004; Vats and Banerjee 2004; Vohra and Satyanarayana 2003).

Natural phytases are distinguished into constitutive and inducible phytases in terms of expression pattern (Shieh and Ware 1968; Vohra and Satyanarayana 2003).

Fungal extracellular phytase is induced and produced at low concentrations of mineral phosphate in the growth medium (Vohra and Satyanarayana 2003). Unlike fungal phytase, *B. subtilis* phytase is induced in the presence of phytate (Kerovuo 2000).

Many fungal and bacterial phytases have been studied. The enzyme phytase produced by gram-positive bacteria and fungi is extracellular (Ariyan Nezhad et al. 2013). Phytase-producing bacteria and fungi have been extensively studied, including gram-negative bacteria such as *E. coli*, *Pseudomonas* sp., *Klebsiella* sp. and from gram-positive bacteria to *Bacillus* sp. (Haefner et al. 2005; Oh et al. 2004; Vats and Banerjee 2004). Cloning, sequencing, and expression of the acid phosphatase/phytase gene (*appA2*/*appA*) have also been identified in pigs (Liang

et al. 2020). The dual function of these enzymes makes them attractive for dissolving soil organic phosphorus. However, isolation of phytase genes from *Aspergillus Niger*, *Emericella nidulans*, and several other species has been previously reported. Alkaline to neutral phytase genes also derived from *B. subtilis* and *B. licheniformis* have been cloned (Kerovuo 2000).

Due to the importance of phytase enzyme in the dissolution of organic phosphate and the need of monogastric animals to use these food sources, the production of phytase and its addition as a food additive to the diet [feed additive] is considered (Kour et al. 2020; Pandey et al. 2001). In a study, isolation and sequencing of the *phyA* gene from the Obesumbacterium proteus genomic library was reported, and then cloning and expression of this gene in *E. coli* were investigated, and the characteristics of the produced phytase enzyme were evaluated (Zinin et al. 2004). In another study, after establishing the genomic library of *P. putida* strain P13 in *E. coli* strain DH5α strain, they screened in at least Sperber medium in the presence of BCIP and then isolated two genes related to organic phosphate mineralization called PPP1 and PPP2. Examination of the enzymatic properties of the mentioned genes has shown that PPP1 has a prominent phytase property, while PPP2 has shown more sugar phosphatase properties (Malboobi et al. 2013; Sarikhani et al. 2014).

19.7 The Role of Different Microbial Genes in the Synthesis of Bioethanol

Bioethanol (bioethanol, bio-ethyl alcohol) is a clear, colorless, bicarbonate (C_2H_5OH), with low toxicity, biodegradability and causes less pollution to the atmosphere. Ethanol combustion absorbs carbon dioxide and water. The biological raw materials used in bioethanol production are primarily plant-based. Bioethanol (Champagne 2007; Yu et al. 2020) may also be produced in some raw materials of animal origin. A large part of agricultural products, wastes and wastes of agricultural products, by-products of agricultural conversion industries, products and wastes of by-products of forest and related industries, and of course urban and industrial wastes and biological wastes are used for bioethanol production. Increasing the yield of ethanol along with improving production economic processes and technological advances is key parameters in ethanol production. In order to achieve a high yield of ethanol, strains must be produced that produce fewer unnecessary products and are able to metabolize all major sugars. One of the main obstacles to the development of bioethanol production has been the lack of suitable industrial microorganisms to convert biomass into ethanol fuel. Quantitative conversion of glucose to alcohol is done by yeasts as well as a number of bacteria. Lignocellulosic biomass contains complex carbohydrates that necessitate the use of associated microorganisms; for the yeast to ferment non-fermentable sugars (Zeinali et al. 2016).

The pyruvate decarboxylase gene from the bacterium Zymomonas mobilis is the primary and main gene for the development of bioethanol and, among all related enzymes in other microorganisms, the enzymes it produces have the highest

enzymatic activity. It was ethanol (Zeinali et al. 2016). Also, the enzyme *Cel6B* from the bacterium *Thermobifida fusca*, a CBHII belonging to the family of celluloses that is very resistant to heat, which is ultimately effective in the production of ethanol (Heidari-Gharehsoo et al. 2018).

The hexose transporter family in the yeast *Saccharomyces cerevisiae* includes the proteins *Hxt1p-Hxt17p*, *Gal2p*, *Snf3p*, and *Rgt2p*. As the activity of these transducers increases, the accumulation of ethanol or lactic acid in the cells increases. *Hxt1* to *Hxt17* transducers are involved in glucose transport, *Gal2* acts as a glucose sensor for galactose transport, *Snf3* and *Rgt2*. This gene family has different expression patterns and their regulation is strongly influenced by the kinetics of transmitters and glucose is the first-factor controlling expression. *HXT1-HXT7* are among the most important transporters and are metabolically similar and interrelated. The effect of maximal expression of these genes in yeasts has been studied and ethanol production in the wild yeast strain has been compared with the engineered strain. The obtained data showed that overexpression of hexose transporters leads to increased glucose uptake. The researchers showed that by regulating the first stage of the glucose biosynthesis pathway, lactic acid accumulation could be observed, which observed a 15% increase in ethanol production compared to the wild strain (Azizi et al. 2016).

19.8 Use of Microbial Genes in Plant Genome Editing

Ensuring society's food security and the need to increase agricultural production on an ongoing basis depends on arming and efficiently integrating conventional plant breeding with modern biotechnology and powerful tools for genetic engineering (Kirillova et al. 2020; Yadav et al. 2019). The most complex branch of biotechnology is genetic engineering, which involves methods to pick the target gene, locate, isolate, purify, replicate, and transfer genes and test their expression in living organisms (Kim et al. 2020; Sedeek et al. 2019).

At present, the great importance and ability of genetic engineering by making purposeful changes in the genomes of plants and animals to remove many limitations of traditional plant breeding, create new characteristics, and improve the quantity and quality of food products by producing crops and gardens. Transgenics are not hidden from anyone. However, modern biotechnology has been accepted as a way of producing products that have wide applications in medicine, agriculture and industry. However, the safety aspects of genetically modified or transgenic organisms (LMOs) and their products must also be carefully considered before use. (Gabriel 2019; Haribabu 2019).

Selective genes such as antibiotic resistance and herbicide genes are mentioned as the most important considerations influencing the utilization of transgenic products, penetration of non-plant DNA fragments into the parent plant genome, gene escape, and vertical transfer of the target gene (Mackelprang and Lemaux 2020; Mathur 2018; Parray et al. 2019). Therefore, the need to use advanced methods of producing transgenic plants such as short intervals of the regular clustered palindrome

(*CRISPR*/*Cas9*), which is a genome editing method, to improve the quality and effective yield of high-yield crops is felt more than ever. So that this system can accelerate plant modification without transferring external genes by making mutations in multiple gene sites and creating large deletions. This method can improve the function and activity of plant genes and create new traits. Over the years, the process of this advanced technology has been extensively studied by numerous examples of targeted mutations and regulation of the copying process in a variety of crops, and this has demonstrated the effective aspect of this new system. To date, the *Cas9* system has been widely used in gene silencing, gene replacement, multiple gene editing, gene function identification, and regulating the replication process in animals and plants (Artegiani et al. 2020; Cunningham et al. 2018; Mackelprang and Lemaux 2020; Molla and Yang 2020; Si et al. 2020).

19.8.1 Overview of the *CRISPR*/*Cas9* System Mechanism

In the Escherichia coli genome, the *CRISPR*/*Cas*9 system was first identified in 1987 as an acquired immune system against the invasion of bacteriophages and the entry into the bacterial cell of foreign DNA such as plasmids. In all prokaryotes, 2000 *CRISPR* gene families were described (Si et al. 2020; Zhang and Zhang 2020). According to the *CRISPRdb* (http://www.crispr.i2bc.paris-saclay.fr) database, the locus of the *CRISPR* gene is approximately 84% in the archaeal genome and 45% in the bacterial genome (Makarova et al. 2020).

The *CRISPR* system consists of two regions, including genes encoding Cas nuclease enzymes and the gene locus of *CRISPR* arrays containing repeat sequences and spacer sequences between them. The length of duplicate sequences is about 25–50 bp and more than 249 bits and the spacing area containing non-duplicate sequences is about 26–72 bp (Arslan et al. 2014; Kunin et al. 2007; Pourcel et al. 2020). The leader sequence is approximately 200–500 bp in length, consisting of AT-rich sequences that are necessary as a promoter sequence for copying *CRISPR* locus arrays. The 4 (*Cas*) associated *CRISPR* genes (Cas 1–4) are located near the CRISPR array region, encoding proteins essential for inducing an immune response by bacteria against virus attack (Fonfara et al. 2016; Richter et al. 2012; Zhang and Ye 2017).

Streptococcus pyogenes has been widely used to edit the genomes of various species and cell types such as human, bacterial, yeast, laboratory mice, vinegar flies, nematodes, crops, insects, and monkeys. In the crRNA processing mechanism type II, the Trans-activating crRNA sequence binds to duplicate sequences to form the crRNA/tracrRNA complex. It then produces mature crRNAs with the *Cas9* protein and RNaseIII enzyme activity. This processing system is well known and widely used in genetic engineering and genome editing. The generated *crRNAs* have 20 nucleotide sequences at their 3′ end. In this system, adult *crRNAs* are generated after transcription of pre-crRNA sequences from the *CRISPR* gene locus by the *Cas9* protein (Fonfara et al. 2016; Hynes et al. 2017; Marraffini 2016; Zhang and Ye 2017).

CRISPR/Cas9 achievements in plants: The *CRISPR/Cas9* system produces stable and inherited mutations that can be easily distinguished from the *Cas9*/gRNA structure for further modification by *CRISPR/Cas9*. This leads to the development of non-transgenic homozygous plants that have been produced in only one generation (Fauser et al. 2014; Xu et al. 2015). A non-transgenic rice cultivar with a mutation in the target gene has been successfully produced by transgenic cleavage by causing T1 generation self-healing (Xu et al. 2015). Also, a series of dual vectors based on *CRISPR/Cas9* system with stable expression capability in plant systems and a series of vectors containing gRNA module have been designed. Therefore, the transfer of only Cas9 nuclease protein and gRNA into the host cell by genetic transfer methods is the only necessity for plant genome editing (Xing et al. 2014).

It has been suggested that viral glycemic replicates (GVRs) can be used to transfer the *Cas9*/gRNA structure into the host cell when the virus replication initiation protein (REP) gene is transferred along with the Cas9/gRNA structure (Baltes et al. 2014). In addition, in order to use this system in upgrading and discovering genetic traits, high-performance transfer methods such as DNA-based replication viruses are used to transfer genome engineering materials without the need for genetic engineering transfer methods (Ali et al. 2015; Yin et al. 2015). Based on the researches, direct transmission using tobacco rattle virus (TRV), cabbage leaf complex virus (*CaLCuV*), *Cas9*/gRNA transferability by different viruses in editing the genomes of different plants have been clearly shown (Yin et al. 2015).

19.9 Conclusion

In today's world, there are other significant issues such as climate change and environmental problems, and the loss of genetic resources, in addition to the issue of human communities supplying food and producing more food, one of the reasons for the improper use of chemical products in agriculture (such as toxins and chemical fertilizers, etc.).

Although pesticides and herbicides play an important role in controlling plant diseases and weeds, they have negative side effects on living organisms and their environment. They also cause resistance to pests and weeds and have adverse effects on desirable non-target microorganisms. Also, the limitation of the use of physical and chemical cleaning methods of plant toxins, such as reducing the quality and nutritional properties of the product and the high cost of the required equipment, has led researchers to focus on biological methods. In order to minimize the use of these toxic chemicals, the use of biological agents such as fertilizers and biotoxins can play a very important role in the protection of the environment and of agricultural farms. Moreover, by using modern biological technology, such as genetic engineering, increasing the performance of these biological factors will also improve their efficacy. However, the transfer of new genes to these biological agents and their release into agricultural environments can be associated with certain potential environmental hazards. Therefore, due to the acquisition and development of new molecular technologies to track and study the effects of such recombinant

microorganisms in nature, it is necessary to carefully study the possible effects to safely and efficiently use the definite benefits of these organisms in agriculture.

It is suggested that given the standards for assessing the potential hazards of recombinant microorganisms as well as microbial compounds (genes, proteins, or metabolites) used, possible risk assessment on a case-by-case basis for each microorganism or microbial compound according to the purpose and type of environment used and done in a completely scientific manner and if there was a possibility of specific hazards for that microorganism or the microbial compound used, scientific and managerial solutions should be provided to prevent or reduce those risks so that we can take full advantage of the environmental and economic benefits of such biological products.

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