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REVIEW

Study on Artificial Seeds of Plants

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ABSTRACT

The establishment and development of artificial seed technology is to quickly reproduce excellent varieties or hybrids, which can be applied to hybrid generation seeds produced by the three-two line method. For some varieties that are difficult to propagate with seeds or plant species with unstable genetic traits and poor fertility, artificial seed technology can also be used for mass reproduction. In particular, some new plants created through genetic engineering, such as somatic hybrids or transgenic plants, can be propagated or maintained by artificial seed technology. In addition, artificial seed technology can be used for the maintenance and rapid propagation of virus-free seedlings. Compared with ordinary test tube seedlings, artificial seeds have low cost, convenient transportation, and to a certain extent reduce vitrified seedlings.In particular, the production of artificial seeds does not occupy a large amount of soil. It can be produced all year round. Therefore, the research on artificial seeds has developed rapidly in the world.

1. The Concept of Plant Artificial Seeds

The general concept of plant artificial seedsThe concept of plant artificial seeds was first proposed by Murashige (1978). It refers to the somatic embryos produced in the culture of plants in vitro or meristems that can develop into complete plants (buds, Callus tissue, embryoid body, etc.) are embedded in the shell containing nutrients and protective function to form granules that can germinate and emerge under appropriate conditions.

Since artificial seeds are asexual reproduction in nature, compared with natural seeds, they have the advantages of large-scale factory preparation, storage and rapid promotion of excellent germplasm resources, so they have been valued by many countries. After being buried in a certain capsule, the body has the function of seed and is directly used for sowing in the field.

The artificial seed technology in a broad sense includes the production and packaging of somatic embryos, storage of artificial seeds, artificial seed making equipment, etc., but in a narrow sense, artificial seed technology includes the production of somatic embryos and somatic cells Embryo wrapping (artificial endosperm and seed coat). The complete artificial seed includes three basic parts: somatic embryo, artificial endosperm, and artificial seed coat. Because artificial seeds are asexual in nature, they can propagate some plants that are not strong under natural conditions or have expensive seeds, fix

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the heterosis, make Fl hybrids available for multiple generations, and enable excellent single plants to quickly reproduce. Clonal varieties, which greatly shortens the breeding period [5].

Plant artificial seed is a bioengineering technology that is being created because of its great practical significance and economic value. At present, the United States, Japan, and France have carried out this research in succession. It has initially achieved success in plants such as carrots, celery, alfalfa, camellia, and tomatoes, but it is still in the laboratory stage and has not yet been applied to production practice.

2.The Production Process of Artificial Seeds

There are three major steps in making artificial seeds: ① embryoid body culture; ② wrap seed coat; ③ germination test.

Basic process: disinfection of the explant surface → induction of embryogenic callus → development of embryoid body → synchronization and screening of embryoid body → encapsulation of calcium alginate capsules by beading method → wrapping outer membrane → germination and seedling experiment.

To make artificial seeds, you must first have high-quality embryoid bodies. Some plant tissue cultures are not easy to obtain embryoid bodies, but can also be replaced with adventitious buds, small bulbs, hairy roots, etc. But artificial seeds encapsulated with embryoid bodies are better. High-quality embryoid bodies can germinate and emerge in time after sowing, and the roots and shoots grow almost simultaneously. It has sufficient nutrients, has a certain resistance, and has strong vigor. The hypocotyl is not enlarged and there is no callus organization. The synchronization of development is good, and the size is basically the same after sorting. The germination and seedlings are neat, normal and non-deformed [6].

3.Embedding and Seed coat of Artificial Seeds

The embedding methods of artificial seeds mainly include: liquid gel embedding method, dry wrapping method and hydrogel method.

The liquid gel embedding method is to directly suspend the embryoid bodies or small plants in the soil with a viscous fluid gel. Drew (1979) used this method to place a large number of carrot somatic embryos in a sugar-free nutrient matrix to obtain 3 plantlets. Baker (1985) then added sucrose to the liquid gum, and 4% of the embryos survived for 7 days. The drying method is a method of burying somatic embryos together with polyoxyethylene and other polymers. Although the method reported by Ktto et al. has a low plant formation rate, it demonstrates the effectiveness of drying and embedding somatic embryos. The hydrogel method refers to a method of encapsulating material formed by ion exchange or temperature mutation. Redenbaugh et al. (1987) used this method for the first time to obtain artificial seeds from somatic embryos of a single alfalfa plant, and the plant formation rate in vitro was 86%. Later, this method was widely adopted by other artificial seed research groups [10].

In order to solve the problem of human endosperm, people began to study the outer seed coat to add a protective layer. The ideal artificial seed coat should have: (1) Non-toxic and harmless to artificial seed embryos, biocompatible, and support artificial seed embryos; (2) Has a certain permeability and water retention, does not affect the storage and preservation of artificial seeds, Make the artificial seeds grow normally during germination; (3) Have a certain strength, can maintain the integrity of the capsule, which is convenient for the storage, transportation and sowing of artificial seeds; (4) Keep the nutritional ingredients and other auxiliary agents from leaking; (5) It can be degraded by some microorganisms (selective biodegradation), and the degradation products are not harmful to plants and the environment.[2]

RedenbaughThe others successfully used ElvexTM 4260 as the outer seed coat with a germination rate of 80%.

Artificial seeds of Brassica oleracea were prepared with chitosan as the outer seed coat, and their germination rate was measured.But in the presence of bacteria, the germination rate is still not high. At present, most of the artificial seeds developed by the researchers use sterile agar germination test. Artificial (outer) seed coats are not necessary, so most artificial seeds do not have seed coats [9].

4.Conversion of Artificial Seeds under Soil Conditions

Although the theory of somatic embryogenesis is important to restore a complete plant from a cell embryo under test-tube conditions, artificial seeds will eventually grow into a complete plant in a greenhouse or field. Many researchers have successfully obtained the same germination rate as the production conditions through experiments.

The germination of seeds in human soil, whether in a controlled box temperature environment or an uncontrolled greenhouse and field environment, requires the endurance of somatic embryos and the formation of its own roots, stems and leaves, also depends on the quality of the seedlings, high quality , Then the conversion of artificial seeds in the soil is high, and the subsequent

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development is also stable.

After the conversion of artificial seeds (growing seedlings), the main limiting factor for continuing to grow into a complete plant may be the availability of nutrients (salt and carbohydrates). To solve this problem, embryos must have their own nutrient reserves and external nutrition supplies. At the same time, these components are required for the entire growth period of the artificial seed rest system, so the nutritional structure library (seed coat, matrix) of the somatic embryo must have both endogenous (starch and protein) and exogenous (artificial endosperm)[1].

5. Somatic Clone Variation of Artificial Seeds

In plant cell tissue culture in vitro, tissue culture will produce clonal variation. Chromosome abnormalities and morphological variations also exist in the regenerated plants of wheat somatic cell culture. Vasil (1986) believed that the chromosome number and structure of the regenerated plants obtained by the somatic embryo route were stable. However, Hartman et al. (1987) found significant changes in the DNA of wheat embryonic cell lines. Bogini (1988) believed that the propagation of axillary buds could overcome somatic clone variation. The mutant was discovered during the germination of carrot artificial seeds. In addition, after the artificial seeds are dried, low-temperature ABA treatment will increase the proportion of abnormal cotyledons. Japan Kirin Beer Company (1989) transplanted 20,000 seedlings of F1 generation of artificial seeds, celery and lettuce into the field, observed the offspring, and found no changes in the production and quality of artificial seed germination seedlings.

Artificial seed germination seedling variation is strived to avoid in the application of artificial seeds, but did not overcome the changes of effective means. However, if the mutation trait is not important to the crop, or if the mutation frequency is lower than the allowable value that affects yield, this artificial seed is still valuable.

For most crops, artificial seeds are not as good as natural seeds, but with the advancement of artificial seed technology, in some crops, it is possible to replace natural seeds with artificial seeds [8].

6. The Significance and Prospect of Artificial Seeds

Seeds are the basis for plant seed generation and human reproduction. Artificial seeds not only can be stored, transported, sown, germinated and grown into normal plants like natural seeds, but also have many unique advantages.

(1) Plants that are not strong or have expensive seeds can be propagated under natural conditions. (2) Fixed heterosis. (3) Fast and efficient breeding method. (4) The research results of genetic engineering can be transformed into productivity as soon as possible. (5) The growth and development and stress resistance of plants can be controlled artificially. (6) Low cost, convenient storage and transportation [7].

Artificial seeds have shown attractive prospects to humans since their birth, but there are still many problems (including theoretical, technical, and commercial) in large-scale application in production that need to be solved urgently. Artificial seeds must really enter the commercial market and allow nature Seed competition, the most important thing is to reduce production costs, because the cost of artificial seeds of most crops is still much higher than natural seeds, but one thing is clear, that is, artificial seeds and test tube seedlings use less medium , Small size, fast reproduction, fast germination and seedling generation, convenient transportation and storage, the prospect of the development and utilization of artificial seeds is attractive. It is foreseeable that this biological high-tech will play a huge role in crop genetic breeding, improved breeding and cultivation [3].

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REVIEW

Summary of the Frontier Introduction of Preparation of Secondary Metabolites in Plant Cell Culture

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ABSTRACT

Plant cell culture technology is a technology that applies the research results of cell engineering to produce plant biological products at the cellular level. In recent years, the secondary metabolites of plants have attracted more and more attention. The use of plant cell culture technology is a fast and efficient method of producing secondary metabolites.

1. Introduction

Plant secondary metabolites occupy a large position in people's lives and exist in all aspects of our lives, such as paclitaxel and vinblastine in industry [1], and coffee and cocoa in daily life. People's use of plant secondary metabolites can be traced back to ancient times, but it has always been restricted by various natural factors, such as the destruction and restriction of the ecological environment at this stage, the influence of natural conditions in the process of plant cultivation, and the yield and quality are difficult to control However, the chemical synthesis method is complicated and expensive, and it is easy to cause great environmental pollution, and its development prospects are not satisfactory. Therefore, the use of plant cell culture to produce secondary metabolites has become an important way to solve the problem.

2. Introduction to the Preparation of Secondary Metabolites from Plant Cells

Secondary metabolites refer to a large class of small molecular organic compounds that are not necessary for cell life activities or normal plant growth and development in plants. Compared with other methods, the use of plant cell culture technology to prepare secondary metabolites has great advantages:

(1)Under the conditions of complete manual control,

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continuous production can be achieved, regardless of region and season, saving land and facilitating industrial production;

(2)Obtain metabolites that exceed the yield of the original plant by changing the culture conditions and selecting superior lines;

(3)Plant cell culture technology is completed under aseptic conditions, which can eliminate the intrusion of germs and insect pests on medicinal plants;

(4)Genetic manipulation of the synthetic route of active ingredients can increase the production of required secondary metabolites on a large scale;

(5)Plant cell culture technology is also an important way to solve resource problems.

After a long period of development, plant cell culture technology has established a standard operating procedure.

2.1 Selection of Highly Expressing Cell Line

Screening high-yield and stable cell lines can greatly increase the yield and quality of secondary metabolites. At present, the stable expression system and transient expression system in transgenic technology [2] are used to produce high-yielding cell lines with the target gene. After a large number of screenings, cell lines that can produce specific secondary metabolites can be obtained.

Transgenic technology produces cell lines with low investment, low cost and safe use. The plant cell culture conditions are simple, easy to survive, and easy to operate. The main methods used are: Agrobacterium-mediated method, recombinant virus infection of plants, and gene gun transformation method And pollen tube channel transformation method [3].

2.2 Suitable Environmental Conditions

When different plants produce secondary metabolites, the required environmental conditions are different. When plant cell culture is carried out, the medium components such as carbon source, nitrogen source, organic matter, exogenous plant hormones, plant growth regulators, inorganic The requirements for salt and pH are different. At the same time, proper light and temperature are important factors to increase the production of secondary metabolites of plant cells. For example, when roselle suspension cells synthesize anthocyanins, blue light is the most effective monochromatic light that promotes the production of anthocyanins by roselle cells. The output is 416mg/L, which is similar to panchromatic light. Red light and orange light are ineffective. Other monochromatic light As its wavelength approaches blue light, the positive effect increases [4].

2.3 Production of Secondary Metabolites

(1) Precursor feeding and biotransformation, and insufficient precursor compounds will affect the anabolism of secondary metabolites in plants. Reactions such as saponification, esterification, demethylation and double bond reduction can occur in plants. For example, adding farnesol to Tripterygium wilfordii cell culture can increase the production of tripterygium hydroxylactone by more than 3 times. The addition of L·phenylalanine can increase the yield of shikonin by 3 times[4].The type, concentration and time of the added precursor will also affect the preparation of secondary metabolites.

(2) Cell fixation system, the cell fixation system can maintain the amount of cells in the reaction tank, immobilization makes the reaction activity stable, and can be produced continuously for a long time, and is easy to separate from the cells as the catalyst [5], At the same time, it is easy to control the environmental conditions and substrate concentration in production.

However, when the fixed system is used for plant cell culture, the cell culture density is high, the supply and delivery of oxygen and nutrients are a difficult problem, resulting in relatively slow growth of cultured plant cells, and even inhibiting cell growth and development, cell genetic stability, and non-secretion. Form release of secondary metabolites and other issues, these issues still need us to further study.

(3)The two-phase culture method prevents the metabolic effects of secondary metabolites from inhibiting their biosynthesis and protects them from the influence of enzymes or acids in the medium. For example, only 1% of the thiophene synthesized by the hairy roots of maidenhair can be secreted into the culture medium. Adding hexadecane to the culture system can promote the secretion of 30% to 60% of thiophene[6].

(4) The two-step culture method better solves the contradiction between the growth of cell biomass and the accumulation of secondary metabolite products, and greatly improves the yield of the target product [7]. Coptidis cells were cultured in the culture medium for 3 weeks, and then cultured in the production medium for another 3 weeks. Each liter of culture solution can obtain 556 mg of alkaloids, and the yield is 1.72 times that of the one-step culture method.

(5) The use of inhibitors and antisense technology. Antisense technology is the use of modern molecular biology technology to introduce artificially synthesized antisense RNA into the plant genome, and combine with the key enzyme gene RNA of the metabolic pathway to be inhibited to form double-stranded RNA to block

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the normal expression of genes, thereby promoting The expression of the target gene. Nowadays, antisense technology is more and more widely used in the production of plant secondary metabolites. For example, after the antisense RNA of ethylene synthase in tomato is transferred into tomato, ethylene synthesis is reduced by 97%[8].

(6) Application of elicitor. Elicitors can enhance the respiration of cells, provide the energy required to produce secondary metabolites, change cell structure, and facilitate the formation, transportation and accumulation of secondary metabolites. The elicitors used in plant cell culture mainly include glycoprotein elicitors, protein elicitors, polysaccharide elicitors, and microbial elicitors[9].Among them, the factors that affect the elicitor induction effect include the type, concentration and inoculation time of the elicitor.

3. Summary and Outlook

Although compared with other methods, plant cell culture technology has obvious advantages, but there are still many problems that need to be solved to truly realize the large-scale industrialization of plant cell culture. With the development of molecular biology, it will help plant cell culture to solve many theoretical and practical problems.

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ARTICLE

Ontogenetic Structure of Ceonopopulations of *Tulipa korolkowii* Regel in Uzbekistan

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ABSTRACT

Ontogenetic structure of eight coenopopulations of *Tulipa korolkowii* Regel were studied in Uzbekistan. Resistance mechanisms of *Tulipa korolkowii* coenopopulations are shown: seed and vegetative methods of self-maintenance of coenopopulations. Coenopopulations (CP) of *T. korolkovii* studied in normal. CP 1, 2, 6, 7,8 complete, and the rest (3, 4, 5) are incomplete, no senile individuals. Absence of old specimens in coenopopulation connected with die-off great number plants in generative period of ontogenesis.

1. Introduction

Due to the growing anthropogenic impact on ecosystems, there is a need to conduct research to identify and preserve biological diversity. Much attention is paid to rare communities and the species that make up them, as well as species that grow on the edge of the range. The ontogenetic structure is one of the essential features of a population; this side of the structural organization provides the ability of the population system to self-support and determines its stability. The analysis of the ontogenetic structure of plants gives an idea of the future fate of species populations [1,2].

During the study, the ontogenetic structure of 8 ceonopopulations of the *Tulipa korolkowii* in Uzbekistan was studied (Figure.1). To date, the ontogenetic structure on this species has not been studied [3-5]. *T. korolkowii* is included in all editions "Red Book" of Uzbekistan [6-9]. This species is one of the rare species in the flora of Uzbekistan. Currently, the population is declining.

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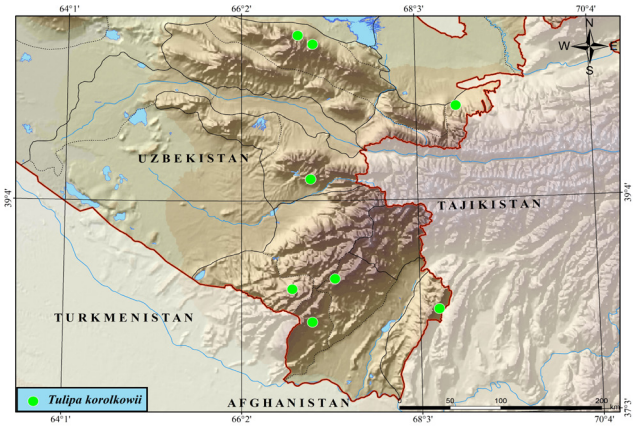

Figure 1. Map of the location of *Tulipa korolkowii* coenopopulation

Different indicators of each coenopopulation were identified. The plants and dominant species in it were identified under laboratory conditions (**Table.1**).

During the study period in Uzbekistan, the population of the species was not found Bukhara region (Kyzylkum desert). The species is known for herbarium collections from several two points of the Bukhara region: 1. Between Shafirkan and the desert station Institute of Botany (herbarium specimen, 1964, geographical coordinate N 40.40.13.3 E 063.47.566) and 2. Central Kyzylkum, Kokchatau (herbarium specimen, 1905, geographical coordinate N 40.31.82.2 E 065.14.083).

Table 1. Phytoceonotic characteristics of cenopopulations

№ of CP	Geographical location of coenopopulation	Geographical coordination	Altitude, m	Plant community	Total projective cover of vegetation, %	Projective cover of species, %
1	Jizzakh region, Turkistan ridge near village Turkman	E 68,510837 N 39,940269	840	*Crataegus turkestanica - Artemisia sogdiana - Poa bulbosa*	50-55	2
2	Navai region, Nurata ridge, village Sentabsay	E 66,690414 N 40,615935	970	*Amygdalus spinosissima - Allium altissimum*	55-60	3
3	Navai region, Nurata ridge, Ustaxon	E 66,863255 N 40,532965	1020	*Amygdalus spinosa - Erodium ciconium - Carex pachystylis*	35	1
4	Kashkadarya region, Zerafshan ridge	E 66,825131 N 39,26838	1223	*Amygdalus spinosissima - Ferula varia - Allium suvorovi*	30	1
5	Surkhandarya region, Baysuntau, Yuqari Machay	E 67,092985 N 38,332464	1516	*Alhagi pseudalhagi - Onobrychis chorassanica - Poa bulbosa*	35-37	2
6	Kashkadarya region, Western Gissar, Tarkapchigay	E 66,608504 N 38,23705	1293	*Amygdalus spinosa - Ferula sp - Carex pachystylis*	65	3
7	Surkhandarya region, Kuhitang, Surxan reserve	E 66,831654 N 37,927275	1230	*Amygdalus bucharica - Geranium collinium - Ferula sp.*	35-40	1
8	Surkhandarya region, Babatag	E 68,263868 N 38,03785	1436	*Crataegus sogdiana - Alhagi pseudalhagi - Poa bulbosa*	40-45	1

2. Material and methods

Our research was conducted in Uzbekistan. Object of research – *Tulipa korolkowii*. Commonly accepted methods were used to assess ceonopopulations [10-14].

3. Results and Discussion

In this article, we talk about the ontogenetic structure of various ceonopopulations of *Tulipa korolkowii* Regel, distributed in Uzbekistan. During the study, the age structure of *Tulipa korolkowii* in different ceonopopulations was analyzed. The age structure of the plant was divided into 5 (juvenile-j, immature-im, virginile-v, generative-g and senile-s). I was noted that isolated ceonopopulations are specific to 3 different types. Left-sided, centralized and bimodal ontogenetic spectrum.

Left-sided ontogenetic spectrum. Was found to be a peak in most cases, and the peak (or peak) to virginal plants (CP, 2, 6, 7). The predominance of virginal-age tufts in these ceonopopulations is explained by the fact that this stage lasts longer than the earlier stages. The duration of the juvenile and immature phase is 1-2 years. The duration of the virginal phase is relatively - longer, lasting up to 5-7 years. Left-sided ontogenetic spectrum specificity-virginal stage period in ceonopopulations were noted to be in range of 35.3-39.31 % (Figure 2)

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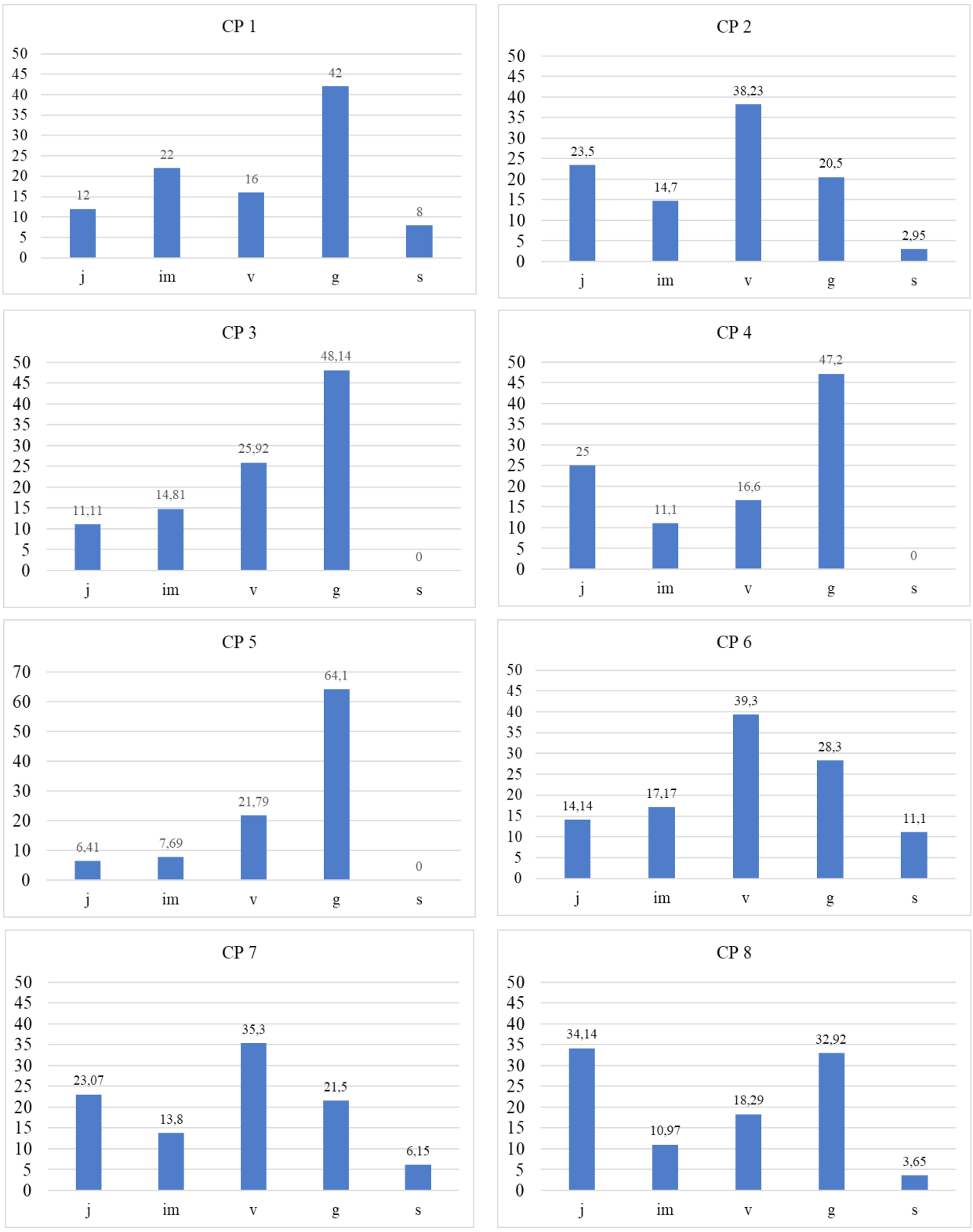

Figure 2. Ontogenetic structure of *Tulipa korolkowii* coenopopulations

In addition to a number of ecological and phytocenotic factors, the large number individuals belonging to the young fraction in ceonopopulations is also related to the biology of the species. *T.korolkowii* have a high seed productive. According to the analysis of the obtained data, 180-320 seeds are formed in the generative period of the

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species, which is turn has a direct impact on the recovery of the number of young fractions in the ceonopopulations (Table 2).

3.1 Centralized Ontogenetic Spectrum

T.korolkowii ontogenesis – enters the flowering stage in 4-5 years, and the duration of this stage is 11-14 years. This is turn means that the main part of the ontogenesis of Tulipa L. species belonging to generative ceonopopulations, seed multiplication does not exceed one norm and the length of the generative phase allows the structure of some ceonopopulations to be centralized. During the observations, it was noted that in the ceonopopulations of 1, 3, 4, and 5 the generative stage period was more than in the rest of the age period, their share was around 42-64 %.

3.2 Bimodal Ontogenetic Spectrum

9 ceonopopulations were observed to be specific to the bimodal ontogenetic spectrum. The ontogenetic spectrum has two peaks: the first peaks to the juvenile period (34,14%) and second to the generative period (32,92%). Such spectra are usually formed in ceonopopulations where reproduced from seed is moderate. The success of grasses formed in previous years due to mass germination has led to the prolongation of the generative period under favorable conditions has led to an increase in the number of periods of the same age.

The average of ontogenetic structure of ceonopopulations isolated from different ecological-geographical conditions was compared (Figure 3).

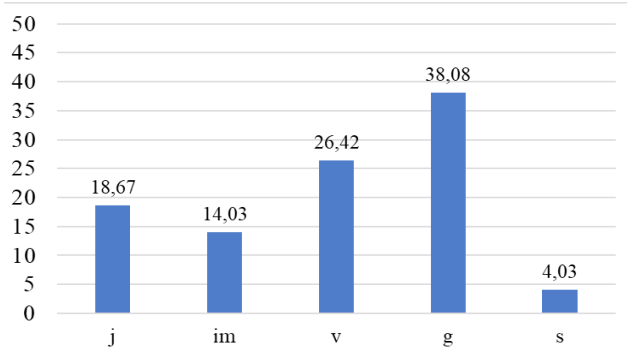

Figure 3. Averaged ontogenetic spectrum of *T. korolkovii*

The results showed that the mean value of the ontogenetic structure was a peak and was specific to the centralized (percentage of generative period 39,6%) ontogenetic spectrum. The mean value of the ontogenetic structure does not correspond to the characterictic spectrum. The predominance of generative bushes in ceonopopulations is often explained by the high elimination of young bushes (water erosion, use of pastures during the development of grasses in the early spring, or high projective cover).

Due to different ecological and phytoceonotic conditions, the density of individuals in ceonopopulations was 1.8-4.94 per $1m^2$, ecological density was 2.11-5.82. According to the results to the analysis, the total number of individuals in the ceonopopulations (99) and their density $1m^2$ was higher in the coenopopulation isolated from the hills around the village of Tarkapchigay (CP-6). The lowest rate was observed in the coenopopulation isolated from the rocky, gypsum soils around the Zerafshan ridge of Kashkadarya region. The total number of individuals in this coenopopulation is 36, the density is 1.8 per. This coenopopulation around the village.

Table.2 Age structure of T. korolkovii ceonopopulations

№ of CP	Age structure, pcs. (%)					Density of individuals, pcs, 1m2	P есол (1m2)	Ir	Ia	Total number of individuals, pieces
	j	*im*	*v*	*g*	*s*					
1	12	22	16	42	8	2,5	3,33	1,19	0,08	50
2	23,5	14,7	38,23	20,5	2,94	3,7	4,35	3,72	0,03	74
3	11,11	14,81	25,92	48,14	0	2,8	3,5	1,07	0	56
4	25	11,1	16,6	47,2	0	1,8	2,11	11,1	0	36
5	6,41	7,69	21,79	64,1	0	3,9	5,2	0,55	0	78
6	14,14	17,17	39,3	28,3	11,1	4,95	5,82	2,49	0,12	99
7	23,07	13,8	35,3	21,5	6,15	3,25	4,06	3,35	0,06	65
8	34,14	10,97	18,29	32,92	3,65	4,1	4,4	1,9	0,04	82

Note:
$P_{есол}$ – ecological density. I_r– recovery index I_a- aging index

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Recovery and aging indices showing the dynamic process of ceonopopulations were also studied. In the studied ceonopopulations, the recovery rate of the species was found to be around 0,55-11,1. The high value of the recovery rate is explained by the high seed productive. The low value of the aging index (0-0.12) in all ceonopopulations studied is due to the fact that most the of the individuals die during the generative period.

4. Conclusion

The mean value of the ontogenetic structures of the studied ceonopopulations is centralized and does not reflect the biology of the species. The ontogenetic structure of ceonopopulations is normal, in most cases incomplete due to the absence of senile individuals. This suggests that, despite the high recovery index of ceonopopulations, the current state of the species population has become a matter of concern and the need for systematic protection of areas where *T.korolkowii* is distributed. The deviation of the ontogenetic spectrum of specific ceonopopulations from the characteristic one is associated with the ecological and phytoveonotic conditions of the habitat.

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ARTICLE

Uses of Different Techniques for the Production of Sustainable Soil and Food

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ABSTRACT

Due to the rapid increase in population, it is estimated that the human population will increase to 9.7 billion in 2050. Hence the demand for food production will also increase. That is why there is a need to solve problems regarding food production. Major problems in food production are the shortage of land due to bad soil structure and quality of the soil. Soil erosion is one of the major issues caused by the use of different chemicals, pesticides and fertilizers that mainly used for plant growth and protection, but at the same time, they also pollute the soil. Therefore, new technology is needed for improving the soil structure, quality, fertility and its decontamination, that should be eco-friendly having no adverse effects on the environment. In this study, the role of different techniques like genetic engineering, Nanotechnologies, soil and crop management strategies, integrated pest control management strategies, sustainable remediation techniques, microbial management strategies and the different management strategies are taken into account. All these techniques aim to produce plants and microbes that are effective against plant disease management. The aim is to use nano agrochemicals and nanosensors for sensing environmental and pathogen conditions against disease management. The primary purpose is to develop disease resistance in plants and to provide balanced nutrient supplements to the soil for the improvement of soil condition and its fertility. These techniques are of economic importance owing to the use of the nano agrochemicals that have low cost, are more effective and also reduce the use of chemical substances that have an adverse effect on soil fertility. Many sustainable remediation techniques used for decontamination of soil are also discussed. The main focus of this study is to improve and increase soil fertility for enhancing the growth of the plants as well as the production of crops. Stress and degradation resistance microbes are found to be essential factors for the protection of soil from degradation or contamination in this study. All the techniques which are used in this paper have no adverse effect on the environment and are also helpful in developing stress resistance.

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1. Introduction

Nutritional deficiency due to the shortage of food supply has to descend to 1 billion in 2009 [1] from 850 million [2], which is alarming. Statistics have revealed that frailty development in people due to lack of food availability has increased to 263 million for South Asia (SA), 212 million in sub-Saharan Africa (SSA), 268 million in China and Southeast Asia, 60 million in South and Central America and the Caribbean and 50 million for rest of the world. The most significant contributing factor that is often ignored in this regard is soil richness [3]. In contrast to other business, horticulture needs more attention, efforts and hard work [4].

Due to the increase in the population, the demand for food is also increasing, as the estimated population growth by 2050 is 9.7 billion. To meet food demand of the growing population, food production will have to be increased by 50%. Keeping this in mind, there is a need to devise techniques to resolve the issues related to food production [5]. Food protection and the development of sustainable food resources offer a new challenge due to depletion of natural resources because of soil contamination, environmental pollution and species extinction [6].

For the production of food, water availability and soil quality are major factors. This cultivable land covers around 12% area of earth' area [7]. Soil degradation is one of the major issues present in many regions of the world. Soil contamination removes 75 billion tons of soil per year. Soil contamination affects nearly 80% of cultivated land due to improper agriculture practices [8] which in turn has a negative impact on food production [9]. For the production of food, the improvement of soil condition is needed, which in turn is a big challenge because of the increasing population.

The organic content of soil decreases due to soil erosion which is responsible for the reduction in hydrolytic properties of soil and also affects the soil water holding capacity and its stability. Development of successful strategies of soil management and productivity of agriculture requires improvement and maintenance of efficient nutrient cycle and soil organic matter. These practices make use of fertilizers which also play an essential role in crop production [8,9].

Different organic resources are used for the improvement of soil fertility and play an essential role in the growth of plants and the high yield of the crop. There are different techniques which are used for the production of sustainable soil and food. When soil fertility is right, then ultimately the productivity of food is also good. The soil has a significant impact on the productivity of food. For the production of sustainable food, there is a need to determine efficient resources [10].

Different practices that are being carried out including the use of chemicals, fertilizers and pesticides negatively affect soil's quality that's why there is need of eco-friendly resources for the improvement of soil quality, making it a healthy medium for food production [11]. The use of microbial inoculants as an alternative of chemicals maintain soil's integrity as well as improves plant growth providing sustainable means agriculture [12,13].

Different genetic modification techniques are being practised including horizontal gene transfer technique which involves the transfer of the genetic material between different microbes and making them more efficient through conjugation, transformation and transduction for the exchange and transfer of different genes which in turn play an important role in the improvement of soil fertility and production of the crop [[14].

2. Use of Different Approaches for the Production of Sustainable Soil and Food

There are different techniques which are used for the production of sustainable soil and sustainable food. These techniques play an essential role in the improvement of soil condition and growth of plants which are responsible for high crop yield, whereas soil erosion reduces food productivity and nutrient content of the soil.

2.1 Genetic Engineering Techniques

Different bacterial species are present in the root of plants increasing soil fertility and plant health, including tolerance against extreme climatic conditions. These bacterial communities evolve through the mobile genetic elements participating in the exchange and transfer of genetic material. They are used as a vector in the process of (HGT) horizontal gene transfer via transformation, transduction and conjugation, which make the bacteria more tolerant against stress conditions. These modifications occur by the expression of genes acquired through conjugative plasmids and other genetic elements [15].

Genetically modified microbes, as well as genetically modified plants, play an essential role in the sustainable production of soil which makes plants more resistant against pests and insects and also increases the productivity of sustainable food. Contaminant degradative genes transferred to rhizosphere lead to improved plant growth [16]. Gene transfer in soil system as well in biofilms has been reported. All these gene transfer techniques enhanced the soil-plant-microbes relationship [17].

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Different transgenic crops are monitored for the horizontal gene transfer from crop to soil microbes which have two implication for the field trial assessments in which plant to soil microbes horizontal gene transfer still have an environmental impact as well as present methods which are used for capturing traits and sampling in recombinant are insensitive for monitoring of the evolution of horizontal gene transfer. The HGT model involving short summarized events explains how HGT can occur at high frequencies but seen at relatively low frequencies [18].

Genetic engineering techniques are used for the production of genetically modified crops which play an essential role in the production of food. Genetically modified crops are resistant to targeted pathogenic disease. Due to these modified crops, the need for pesticides is also reduced. Genetic engineering provides one to one basis for improving the stability of plant disease management. GE has beneficial effects for the long term and sustainable production of crops [19].

2.2 Effects of GM Crops on Rhizosphere Soil Bacteria

Hereditarily adjusted plants can conceivably modify soil microbial networks and thus imperative environment capacities, including carbon cycling, nutrient solubilization and the occurrence of soil-borne plant malady [20]. Be that as it may, it is not evident whether these effects are straightforward because of the recently acquainted quality or by implication due to the change of the rhizosphere science of the GM plants.

Numerous constituents of soils, particularly colloidal particles including dirt minerals and sticky substances, have a high capacity to adsorb organic atoms, for example, DNA and proteins starting from soil microorganisms [21]. Growing logical proof show that dirt would safe be able to monitor such bioparticles from natural disintegration [22]. Consequently, the soil colloid-intervened security component may empower soils to retain concerning specific molecule's genetic and toxic properties for a long time [23].

In creating countries, GM crops can satisfy the food request of an ever-developing population and make nations independent in horticultural creation While a few investigations demonstrated that GM plants caused consider capable changes in the structure and elements of indigenous soil microbial network, the dirt heterogeneity, fluctuating dietary prerequisites of GM plants lack of appropriate controls and other environmental set-up forced significant challenges in deciphering the genuine effect of GM plants on soil microorganisms [24].

2.3 Nano-technologies

There are different nano-based which alternative to other conventional technologies. For production and protection of plant and crops yield, there are different nano-enabled agrochemicals and nanosensors are used. Different nanosensors are used for the sensing undesirable conditions of plants due to pathogens and stress factors [25].

There are different agrochemicals used for protection and production of crops yield. These agrochemicals have industrial importance due to their low cost and also have importance to increase productivity. These agrochemicals are sprayed on leaves of young plants of potatoes as well as on (eggplant) seedlings which make them effective against the diseases which enhance the yield of plants [26].

Nanoscale materials improve tolerance of plants to natural factors, for example, dry season, saltiness and temperatures. In nursery conditions, nanoscale CeO2 salt was used in canola, the uncovered plants developed apoplastic root boundaries and had upgraded photosynthetic yield [27]. Carbon-based nanomaterials can enhance germination and biomass [28].

A few examinations report that nano molecule co-introduction can diminish heavy metal and organic pollutants accumulation in crops [29]. The main focus on the application and preparation of agrochemical depends upon the risk evaluation. There are different proposals and capital investment in the development of new technologies [30].

Nanotechnology plays an essential role in the agri-food system for packaging and long term preservation providing means for sustainable food production, and pathogens control, enabling food preservation, thereby minimizing food wastage [31].

2.4 Soil and Crop Management Strategies

There are different practices which are used for the production of crops by the use of chemicals; fertilizers but these practices have a negative effect on the environmental conditions that's why there is need of the new practices which play an essential role in high production of the crop as well as they have eco-friendly nature [32]. In this way, consideration must not be paid to shielding soil from disintegration (which straightforwardly prompts land deficiencies).

Soil disintegration and land debasement are primary dangers to biological system administrations and horticultural profitability, and such loss in capital resources mostly happens in semi-dry and tropical locales, where agronomic sources are low, and vegetation spread is poor. The disintegration of soil by water and wind is

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vital procedures changing the surface structure of bare soil by which soil is lost, which impedes soil fruitfulness and results in unreasonable agriculture [33].

Different Soil and crop management strategies or other moderating alternatives have been identified and rehearsed by researchers for the ideal utilization of asset with natural control. All these soil and crop management strategies intend to improve crop efficiency and diminish crumbling of land by improving different attributes of soil, for example, its nature, physical substance and hydrological properties [34].

These strategies keep the balance between supplement information sources and yields through two essential standards:

(1) Matching the information with the crop of interest.

(2) Synchronization between crop development and application timing.

These soil and crop management strategies upgrade the yield of crops and simultaneously save soil assets, thereby securing the environment. Different efforts are being made for the management of soil and crop for providing balanced nutrients, improving soil fertility and improving both soil and crop system which have long-term effects. Furthermore, these techniques reduce the uses of chemicals, fertilizers and also reduce emissions of greenhouse gases, consequently protecting the environment [35].

2.5 For Sustainable Crop Production and Disease Control Management Technique

Numerous specialists have recommended that disruption in agro environments is because of changes in harvesting and farming practices since World War II especially with the utilization of agrochemicals [36]. Soil is the primary medium for the production of crops. Besides the state of soil also have a negative effect on yields of the crop by causing disease in crops. High or low factors of soil and plants are the leading cause of the production of disease-borne crops directly or indirectly.

Soil physical properties, for example, temperature, dampness and structure have been found to influence soil-borne diseases through soil compaction, seepage and soil temperature. That is why there is need of proper integrated soil crop disease control management techniques which play a role in an assortment of natural materials, for example, fertilizer, excrement, can be utilized to improve soil structure, food web and mineralization of supplements in the root zone to oversee crop sicknesses in all cropping frameworks. A relative investigation of field soils from natural and regular farms is expected to take into account organically, physical, and compound properties of soil and their effect on plant diseases through (IPM) integrated pest management program [37].

Microbes play a significant role by altering the physiology and development of plants. Although some members of rhizosphere microbiome are advantageous to plant development but still plant pathogens can frequently disrupt the root framework by surpassing defensive microbial shield in the rhizosphere and can overcome the intrinsic plant defence systems [38].

Soil-borne pathogens inhabit rhizosphere of the majority of crop plants. Attention is being paid to them because of their ability to destroy crops affecting yield and quality. A few parasitic pathogens (Fusarium, Verticillium, Alternaria, Phytophthora, Didymella, Rhizoctonia, Sclerotium, Pythium, and Rhizopus) are most prevalent in soil and responsible for most of the diseases [39].

Soil-borne pathogens produce diseases in different parts of plants by decreasing the transport of water and nutrient to these parts; therefore, cause damage and affect the quality of plant and crops. IPM (integrated pest control management) is a technique which is used to an enhanced natural mechanism of pest control and production of healthy crops without affecting agro environments. Three main components of IPM are development, checking and anticipation [40].

2.6 Sustainable Remediation Techniques

Pesticides are being used in agribusiness and are meant for protecting crops for long terms. Pesticide application is considered the best method for plant crop protection.[41] However, 1% of applied pesticide kills target while rest of it kills other non-target insects present as well as humans and other plants.

80 to 90% of pesticides that are applied to crops affect non-target vegetation and can also enter the air and soil. About 80% of every single applied pesticide could be recognized, with half of these buildups found as change items (TPs) with an industriousness of over ten years.

There are following remediation techniques.

(1) Ex-situ strategy, in which sullied soil is unearthed and shipped to another area for treatment.

(2) On location, in which polluted soil is treated to recover its original state.

In situ, in which damaged is treated without digging the polluted soil.

The selection of methods depends upon contamination of soil [42]. Ex-situ strategy was utilized as a soil remediation technique earlier, but it has a few drawbacks, including the significant expense of soil exhuming and transport. Owing to this, in situ strategy has become a method of choice [43]. Soil remediation techniques

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are chemical, physical and biological and include bioremediation, phytoremediation and chemical oxidation.

On the one hand, pesticides protect crops against pests, but on the other hand, they contaminate the soil. These pesticides are mixed with food and have a negative effect on the environment and human health. Therefore there is a need for degradation of these pesticides from the soil by using different techniques of remediation. Different soil microbes play an essential role in the degradation of soil contamination through the process of bioremediation or phytoremediation. Mostly in-situ remediation is used for removal of pesticides. As pesticide has a harmful effect on growth as well as productivity of crop is reduced so for better crop yield and soil fertility, phytoremediation and bioremediation are used as an alternative of chemicals and physical techniques for removals of these contaminants [44].

2.7 Role of Different Microbial Management Strategies

Different microbial species are used for improvement in soil structure, fertility and quality. Association between plants and the soil microbes is used for removal of toxins, therefore, purifying the soil.

2.7.1 Plant Growth-promoting Bacteria

It has been assessed that a milligram of soil contains around 90-100 million microbes with most of these living beings being situated around the base of plants. This reflects that plant roots emit a large amount of carbon that they fix during photosynthesis, and soil organisms use this carbon as a food source. The interaction between plant and soil microbes might be beneficial, harmful or does not affect either of them. Microscopic organisms that are beneficial for plant development and advancement are generally called plant development advancing microbes (PGPB). Due to interactions of these bacteria and fungi, many researchers utilized them in the field of agriculture and for environmental decontamination purposes [45]. PGPB may encourage plant development either directly or indirectly. Direct advancement of plant development occurs when PGPB supply nutrients from the earth, including nitrogen, iron, and phosphate, or balance levels of plant hormones. In the Indirect method, these microbes protect plants against plant pathogens. Due to the rapid increase in population supply does not fulfil the demand of population so in order to increase food production chemicals methods are being replaced with eco-friendly methods that are also more cost-effective [46].

2.7.2 Soil and Root Related Bacteria

These yield systems antagonistically influence type and physiology of valuable soil-and root-related micro-biota. The subsequent loss of soil structure reduced water and nutrient supply negative effect on soil richness and its tendency to support plant growth. Protection is conferred by abundant glycoprotein and glomalin, delivered by root mycorrhizal organisms [47].

2.7.3 Microbial Inoculants

Microbial vaccination is one of the significant horticultural practices that have been utilized to achieve the desired texture of the soil. Microbial inoculants are advantageous living microorganisms that when added to the soil, improve the accessibility of nutrients subsequently improving plant's development [48]. The vast majority of microorganisms that are utilized for the production of microbial inoculants possess the ability to inhabit soil to perform desired tasks. Microbial inoculants are applied, independently or in blends, to seeds, plants and soil to upgrade their efficiency. Microbial inoculants have offered eco-accommodating control system against plant pathogens. Microbial inoculants produce auxiliary antifungal metabolites. These inoculants act as a bio-control agent against disease. These inoculants can tolerate stress conditions, whereas other chemical fertilizers effect soil conditions. Different inoculants are used in combination with other fertilizers or other strains of microbes which play a role in the improvement of soil quality, its structure, growth of the plant, make the plant resistant to other factors and help to produce crops in high yield [49].

2.7.4 Plant-growth-promoting Rhizobacteria

Beneficial microbial association improves plant stature, development, accessibility of nutrients, osmosis and also enhance efficiency of plant against a few infections causing microorganisms [50]. Different processes, for example, development, differentiation, advancement, and stomatal development, are additionally directed by phytohormones [51]. It is found that two or more than two hormones are acting together. The effect of these hormones delivered by PGPR can stimulate or hinder the plant's development. Plant hormones are the most pivotal development controllers; they are known for having a significant impact on plant's auxiliary digestion and are also has a real job in the initiation of plant defence response against stresses. One of the systems for improvement of plant development and stress resistance by beneficial microorganisms is their capacity of phytohormone combination in the rhizosphere or root tissue.

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Ongoing reports recommend that PGPR upgrade the resilience of plants to abiotic stresses, for example, chilling injury. Plant development can be restrained by different factors like salt, dry season, toxic metals, flood, pathogens, temperature etc. Stress resistant PGPR can limit these factors by utilizing different mechanisms, for example, phosphate solubilization, nitrogen fixation, ACC deaminase creation, and siderophore creation.

PGPR can affect the plant in two different ways either directly by the release of phytohormones or indirectly by. The direct method involves the production of phytohormones, fixation of organic nitrogen and phosphate solubilization. The indirect mechanism involves protecting against phytopathogens by the synthesis of HCN, siderophores, anti-infection agents, unstable metabolites, and smelling salts etc. Utilization of PGPR in horticulture has improved dramatically in different parts of the world to decrease the usage of manure and pesticides. Stress tolerant PGPR is multifunctional involved in the improvement of harvest yield, control of natural contamination, condition eco-accommodating under practical advancement through an assortment of components like activating nitrogen fixation, phosphate solubilization, giving development hormones, siderophores, osmotic reaction, and supplements and going about as biocontrol specialists. The use of these microbes reduces the use of the chemicals and other fertilizers. They also play an essential role in the improvement of soil nutrients [52].

2.7.5 Bacterial Endophytes

The interrelationship of every single living thing on earth is considerably more clear in plants world. The plant exists in associations with microorganisms. The connection between plant host and different microorganisms is often beneficial. There are epiphytic, rhizospheric and endophytic microorganisms that occupy the different ecological localities. Amongst these species of microorganisms that exist in profitable relationship with their plant, endophytes have the strongest association than others. Endophytes can be essentially defined as microbial structures that colonize plant tissues without bringing about any unfriendly effect [53]. These endophytic microbes benefit their host plants by helping them to develop resistance against biotic and abiotic stress [54]. They promote the plant's development. Different investigations have suggested the utilization of endophytic microscopic organisms as bio-inoculants to accomplish a practical, eco-accommodating agrarian development framework.

2.7.6 Fungal Endophytes

Fungal endophytes have beneficial relationship with plants, offers biological help to host plants to endure biotic and abiotic stresses. Endophytes get nutrients. They colonize in tissues of, for example, stems, natural products, flowers, roots, leaves and branches; this is asymptomatically.

Endophytic microscopic organisms transmitted in different ways plan or soil to plant, vertically (parent plant to seed), or in a blended way. Most parasitic endophytes are vertically transmitted through the seed [56].

Additionally, these microorganisms enhanced production of nutrients in the plant by utilizing different systems. Such techniques separate inorganic supplement from dirt and absorbed by host plant for the production of proteins and other secondary metabolites. They played a role to improve efficiency by protecting plants from other pathogens for producing significant phytohormones by influencing the physiology of the host plant [57].

3. Management Practices

Different management practices were used to improve soil conditions for sustainable production.

3.1 Tillage Practice for the Improvement of Soil Conditions

Condition of the soil is changed for tillage. Culturing either releases or compacts the dirt and changes its volume and mass relationship. One property of soil is probably going to change by culturing is mass thickness. Lessening mass thickness becomes penetrable and macropores. In this way, every single physical boundary influencing seedling development and root development, for example, soil wetness, air circulation, temperature and entrance obstruction are influenced by the culturing.

3.2 Mulching Practice for the Improvement of the Soil Conditions

Mulch implies a layer of unique material isolating the dirt surface from the air, and mulching is the counterfeit utilization of mulch, to get the changed physical condition. Mulching improves states of substance and soil. Suitable adjustment of the aqueous dirt system, improvement of soil accumulation and impediment of disintegration and soil misfortune, improve the state of soil under mulch. Mulching well impacts the dirt dampness system by controlling dissipation from the soil surface, improving penetration and soil dampness maintenance and encouraging.

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Mulch impacts the warm dirt system and fluctuates soil atmosphere, mulch materials utilized and pace of utilization. It enhanced soil temperature during cold climate and diminished it during hot. Mulching improves auxiliary soil properties straightforwardly and in a roundabout way by advancing the natural movement. Natural mulching improves porosity and means weight distance across of water-stable totals, due to natural issue decayed by soil microorganisms. The mean weight distance across of water-stable totals increments with increment in the mulch rate [58].

4. Personal Analysis

From my point of view, there is need of complete understanding and mechanisms of techniques which are mentioned above, in this study, horizontal gene transfer used for the transfer of genetic materials to plants and microbes. Due to this transfer of genes evolution occurred in genome of plants and microbes which make them more resistant to environmental conditions or factors including stress and others make it more fertile that is necessary for food or crop production. Discuss those technologies which play the role for improvement of soil structure. Because when the soil medium is healthy, the healthy plants were grown and the production of crop or food in high yield with high quantity and quality. Different techniques needed for improvements. Because the population of humans are increasing day by day and food demands are also increased. That is why to meets the future demands of food, more innovative techniques are needed in the more effective agriculture sectors, less time consuming and eco-friendly to the environment. Genetically modified plants and microbes reduced the need for different chemical and pesticides, which have an adverse effect on soil condition. Use of new nanotechnology which is significant, through this technology production of resistance plants by nano agrochemicals, have no adverse effect on environment and nanosensor for sensing the effects of a plant due to undesirable factors like pathogen. However, there is a need for more knowledge for exploring this technique. The techniques which are used in this paper are not enough more techniques needed for the production of sustainable soil and food.

5. Conclusion

The increasing population have more demands for food. However, in present condition, due to shortage of land, soil infertility problems and for solutions of these problems different fertilizers, chemicals and pesticides are used for soil and plant growth, but these organic matters have a negative effect in soil condition and produced soil pollution. Different techniques used for the improvement of soil and food production in which genetically modified technique is used for the production of genetically modified plants and microbes through horizontal gene transfer which is used for the transfer of the genetic materials and the production microbes and plants which are resistance to pathogen disease. This technique provides an option for improving the stability of plant disease management. Nanotechnology is the use of nanoparticles of agrochemicals and nanosensors which are used for sensing undesirable conditions and production of yields which are effective against the disease. This technology also has importance for safety opportunities for food. Soil and crop management techniques used for better soil conditions by providing essential balanced nutrient supplements. Integrated pest management is used for improved soil structure and food web. Sustainable remediation techniques are used for decontamination of soil pollution by use of bio and phytoremediation which have more importance in agriculture sectors Different microbial strategies used for the use of the different microbes and microbial inoculants which are used for the enhancement of soil and plant conditions by reducing the stress. Improvement of soil conditions by using tillage and mulching management practices that will be helpful for better soil fertility, which is the primary medium for the production of the high yield crops.

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ARTICLE

Genetic Evaluation of Starch Synthesis-Related Genes and Starch Quality Traits in Special Rice Resources

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ABSTRACT

The genetic diversity of 36 rice landraces and 43 breeding materials in the upper reaches of the Yangtze River in China was studied by intragenic molecular markers of 26 starch synthesis-related loci. And research on quality traits such as the amylose content (AC), gel consistency (GC) and alkali spreading value (ASV) to analyze genetic differences in quality traits. The results showed that the number of alleles, average gene diversity and polymorphism information content values of landraces were higher than those of breeding materials. The genetic similarity coefficient (GS) of 79 rice materials ranged from 0.392 to 1, with an average of 0.757.There were significant variations in the quality traits of rice landraces and breeding materials, and the high-quality compliance rates were low, only 6.3% of the varieties have an amylose content that reached grade 1. The results of cluster analysis and population structure analysis are generally consistent; that is, the two resource types are closely related and cannot be clustered independently. This study can provide a basis for genetic improvement of rice starch quality. Make full use of the quality genetic diversity of landraces in modern breeding work, further broaden the genetic base of rice and improve rice quality.

1. Introduction

Rice (*Oryza sativa L.*) is the first of the three major grain plants in China, and also one of the most adaptable cultivated crops in the world, supporting more than half of the world's population[1]. With the rapid development of rice breeding technology in China, rice yield has been dramatically increased, while the market demand for rice quality improvement has been increasing [2-3]. As one of the main objectives of rice breeding, high-quality compliance rate of rice in China has shown an apparent upward trend in the past ten years. However, as quality traits tend to be high quality, it is bound to narrow the quality difference between varieties and reduce their genetic diversity[4-6], making the genetic basis gradually narrow. The content of starch in the endosperm is closely related to rice quality. A large number of studies have shown that there are abundant allelic differences between starch synthesis related genes in rice germplasm resources, and these allelic variations are an essential reason for the difference in cooking and eating quality between different rice varieties [7].

Rice landraces are the main components of rice

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Table 1. Name, type and serial number of the 79 rice varieties in this study

Serial number	Variety type	Country number	Variety name	Serial number	Variety type	Country number	Variety name
1	Landrace	32-00080	197	41	Breeding material	ZD06675	89-1/527
2	Landrace	32-00081	Shilixiang	42	Breeding material	ZD06676	89-1/CDR22
3	Landrace	32-00082	Sanjiujiu	43	Breeding material	ZD06677	89-1/Gui 99
4	Landrace	32-00083	Sanbaibang	44	Breeding material	ZD06678	184Yi
5	Landrace	32-00084	Fengyou 788	45	Breeding material	ZD06679	355-2
6	Landrace	32-00085	Wujiehuanggu	46	Breeding material	ZD06680	481 Xuan
7	Landrace	32-00086	Kaizhou No. 2	47	Breeding material	ZD06681	486 Yi
8	Landrace	32-00087	Wuminggu	48	Breeding material	ZD06682	CDR22
9	Landrace	32-00088	Niushizhan	49	Breeding material	ZD06683	D62B
10	Landrace	32-00089	Xianxiang B	50	Breeding material	ZD06684	D62B Yi
11	Landrace	32-00090	Banbianzhan	51	Breeding material	ZD06685	G2480B
12	Landrace	32-00091	Baishuigu	52	Breeding material	ZD06686	Wanhui 86
13	Landrace	32-00092	Zaohuangai	53	Breeding material	ZD06687	Wanhui 910
14	Landrace	32-00093	Hongmiaoxiang	54	Breeding material	ZD06688	Lehui 101
15	Landrace	32-00094	Yangcenggu	55	Breeding material	ZD06689	Lehui188
16	Landrace	32-00095	Qimiaoxiang	56	Breeding material	ZD06690	Ranhui
17	Landrace	32-00096	Yijudao	57	Breeding material	ZD06691	Shan B
18	Landrace	32-00097	Zhongzhou No.1	58	Breeding material	ZD06692	Jianghui 151
19	Landrace	32-00098	Beizinuo	59	Breeding material	ZD06693	Yangfu No.6
20	Landrace	32-00099	Honggu	60	Breeding material	ZD06694	Yihui 1577
21	Landrace	32-00100	Kehui 675	61	Breeding material	ZD06695	Yixiang B
22	Landrace	32-00101	Yapengzi	62	Breeding material	ZD06696	Luhui 17
23	Landrace	32-00103	Fuyin No.1	63	Breeding material	ZD06697	Yu 69-1
24	Landrace	32-00104	Fuyin No.2	64	Breeding material	ZD06698	Zheng B
25	Landrace	32-00105	Fuyin No.3	65	Breeding material	ZD06699	Jin 23B
26	Landrace	32-00106	Fuyin No.4	66	Breeding material	ZD06700	Kehui 10
27	Landrace	32-00107	Jinmazhan	67	Breeding material	ZD06701	Kehui 26
28	Landrace	32-00108	Zhandao	68	Breeding material	ZD06702	Kehui 36
29	Landrace	32-00109	Zhandao 69-1	69	Breeding material	ZD06703	Gui 99
30	Landrace	32-00110	Zhandao 69-3	70	Breeding material	ZD06704	Miyang 46
31	Landrace	32-00111	Huangkegu	71	Breeding material	ZD06705	Mianhui 527
32	Landrace	32-00112	Huangbianzhan	72	Breeding material	ZD06706	Fuhui 130
33	Landrace	32-00113	Xinxiangdao	73	Breeding material	ZD06707	Fuhui 838
34	Landrace	32-00114	Aihuanggu	74	Breeding material	ZD06708	Shuhui 162
35	Landrace	32-00115	Nuo 89-1	75	Breeding material	ZD06709	Shuhui 527
36	Landrace	ZD06673	77D	76	Breeding material	ZD06710	Qianhui 15
37	Breeding material	ZD06670	44D(1)	77	Breeding material	ZD06711	Qianhui 718
38	Breeding material	ZD06671	44D(2)	78	Breeding material	ZD06712	Nuo 89-1/725
39	Breeding material	ZD06672	46B	79	Breeding material	ZD06713	Nuuo 89-1/838
40	Breeding material	ZD06674	88/Jinmazhan				

germplasm resources in China, and they are rich in genetic diversity and contain a large number of excellent genes such as stress resistance, high yield, high quality and wide adaptability [8-9]. Therefore, to study the genetic diversity of the resource quality genes of the distinctive landraces of rice, and to fully exploit and utilize the genetic potential of the local germplasm resources of rice is an critical way to improve the quality traits of rice and realize the transformation of germplasm resources to genetic resources [5].

At present, there are many studies on genetic variation and genetic structure of local varieties and selected varieties at the molecular level in China [10-12], but the comparative analysis on genetic diversity of genes related to starch synthesis of different resource types is rarely reported. In this study, genetic variation, population structure and quality characteristics of genes related to starch synthesis were analyzed for 36 local varieties and 43 breeding materials in the upper reaches of Yangtze River in China by using internal molecular markers

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Table 2. The molecular markers of starch synrhesis related genes

Gene	Molecular markers	Sequence of primers(5'~3')	Marker type
GBSS Ⅱ	*GBSSII* M1	F:TTGCTGCGAATTATCTGCG; R:ACCTCCTCCCACTTCTTTGC	STS
Wx	*Wx* M1	F:CACAGCAACAGCTAGACAACCAC; R: CACGACGACGGAGGGGAAC	STS
SBE2	*SBE1* M2	F:GTGGGGAAAACAAGTAAGTCTG; R:AGTTCCATCAGAAGAATCAGGG	STS
	SBE1 M3	F: GGAAATGGGAGTCGCC; R: CGAAGAAACCACGCTCA	STS
SBE3	*SBE3* M1	F:AAGGTTAGCATTGGTTGGTGAG; R:TCTCCTTGAACAGCGACAGC	STS
	SBE3 M2	F:GTGGGGTTCTCAACTTAGC; R:CATCAGCATTGTTAGGCAG	STS
SBE4	*SBE4* M1	F:CACCAATTATATTAGCGTGCTCC; R:CGTGGCTCTTGGCTCTCTTG	STS
	SBE4 M2	F:CCATCACCTCAAATACATCACTC; R:AGACTGGAATGCCCCTTAGG	STS
SS Ⅰ	*SSI* M2	F:CTTCTATCCATTCCTTAATCCCA; R:ATGCTATTGATGTTAAGAGGGC	STS
SS Ⅱ *-1*	*SSII-1* M1	F:CACCCCACCGTTCTACTATGC; R:TCCATAGTTTCATTGAGATTGCTC	STS
	SSII-1 M3	F:AGAGATCAAATCGTGGAAC; R:TGGAGTGAAGTAGTGGAAT	STS
	SSII-1 M4	F:ATCTTTAGACGATTAGCG; R:AAGTCACAAGTAGAAGGG	STS
SS Ⅱ *-2*	*SSII-2* M1	F:AGATTTGAACTCAGGACTTGGTG; R:TCTATGGGCTCTATCCTTACTAGG	STS
	SSII-2 M2	F:CGCTCGTTGCCTAGCTAGC; R:GGCGAGGAAGCGATTGCC	STS
	SSII-2 M3	F:ACAGTATGTTTGCCTCAGCG; R:GTAAATCCACCCAGCCAGTC	STS
SS Ⅱ *-3*	*SSII-3* M1	F:CCAATACCGTAAACTAGCGACTATG; R:TACAGGTAGAATGGCAGTGGTG	STS
SS Ⅲ *-1*	*SSIII-1* M1	F:AAGAAGGGAAGGGAGTCAGC; R:GCCATCTCCATTGCCAGC	SSR
SS Ⅲ *-2*	*SSIII-2* M2	F:GAACTTGTGCCTTAAGCTGACTG; R:GGAATAGTAAGCCGAAGGACTT	STS
ISA	*ISA* M1*	F:ATAGATGCTAATGTGATGTGGC; R:TGGTATAGGCACAACCGTAGA	STS
PUL	*PUL* M3	F:CTGTATGGACTGAGTAGTCGATGG; R:TGAGCCTCATCTGCCAGAGT	STS
	PUL M4	F:TACACCATCCTCACTACCA; R:GCAACATCTAAAACACCAA	STS
	PUL M5	F:ATTGGCATTTGTAAGTTTC; R:CAATCTTGGTTTTATCCTG	STS
AGPlar	*AGPlar* M1	F:CGTTCAGGTTCAGGCAATCA; R:GGAAGGGTGGTGATGTGGAG	STS
AGPiso	*AGPiso* M2	F:CAATCGCTGCCATCGGTTG; R:TTCCACATCGTTAGGTACACG	STS
AGPsma	*AGPsma* M1	F:TCTATTCTCAGCCCTCCAACC; R:GTGTGTTTAGAGGTGCTTTTCG	STS

of genes closely related to rice quality, and clustering analysis were conducted for 79 specific rice species resources based on genetic similarity coefficients between materials.

2. Materials and Methods

2.1 Rice Varieties

A total of 368 resources from 31 districts and counties in the upper reaches of the Yangtze River in China were collected and identified and evaluated according to the "rice germplasm resource description specifications and data standards". Eighty-one specific resources were selected for accurate identification, traditional species cataloguing and enter the national long-term germplasm resource database for preservation and utilization http://www.cgris.net (National unified code: ZD06669--ZD06713; 32-00080-32-00115). The experimental study was carried out using 79 rice resources. The specific numbers and resource names are shown in Table 1.

2.2 Field Trial

The experiment was carried out in the rice test base of Chongqing Normal University from 2017 to 2018. Each material was planted in 3 rows with 12 holes per row. The planting density was 16.7 cm × 26.7 cm. The single seed was inserted, and the spacing between the materials was 33.33 cm. Organic fertilizer and chemical fertilizer were applied in combination, and applying fertilizer with massive base and early topdressing. The application rate of pure nitrogen was 120-150 kg/hm^2 and the ratio of nitrogen, phosphorus and potassium was 6:3:1. After harvesting the middle of the middle ten strains, after drying, the rice seeds are dried in a drying oven at 40 °C for 48 h, and stored in a dry environment for later use; the test analysis of the quality traits of the rice materials is carried out on time.

2.3 DNA Extraction

Fresh leaves were taken back to the laboratory 30 days after sowing, and DNA was extracted and purified by the CTAB method such as Murry and Thompson [13] for PCR analysis. The PCR reaction system was 10 μL, including DNA template of 1 ug, adding 2.5 mmol/L dNTP Mixture 0.2 μL and 2.5 mmol/L 10×PCR Buffer (Mg2+) 1 μL, and 0.2 μL mixture of pre-and post-primers (the concentration of primers were 12.5 umol/L-1), and plus 5 U/L Taq DNA polymerase 0.08 μL. Finally, the volume was set to 10 μL with ddH2O. The procedure of PCR was to pre-denature at 94 ℃ for 5 min, denatured at 94 ℃ for 50 s, annealed at 55 ℃ for 50 s, reached at 72 ℃ for 1 min, 30 cycles, and lastly extended at 72 ℃ for 10 min and stored at 4

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℃ . The annealing temperature in the reaction system depended on the specific primers. The amplified products were detected by 8% polyacrylamide gel.

2.4 Molecular Marker Detection

In this study, 26 molecular markers were selected from 16 starch synthesis related genes in rice. These markers were used to analyze the genetic diversity of 79 rice materials in the upper reaches of the Yangtze River in China. All marking information was provided by Professor Tian Zhixi's [14] Laboratory, and the marker information is listed in table 2.

2.5 Quality Measurement Indicators and Methods

The amylose content (AC) of 79 resources was established with reference to the Ministry of Agriculture standard NY/T 2639-2014; the gel consistency (GC) was determined in accordance with the national standard GB/T 22294-2008; the gelatinization temperature (GT) was established by alkali digestion method, and the gelatinization temperature was expressed by alkali digestion value (ASV). The quality grade of traits was classified according to the national standard GB/T17891-1999.

2.6 Statistical Analysis

Data collation and statistical analysis were completed in Microsoft Excel and IBM SPSS Statistics 22.0 software; genetic diversity analysis was carried out using Power-Marker 3.25[15] software; genetic similarity coefficient was calculated using NTSYS 2.1[16] software, and cluster analysis was carried out according to non-weighted pairing method (UPGMA). Structure 2.3.1 software was used to complete the population genetic structure analysis of 79 tested materials. Referring to Evanno et al.'s [17] method, the preset population subsets K ranged from 1 to 10, each K value was repeated 11 times, the Length of burn-in period was set to 10,000 at each run time, and Marko chain monte Carlo was placed to 100,000, and the optimal subsets were determined according to the maximum likelihood principle.

3. Results

3.1 Diversity Analysis of Starch Synthesis-related Genes

Using 26 molecular markers linked to starch synthesis-related genes, different alleles were detected after amplification with different primers (Table 3). A total of 53 alleles were detected in 36 landraces, with an average of 3.31 alleles per locus. Four alleles were detected in 8 alleles, and the least number of alleles were SBE4, SSI and SSII-1, all of which had only two alleles. The average genetic diversity of local varieties was 0.4339, and the variation range was 0.054~0.6157. The variation range of polymorphic information content (PIC) was 0.0526~0.5374, and the average was 0.3767. Three high polymorphic loci (PIC > 0.5) were detected, 11 moderate polymorphic loci (0.25 < PIC < 0.5) and 2 low polymorphic loci (PIC < 0.25) were detected.

Table 3. The genetic diversity of molecular markers in starch synthesis related genes

	Landrace				Breeding material		
Gene	No.of alleles	Gene disversity	PIC		No.of alleles	Gene disversity	PIC
GBSS Ⅱ	4	0.5340	0.4852		4	0.4532	0.4247
Wx	4	0.3657	0.3302		3	0.2455	0.2247
SBE1	4	0.2948	0.2797		3	0.1320	0.1273
SBE3	4	0.5586	0.4884		3	0.4240	0.3653
SBE4	2	0.3457	0.2859		3	0.4002	0.3491
SS Ⅰ	2	0.4614	0.3550		3	0.4932	0.4223
SS Ⅱ *-1*	2	0.0540	0.0526		2	0.1298	0.1214
SS Ⅱ *-2*	3	0.4799	0.3884		3	0.3916	0.3310
SS Ⅱ *-3*	4	0.6157	0.5374		3	0.5733	0.4842
SS Ⅲ *-1*	4	0.3349	0.3137		3	0.4240	0.3653
SS Ⅲ *-2*	4	0.5293	0.4770		3	0.2791	0.2510
ISA	3	0.4954	0.3972		2	0.3310	0.2762
PUL	3	0.4614	0.3776		3	0.4348	0.3584
AGPlar	3	0.5910	0.5141		3	0.4824	0.4244
AGPsma	4	0.5725	0.5116		5	0.5354	0.5002
AGPiso	3	0.2485	0.2335		2	0.0454	0.0444
Mean	3.31	0.4339	0.3767		3	0.3609	0.3169

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A total of 48 alleles were detected in 43 breeding materials, with an average of 3 alleles per locus. Among them, the highest allele mutation rate was AGPsma locus, with 5 alleles; the lowest number of alleles was detected at AGPiso, ISA and SSII-1 loci, all with 2 alleles. The average genetic diversity was 0.3609, ranging from 0.0454 to 0.5733. The variation range of polymorphic information content (PIC) ranging from 0.0444 to 0.5002, and the average PIC value was 0.3169. There was only one high polymorphic locus, 11 moderate polymorphic loci and 4 low polymorphic loci.

Generally, the richness of allele variation is positively correlated with PIC, that is, the richer the allele variation is, the higher the PIC will be. The more moderate polymorphic loci showed that the genetic differences of 79 tested materials were small, and the genetic basis was relatively narrow. The contrast of the average polymorphic information content between the two types of rice germplasms was 0.07, which indicated that there were some differences in genetic variation level between the two kinds of rice germplasms. Though the genetic diversity and PIC of local cultivars were slightly lower than those of breeding materials at some loci, the average genetic diversity and PIC values were higher than those of the breeding materials.

3.2 Performance of Starch-related Quality Traits

There were significant differences in the performance of the three starch-related quality traits in 79 rice resources (Table 4), and the Max-Min of each quality trait is large. The coefficient of variation is variable that represents a unit amount and can be used to compare the magnitude of variation between different characteristics. The ratio of difference of all three quality traits exceeded 30%, and they were alkali spreading value > gel consistency > amylose content from large to small.

Table 4. Analysis of variation of rice quality traits

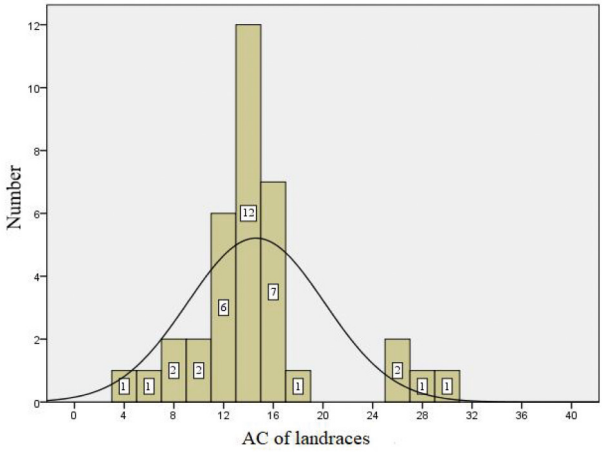
Project	Variation range	Max-Min	Mean	SD	CV/%
Alkali spreading value	1~7	6	3.23	1.85	57.34
Gel consistency /mm	11.2~148.6	137.37	83.08	37.78	45.47
Amylose content /%	3.01~30.72	27.71	15.57	6.08	39.03

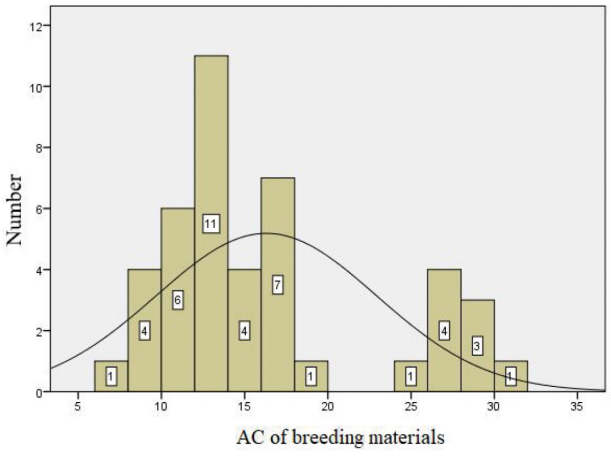
From the high-quality compliance rate of each trait (Table 5), the gel consistency is the best, and the varieties that meet the First-class quality standard are up to 54.4%. The compliance rate of alkali spreading value is low, and the international first-class high-quality rice compliance rate is 8.9%, which is slightly higher than the amylose content. The lowest compliance rate is the amylose content, only 6.3% of the varieties reach the first-class high-quality rice standard, while the proportion of the types that meet the third-grade high-quality rice is only 22.7%.

Table 5. Analysis of excellent rate of rice quality traits

Project	International grade Ⅰ quality rice	International grade Ⅱ quality rice	International grade Ⅲ quality rice	First-class attainment(%)	Secondary attainment (%)	Three-class attainment(%)
Alkali spreading value	6~7	5~6	≥4	8.9	12.7	36.7
Gel consistency /mm	≥70	≥60	≥50	54.4	64.6	77.2
Amylose content /%	17~22	16~23	15~24	6.3	15.2	22.7

3.3 Distribution of Starch-related Quality Traits





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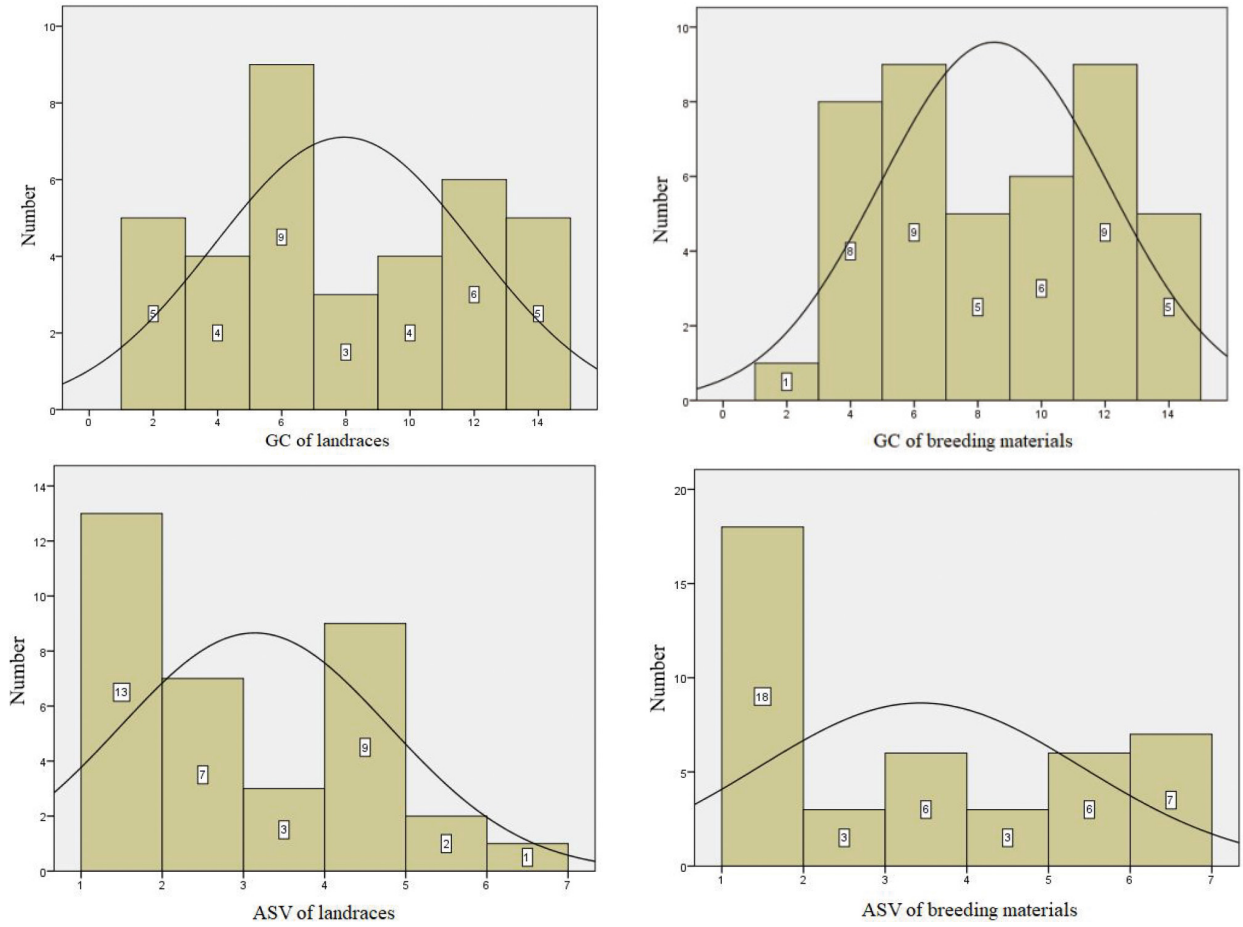

Figure 1. The distribution of several quality traits in different rice varieties

The amylose content (AC), gel consistency (GC) and alkali spreading value (ASV) of 79 rice cultivars in the upper reaches of the Yangtze River in China were determined to determine the quality differences between the tested rice varieties. The results showed that there were significant variations in the quality traits of different types among the 79 certified types (Fig. 1). The AC variation range of 36 rice landraces ranged from 3.0% to 30.0%, of which the AC distribution of 12 varieties was between 14.0% and 16.0%. The AC variation of the breeding materials ranged from 6.0% to 32.0%, a large number of types (including 11 types) distributed between 12.0% and 14.0%. The GC variation of landraces and Breeding materials is between 1.0 and 14.0 cm. The distribution of landraces is scattered, and nine varieties are distributed between 5.0 and 7.0 cm. The breeding materials have nine cultivars in the range of 5.0-7.0 cm and 11.0-13.0 cm, respectively. The rice varieties of the two resource types have similar alkali spreading value (ASV) distributions, with fluctuations ranging from 1 to 7. Rice varieties with ASV values between 1 and 2 are the most.

3.4 Genetic Similarity Coefficient and Cluster Analysis

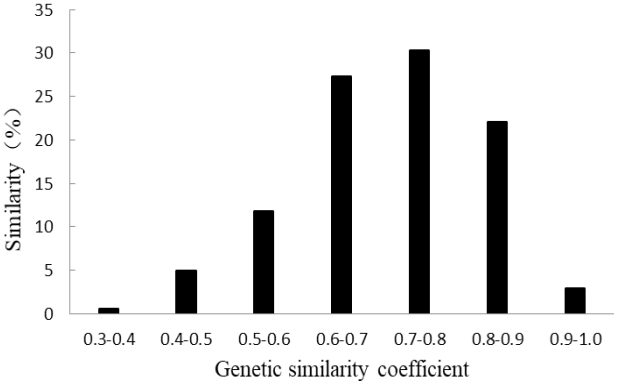

Figure 2. Frequency distribution of similarity coefficients in 79 rice seed resources

Calculating genetic similarity coefficients using NTSYS 2.1, the genetic similarity coefficients of 79 specific rice resources were typically distribution (Fig. 2). Among them, the genetic similarity coefficient was only 0.52 %

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below 0.5, 25.02% was above 0.8, the genetic similarity coefficients of 0.5~0.6 and 0.6~0.7 accounted for 11.85% and 27.30%, respectively, while the genetic similarity coefficient was 0.7~0.8, which was the highest, reaching 30.32%. It indicated that the genes related to starch synthesis showed high genetic similarity among most varieties, and the variation of alleles among most types was small.

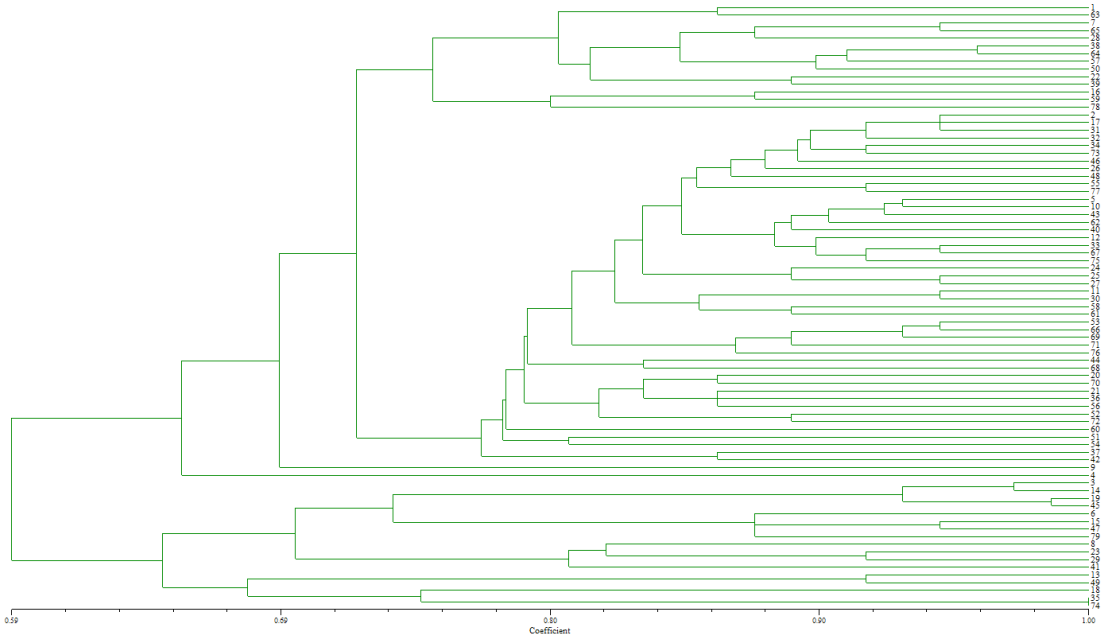

Figure 3. Clustering analysis based on genetic distance

The clustering results of starch synthesis related gene markers showed that the variation of GS of 79 rice resources was 0.324~1, with an average of 0.706 (Fig. 3). Among them, the GS values between Nuo89-1 and Shuhui162 were the largest (up to 1.000), indicating that the two were highly similar in 26 marker sites. If further distinction is needed, some markers need to be added. The GS value between the early yellow dwarf and sticky rice 69-3 was the smallest (0.324), indicating that the genetic relationship was far.

At the GS value of 0. 630, 79 tested materials were divided into two categories (Fig. 3). The first category consists of 11 local varieties and 6 growing materials, and the remaining 62 elements are the second largest category. The second category can be further divided into three sub-categories at the GS value of 0.69. Most of the breeding materials (37 species) are included in the 60 materials of the first sub-category, and the second sub-category and the third sub-category are respectively one local variety (Sanbaibang, Niushizhan). The clustering results of starch synthesis related gene markers showed that local variations and breeding materials existed in different groups at the same time, indicating that there is a specific genetic relationship between the two resource types.

3.5 Population structure of 79 rice varieties

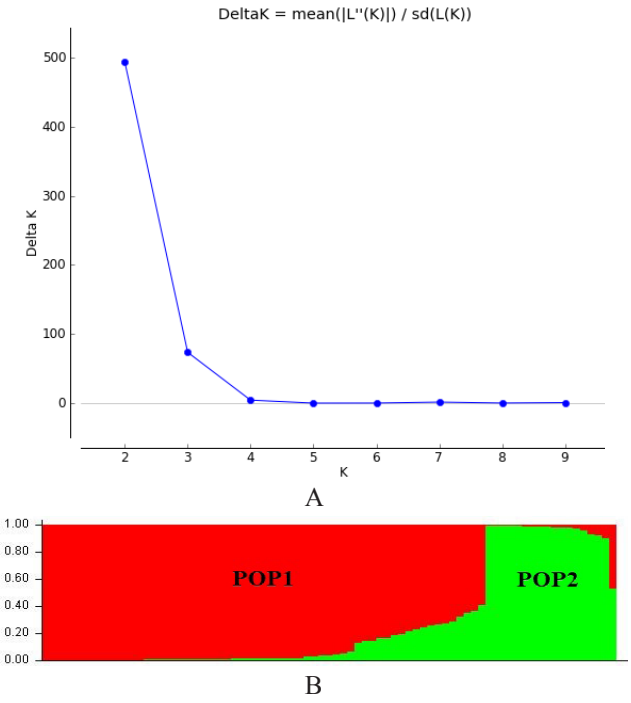

Figure 4. Population genetic structure of 79 rice materials. (A). △K value changes with the number of subgroups; (B). Population structure map based on 26 molecular markers

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Based on the results of molecular marker detection of starch synthesis genes, population structure analysis of 79 specific rice resources was performed using Structure2.3.1 software. As the sample's allelic variation frequency feature type number K (K=1~10) continues to increase, ΔK peaks at K = 2 (Fig. 4A), from which it is concluded that the test material can be divided into two subgroups. The subgroup classification was based on a genetic component value greater than 0.5. The results of the population structure analysis showed that (Fig. 4B), 61 of the 79 rice materials were classified into the first subgroup (POP1), including 25 local varieties and Of the 36 breeding materials, the remaining 11 landraces and 7 breeding materials were classified in the second subgroup (POP2). Landraces and breeding materials are scattered in different sub-groups, and there is a certain genetic exchange between them.

4. Discussion

The success of crop breeding is closely related to the genetic variation of germplasm resources[14] There are many analyses on the genetic diversity of rice population using molecular markers at home and abroad. For example, Deng Hongzhong[18] et al. used 98 pairs of SSR markers to compare the genetic diversity of 202 Chinese rice varieties and selected varieties. Hua Lei[19] et al. used 40 pairs of SSR markers to analyse the genetic diversity of 151 conventional rice varieties in different eras in China. Sun CQ [20] et al. used RFLP markers to evaluate the genetic diversity of ordinary wild rice and cultivated rice in various Asian countries. However, the molecular markers selected for a large number of scientific studies have not been associated with specific phenotypic traits for correlation analysis, and it is difficult to accurately and efficiently play a role in the genetic improvement of essential quality traits of crops [21-22].In this study, 26 intramolecular markers related to rice starch synthesis genes were used to detect allelic variation of rice landraces and breeding materials in the Yangtze River Basin. At the same time, combined with the distribution of three quality traits of amylose content, gel consistency and alkali spreading value, the comparative analysis and evaluation of genetic diversity among different resource types of rice were carried out.

The coefficient of variation of starch-related quality traits and the analysis of international high-quality compliance rates of various characteristics showed that the coefficients of variation of amylose content, gel consistency and alkali spreading value were significant, indicating that these three traits have a full separation range among 79 samples, and the genetic variation is rich. The excellent grade 1 compliance rate of amylose content and gel consistency was lower and less than 10%, indicating that increasing amylose content and gel consistency grade are the key to improving the quality traits of specific rice germplasm resources in the Yangtze River basin. According to the distribution of three quality traits in landraces and breeding materials, the breeding materials show better taste quality, which may be related to the long-term artificial orientation selection of the cultivating materials, and the quality traits of rice varieties are improved and gradually approached to high quality. Among them, the landraces 44D (2) amylose content, gel consistency and alkali spreading value have reached the international level 1 standard, with good starch quality traits, can be applied to the genetic basis of starch synthesis genes and rice breeding.

Many previous studies have shown that rice landraces have higher genetic diversity than modern breeding materials[23-26] The results of this study showed that 26 intramolecular markers of starch synthesis-related genes detected 53 alleles in 36 landraces, with an average genetic diversity of 0.4339 and an average PIC of 0.3767. Although the genetic diversity was slightly lower than the breeding materials at the AGPsma locus, the average genetic diversity and PIC value of the landraces were higher than those of the cultivating materials. In general, the overall genetic diversity of the two resource types is low. According to the results of molecular marker polymorphism detection and genetic similarity analysis, the varieties with a genetic similarity coefficient above 0.7 accounted for 55.34%, the variations below 0.5 were only 5.52%, and the GS range was 0.323~1, with an average of 0.706. It indicates that the genes related to starch synthesis show high genetic similarity among most varieties, and the genetic relationship is relatively close. This may be associated with the geographical location of the test materials in this study, and the number of primers selected is less. These molecular data can be directly used for correlation analysis of starch quality traits, which lays a foundation for excavating the excellent allele loci of rice starch synthesis related genes.

Ao Yan[27] carried out molecular marker-based cluster analysis on 115 rice landraces and 87 breeding materials in Taihu Lake Basin, and found that there were some genetic differences between breeding materials and local varieties. Tang Zhiming[28] et al. carried out cluster analysis based on genetic distance for 50 types of conventional rice in different periods in Guangzhou. It was found that with the increase of years, the genetic distance between variations in each period slowly decreased, and the varieties with distant relatives gradually disappeared. Ma Jing[29] et

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al. UPGMA cluster analysis of 31 selected types in Ningxia showed that the genetic diversity of rice breeding materials in Ningxia has improved in recent years, but the genetic basis is relatively narrow. In this study, the results of cluster analysis and population structure analysis were highly consistent. 36 breeding materials and 43 local varieties existed in different subgroups, and the genetic relationship was relatively close, similar to that in other parts of China.

Among them, the landraces Sanbaibang and Niushizhan have a distant relationship with other types, and the four local varieties of Hongmiaoxiang, Yangcenggu, Zhandao69-1 and Nuo89-1 have good starch-related quality and can be prioritized in the selection of future new breed parent materials. In modern breeding, make full use of the quality genetic diversity of landraces, to broaden the genetic basis of unique varieties of rice breeding in the upper reaches of the Yangtze River in China, and to reduce the loss of beneficial genes in landraces.

Acknowledgments

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ARTICLE

Microbial Biocontrol of Post-harvest Fungal Rot in Apples: Current State of the Science

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ABSTRACT

Our study consists of a careful literature review carried out with the aim of better understanding the models developed in the field of biocontrol of post-harvest fungal rot in apples (PHFRA) over the past two decades. It aims, more specifically, to shed light on the progress made by examining the products developed, their nature, their target pathogens, their effectiveness, theirs modes of action and the stage of their development. The post-harvest biocontrol of apples has made remarkable progress during the last twenty years of research. Several products (yeasts, bacteria, filamentous fungi and actinomycetes) have been selected. Some, are already marketed, others are at different stages of development. However, several points limit the optimal use of microbial antagonists in the bio-management of post-harvest apple rots as an alternative to chemicals. It is, in fact, still necessary to develop appropriate formulations of these microbial biocontrol agents, to better study their mechanisms of action, to test them under commercial conditions and against a broad spectrum of pathogens and hosts. However, although sometimes considered less effective than chemical treatments, biocontrol products based on microorganisms have major advantages for an application in an integrated post-harvest apple protection strategy.

1. Introduction

The apple is among the most cultivated and consumed fruits in the world. Its production has greatly evolved over the past 60 years, from around 17 million tonnes (MTn) in 1961[1], to more than 86 MTn in 2018 [2]. Apple is marketed in more than 100 countries, both in developed countries and in emerging, transition or developing economies [1]. apple is characterized by: (1) its ease of cultivation and consumption, (2) the extent of its commercial varieties (6000 varieties worldwide) [3], (3) its long storage times, (4) its lower production and transport costs, (5) its nutritional value (very rich in water (84.3%), in sugars (12.6%), in vitamins C and E and in mineral salts, mainly magnesium) and its intrinsic health capital (Great source of dietary fiber (2 to 3g / 100g) [4] and polyphenols which are the main source of the high antioxidant potential of this fruit [5]. All these factors have made the apple a global fruit [1].

However, like most fresh fruits intended for human consumption, the apple is subject to constraints that may limit its marketing. Among these constraints, those of a

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sanitary and phytosanitary nature, observed since the mid-1990s, have gained increasing weight [1].

Post-harvest rots are the major constraint that compromises the profitability of the apple sector worldwide. The losses they cause are considerable and can reach up to 25% of the total harvest in developed countries [6], and 50 to 60% in developing countries [7,8].

Several species of fungi are responsible for these rots. They belong to different genera including, *Penicillium, Botrytis, Monilinia, Rhizopus, Botryosphaeria, Alternaria, Aspergillus, Fusarium, Gloesporium and Mucor* [9-13]. But the most important losses are, mainly, caused by the speciess: *Botrytis cinerea, Penicillium expansum* [14-18] and species of the Gloeosporioides group [6].

Although several approaches have been proposed for the management of these diseases, chemical control, in pre-or post-harvest application, is still the most widely used method [6,19,20]. However, growing concerns about fungicide residues in fruit [21-23], the development of resistant strains among pathogens [9,24-26], as well as the environmental risks linked to their continuous use, have stimulated the search for alternative control strategies, safe and effective, but less harmful to human health and more respectful of the environment. One alternative to control post-harvest fungi has been implemented through the application of medicinal and aromatic plant extract. In recent decades, researchers have evaluated the effects this natural compound against main fungi responsible for rotting apples in storage, interesting results have been obtained [12]. However, the most attractive alternative method for the control of post-harvest apple rots remains the biocontrol using microbial antagonists (yeasts, bacteria, filamentous fungi and actinomycetes) [6,8,11,27].

The use of microbial agents in the control of post-harvest apple rot has been the subject of considerable researches, over the past 20 years, and has experienced remarkable progress. Several antagonists have been isolated identified and tested against the main post-harvest diseases of apples and their efficacy has been well established [6,28-33]. Some antagonists have been formulated and marketed, others are currently at different stages of development [6,34,35]. Most of these antagonists have been isolated from the surface of apples [14,36-39], but also from other sources, including soil and seawater [11,32, 40,41].

The present work aims to give a complete overview of the use of microbial antagonists (fungi, bacteria and Actinomycetes), as biological control agents against various pathogenic fungi causing post-harvest rots in apples and to shed light on the progress made by examining the products developed, their nature, their target pathogens, their effectiveness, their modes of action and the stage of their development.As well as the approaches used to improve their effectiveness in the biocontrol of (PHFRA).

2. The Marketed Microorganisms Used in Biocontrol of Apple Post-Harvest Rots

Many microbial antagonists, mainly yeasts and bacteria, have been identified and selected by researchers, many of which had reached advanced levels of development and commercialization.The first generations of registered and commercialized antagonists are shown in Table 1.

Table 1. Main microbial antagonists registred in the post-harvest biocontrol of apples and many other fruits and vegetables

	Antagonists	Trade name	Firm	Reference
Yeasts	***Aureobasidium pullulans, Strains:DSM 14940 ; DSM 14941***	***BoniProtect***	BIO-FERM GmbH, Austria	Lima et al.[42]
	Cryptococcus albidus	***YieldPlus***	Anchor Yest, Cape Town, South Africa	Mari et al. [43]
	Candida sake	***Candifruit***	Sipcam-Inagra, Valencia, Spain	Teixidó et al. [44]
	***Candida oleophila* (Strain O)**	***Nexy***	BioNext sprl, Belgium	Lahlali et al. [45]
	***Candida oleophila* (Strain I -182)**	***Aspire***	Ecogen, Inc. Langhorne, PA, United States	Blachinsky et al. [46]
	Metschnikowia fructicola	***Shemer***	Bayer/Koppert, Germany	Spadaro and Droby [27]
Bacteria	***Bacillus subtilis***	***Avogreen***	University of Pretoria, Pretoria, South Africa	Demoz and Korsten [47]
	***Pantoea agglomerans* strain CPA-2**	***Pantovital***	Domca, Granada, (Spain)	Cañamás et al.[48] ; Plaza et al. [49] Nunes et al. [50] ; Teixidó et al. [51]
	***Pseudomonas syringae* Van Hall**	***Bio-Save***	JET Harvest, Longwood, FL, United States	Janisiewicz and Korsten[8]; Janisiewicz and Jeffers [52]

3. Mechanisms of Action Involved in Applicable Post-Harvest Diseases Biocontrol Systems

Understanding the modes of action involved in biocontrol systems is a prerequisite for the development of post-harvest biological control agents and their registration [35] In fact, it improves the performance and reliability of biocontrol through the development of appropriate for-

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mulations and application methods [27]. Most research on fungal and bacterial antagonists has attributed to biological control four main modes of action: (1) Nutrients and Space Competition, (2) antibiotic production, (3) induction of host resistance, and (4) direct parasitism [8]. Competition for space and nutrients is the main mode of action of microbial antagonists against post-harvest apple fungi [53, 54]. Additional mechanisms of action have been explored, most recently, including: (1) biofilm formation, (2) Quorum detection, (3) Production of diffusible and volatile antimicrobial compounds (4) Competition for iron, (5) Induction of tolerance to oxidative stress, (6) The production of reactive oxygen species (ROS) by the host and the antagonist [11,27].

In studies carried out on the biocontrol of fungal rot in apples, the mode of action of antagonists is rarely studied and when it is, it is not well understood. On the other hand, In the vast majority of studies, each mechanism is generally examined separately. However, it is rare that only one mechanism is involved in the suppression of a disease [27,55-59]. Table 2 presents some modes of action reported in the literature on the biological control of (PHFRA).

Table 2. Modes of action of post-harvest apple biocontrol antagonists

Mode of action		Antagonist	Pathogenic	Reference
Induction of resistance in the host		*Debaryomyces hansenii* AII4b	*Monilia fructicola*	Czarnecka et al. [60]
		Streptomyces rochei A-1	*Botryosphaeria dothidea*	Zhang et al. [61]
		Rhodosporidium paludigenum	*P. expansum*	Lu et al. [62]
		Candida oleophila	*B. cinerea*	Liu et al. [105]
		Candida guilliermondii	*B. cinerea*	Zhang et al. [63]
		Candida saitoana	*B. cinerea*	El-Ghaouth et al. [64]
		Candida saitoana (iso-late US-240, NRRL Y-21022)	*P. expansum*	de Capdeville et al. [65]
		Aureobasidium pullulans L47	*B. cinerea* *P. expansum*	Ippolito et al. [66]
Induction of tolerance to oxidative stress caused by (ROS)		*C. oleophila* (I-182)	*P. expansum* *B. cinerea*	Liu et al. [54]
		M. fructicola	*P. expansum*	Liu et al. [67]
		Cystofilobasidium infirmominiatum,	*P. expansum*	Liu et al. [68]
		C. oleophila (I-182), Metschnikowia fructicola (277)	*P. expansum* *B. cinerea*	Macarisin et al. [69]
		Cryptococcus laurentii LS-28 Rhodotorulla glutinis LS-11	*B. cinerea,* *P. expansum*	Castoria et al. [70]
Nutrient competition	**Nitrates**	*C. guillermondii, Strains:* 3C-1b *and F1*	*P. expansum*	Scherm et al. [71]
	Sugars and nitrates	*Metschnikowia pulcherrima* 2.33 and 4.4	*B. cinerea*	Piano et al. [72]
	Amino acids	*Cryptococcus laurentii Sporobolomyces roseus*	*B. cinerea*	Filonow [73]
		M. fruticola	*A. alternata*	Saravanakumar et al. [74]
		A. pullulans Ach 1-1	*P. expansum*	Krimi Bencheqroun et al. [75, 76]
	Endogenous nutrients pathogen conidia	*A. pullulans* 1113-5	*P. expansum*	El Guilli et al. [77]
		Rahnella aquatilis	*B. cinerea*	Calvo et al. [78]
		A.pullulans LS-30	*P. expansum* *B. cinerea*	Castoria et al. [79]

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Iron competition (production of siderophores)		*M. pulcherrima* *M. fruticula*	*B. cinerea,* *P. expansum,* *A. alternata*	Saravanakumar et al. [74]
Space competition		*P. guilliermondii*	*B. cinerea*	Zhang et al. [63]
		R. glutinis	*P. expansum*	Calvente et al. [80]
Nutrients and space competition		*Pichia anomala*	*B. cinerea*	Kwasiborski et al. [81]
		C. oleophila, O	*B. cinerea* *P. expansum*	Massart et al. [82]
		Pantoea agglomerans	*B. cinerea*	Nunes et al. [50]
Biofilm training on injurie		*Pichia fermentans* 726	*M. fructicola*	Giobbe et al. [83]
		M. pulcherrima, BIO126, GS88, GA102, and GS37	*P. expansum* *B. cinerea*	Spadaro et al. [84]
Antibiotic production		*P. fluorescens strains:* 1-112 and 4-6	*B. cinerea*	Wallace et al. [58]
		Bacillus subtilis 9407	*B. dothidea*	Fan et al. [85]
		Bacillus sp. (UYBC38)	*B. cinerea*	Rabosto et al. [86]
		Bacillus subtilis	*B. cinerea*	Ongena et al. [87]
		Saccharomyces cerevisiae M25	*P. expansum*	Scherm et al. [71]; Ortu et al. [88]
		Trichoderma harzianum	*P.expansum*	Batta [89]
		B. amyloliquefaciens BUZ-14 (Production of Iturin)	*P. expansum*	Calvo et al. [90]
Production of enzymes	proteases	*T. harzianum*	*B. cinérea*	Deng et al. [91]
	chitinases, glucanases	*Amycolatopsis* sp (isolat 521)	*Colletotrichum gloeosporioides*	Sadeghian et al. [40]
	β-1,3-glucanase	*C. oleophila*	*P. expansm*	Guerrero et al. [92]
production of volatile organic compound (VOCs)		*C. sake*	*B. cinerea,* *P. expansum* *A. alternata,*	Arrarte et al. [41]
		A. pullulans **(strain: L1 et L8)**	*B. cinerea Colletotrichum acutatum* *P. expansum*	Francesco et al. [93]
				Mari et al. [94]
		Muscodor albus	*P. expansum*, *B. cinerea*, *A. tenuissima* *A. arborescens*	Ramin et al. [95]
Involvement of several mechanisms				
Production of (VOCs) and direct contact		*Candida pyralidae and Pichia kluyveri*	*B. cinerea,* *C. acutatum and R. stolonifer*	Mewa-Ngongang et al. [96]
Nutrients/space competition, Biofilm and antibiotic production		*P.fluorescens, Strains:* 1-112, 2-28 and 4-6	*B. cinerea*	Wallace et al. [58]
Competition for space ; direct contact ; induction of β-1,3-glucanase in apple ; production of glucanase in apple wounds ; antifungal action of wall chitin		*Rhodosporidium fluviale*	*B. cinerea*	Sansone et al. [57]
Competition for space and nutrients ; (VOCs), secretion of extracellular lytic enenzymes ; inhibition of spore germination		*Aureobasidium pullulans* GE17 and *Meyerozyma guilliermondii* KL3		Agirman and Erten [59]

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4. Microbial Antagonists Used in the Biocontrol of Apple Fungal Rots in Storage

In our study, we identified and analyzed the results of 125 research studies investigating the potential of microbial antagonists in the biocontrol of apple fungal rot. Among these antagonists, yeasts occupy by far the first place with 86 studies and 28 species, followed by bacteria (27 studies and 9 species) then filamentous fungi (10 studies and 2 species) then actinomycetes with only 2 studies and 2 species (Figure 1). Most of these strains have shown significant potential in the fight against the main fungal agents of post-harvest apple rot.

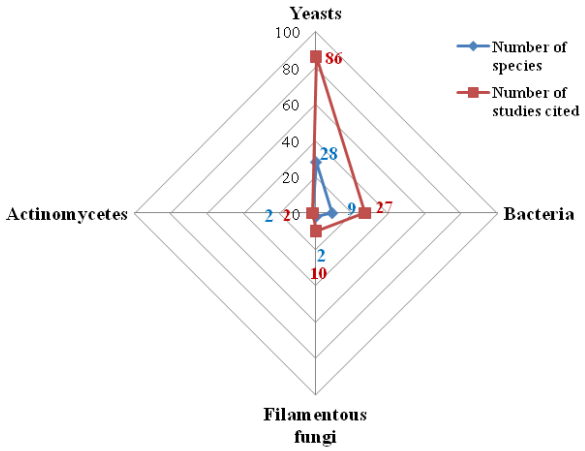

Figure 1. Relative importance of different microbial groups reported in the biocontrol of post-harvest fungal rot in apple

4.1 Fungal Antagonists in Post-harvest Apple Rot Control

In the field of research, considerable efforts have been devoted to the identification of fungi capable of effectively controlling post-harvest fruit and vegetable diseases [53,97,98]. Several antagonistic fungi have shown the ability to protect many fruits against *Botrytis cinerea, Penicillium expansum, Monilinia fructicola* and *Rhizoctonia* [99, 100] and other fungi responsible of apple rots in storage. They are mostly yeasts some are filamentous fungi (Figure 1)

4.1.1 Yeasts

Yeasts have great potential as post-harvest biocontrol agents [11]. They have, in fact, a high tolerance to stressful environmental conditions prevailing in pre-and post-harvest (low and high temperatures, desiccation, broad spectrum of relative humidity, low oxygen levels, pH fluctuations, UV radiation). They are also adapted to the micro-environments present in the tissues of injured fruit (high sugar content, high osmotic pressure and low pH). They can also grow quickly on affordable substrates, in fermenters and are therefore easy to produce in large quantities [101]. Finally, note that yeasts also have simple nutritional requirements that allow them to colonize dry surfaces for long periods, and unlike filamentous fungi, they do not produce allergenic spores or mycotoxins [102]. Isolates of 41 yeast species belonging to 12 different genera: *Candida, Pichia, Aureobasidium, Metschnikowia, Rhodotorula, Cryptococcus, Leucosporidium and Saccharomyces, Sporobolomyces, Rhodospridium, Debaryomyces* and *Wickerhamomyces* have been tested for their biocontrol capacity against the main agents of post-harvest fungal rotting of apples. The most studied genera are in order of importance *Candida, Pichia,* and *Aureobasidium*. Come second, *Rhodotorula, Cryptococcus* and *Metschnikowa*, then third *Rhodosporidium* and *Saccharomyces*. The other genera are very rarely cited in literature (Figure 2)

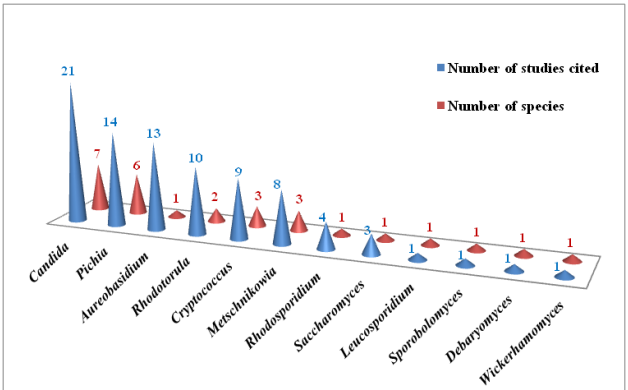

Fig. 2: Number of yeast species in the genera reported in the literature on bio-control of (PHFRA)

(1) Genus *Candida*

Seven yeast species of the genus *Candida,* including *C. oleophila*[45,92,103-105], *C. sake*[36,41,106,107], *C. diversa*[11,108,109], *C. guilliermondii*[110], *C. membranfaciens*[39] *C. saitoana* [64] and *C. pyralidae* [96], have been reported to be effective biological control agents for post-harvest fungal apple rot.

Candida oleophila is one of the most studied species of genus *Candida*.[103] have shown the efficacy against *P. expansum* and *B. cinerea*, In in vitro and in vivo experiments. The researchers have also been able to improve its efficacy against these two pathogens, both in vitro and in vivo on injured fruit, by addition of 0.4% of nisin to the antagonist suspension. Guerrero et al., [92] evaluated three strains of *C. oleophila* (L06, L07 smooth and L07 rough) as biological control agents against *P. expansum* the three strains allowed a significant reduction in the incidence of the disease by 72-82% on "Golden Delicious" apple fruits. Another strain, *C. oleophila* (strain O) has been isolated

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from apple (cv. Golden delicious) and selected for its high and reliable antagonistic properties against *P. expansum* and *B. cinerea*, the two of the most devastating pathogens of harvested apples [29]. Strain (O) was used to control blue rot by Lahlali and Jijakli [104], who have demonstrated that the addition of skim milk, sucrose or sorbitol to the O strain of *C. oleophila* can significantly improves its survival on the surface of apples by 80.8%, 42.26% and 37.27%, respectively, and improve the effectiveness of biological control of *P. expansum.*

Another speciess, *Candida sake*, has been approved for the control of *P. expansum, B. cinerea* and *Rhizopus nigricans* [36]. The antagonistic potential of several strains belonging to this speciess has been demonstrated. Arrarte et al., [41] tested several psychrotrophic, non-pectinolytic yeasts from Antarctica as potential biocontrol agents against several apple pathogens. 34 of them were able to maintain the incidence of rot caused by *P. expansum* and *B. cinerea* at less than 25% on apples stored at a temperature of 0 to 1°C. These researchers, also demonstrated that one strain, C. sake (strain 41E), isolated from marine water, was also effective, in vivo, in the biocontrol of *P. expansum* on "Red Delicious" apples. The antagonistic potentiel of strain *C. sake* 41E was approved in a later study [111], in wich It was also shown that it could also reduce the concentration of patulin in apple juice by almost 80% at room temperature without adsorbing mycotoxin on its cell walls. The biocontrol potential against *P. expansum* was also demonstrated in other strains, C. sake (CPA-1) [106] and *C. sake* (CPA-2) [107].

Another species, *C. diversa,* has also been reported as an effective biological control agent against *Botrytis cinerea* of apples in post-harvest [105, 108, 109].

Strains of *C. guilliermondii* have also been studied for the biological control of grey and blue rot in apples by McLaughlin et al., [110] who demonstrated that the efficacy of biocontrol was directly related to the concentration of the pathogen's spores and the cellular concentration of antagonistic yeasts. Scherm et al., [71] tested the efficacy of two strains of *C. guilliermondii* (F1 and 3C-1B) in the biocontrol of blue rot in In Vivo trials on apple "Golden delicious" and "Fuji" variety. The isolates resulted in a significant reduction in lesion diameter to (14 -38%) and (17 -27%) on the Golden delicious and Fuji variety respectively.

In the study conducted by Gholamnejad et al., [39], Three strains, of another speciess, *C. membranfaciens* (A2, A4 and A5) were isolated from the surface of apples and were evaluated, In Vitro, for the control of apple blue mould caused by *P. expansum*. All 3 yeast strains inhibited the growth of *P. expansum*. The inhibition ranged from 20.6 to 78.9%. In In Vivo trials, the three strains caused a significant reduction in the diameter of the lesions on apples stored at room temperature (20 °C) and cold (5 °C).

El-Ghaouth et al., [64], in turn, studied the efficacy of another speciess of genus *Candida: C. saitoana* against *B. cinerea* apple rot, and showed that the application of this yeast 48 h and 72 h before inoculation with *B. cinerea*, reduced the diameter of the lesions by 50 and 70% respectively, but, when applied at the same time or 24 hours after inoculation with the pathogen, it had no effect.

Another speciess, *C. pyralidae*, was recently tested by Mewa-Ngongang et al., [96] against several post-harvest fungal apple rot (*B. cinerea, C. acutatum* and *R. stolonifer*). Cell suspension has shown growth inhibition activity of up to 100% against all species of pathogens tested. A 100% inhibition against the germination of both three species was observed on the grape pomace extract agar (GPA) plates. The apple bioassay demonstrated the ability of *C. pyralidae* to control spoilage caused by *B. cinerea, C. acutatum* and *R. stolonifer*, by 100, 43 and 52% respectively

(2) Genus *Pichia*

P. anomala is by far, the most studied speciess of the genus Pichia in the biocontrol of fungal rot of apples (6 research studies among the 14 devoted to the genus). One strain of *Pichia anomala* (starin K) was selected by Jijakli and Lepoivre [112] for its great biocontrol activity against infection by *B. cinerea* and *Penicillium* Sp., on injured Golden Delicious apples. Jijakli et al., [113] were able to achieve a good level of protection against *P. expansum* in apple, by spraying strain K 12 days before harvest, the level of protection was higher than the post-harvest chemical treatment but remained below the level of protection obtained by standard pre-harvest chemical treatments. Lahlali et al., [114] tested and evaluated the efficacy of this strain against *P. expansum* in the laboratory and in the field. Very high levels of protection and final yeast densities were obtained when the initial concentration applied was 1×10^8 cfu ml^{-1}. The level of protection correlated positively with the density of yeast determined on the wounds and was influenced by the humidity on the surface of the apple. Jijakli [6] tested another strain P. *anomala* (K) and reported that treating injured sites with 50 µl of yeast suspension (strain K) (107 CFU / ml) was sufficient to completely inhibit the development of rot induced by *B. cinerea* and *Penicillium* sp, at 5 and 25 ° C. Lahlali et al., [115] have evaluated, in vitro and in vivo, the influence of artificial UV-B radiation on strain K of *P. anomala*, and on its potential for controlling fruit diseases after harvest The

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in vitro 50 and 90% lethal dose values were 0.89 and 1.6 Kj/m^2, respectively, whereas lethal values in vivo were 3.2 and 5.76 Kj/m^2, respectively.They also tested the effect of protective substances against UV rays on the efficacy of *P. anomala* strain K in the biocontrol of post-harvest diseases of apples. Several substances (congo red, tryptophan, riboflavin, lignin, casein, gelatin, folic acid, tyrosine) have been tested alone or as a mixture. The results showed that, with the exception of lignin and folic acid, none of the compounds or mixtures significantly increased the capacity of the K strain to control post-harvest *P. expansum* on the injured apples.

Another speciess of genus *Pichia* which has also been well reported in literature in the bicontrol of fungal rot of apples was *P. caribbica.* The effectiveness of this speciess, in controlling post-harvest blue mold in apples has been demonstrated by Cao et al.,[116]. This species reduced significantly the incidence blue mold rot in apples treated compared to controls, and the higher the concentration of *P. caribbica,* the better the effectiveness of the biocontrol. *P. caribbica* significantly controlled the development of apple rot after storage at 20 ° C for 35 days or at 4 ° C for 45 days. Likewise, spore germination and growth of *P. expansum* were significantly inhibited by *P. caribbica* in In vitro assays. The effect of this yeast on the breakdown of patulin produced by *P. expansum* has also been determined in In vitro tests. In another study, Mahunu et al., [117] also showed that *P. caribbica* significantly reduced the incidence of blue mold on apples after 10 days of storage at 20 ° C and 95% RH. The efficacy of *P. caribbica* in the biocontrol of blue mold in apples was also evaluated by Zhang et al. [118], who showed that yeast causes a reduction in the severity of the disease by almost 50% compared to the control. In addition to the antagonistic effect of *P. caribbica*, the effect of this yeast on the breakdown of patulin produced by *P. expansum* has also been determined In vivo. After incubation with *P. caribbica* at 20 ° C for 15 days, the production of patulin by *P. expansum* inside the apples was significantly reduced compared to the control [116].

One of the species of the genus *Pichia* which is also well reported in the literature in the biocontrol of fungal apple rot in storage is *P. guilliermondii.* Scherm et al., [71] tested the efficacy of a strain *P. guilliermondii* (5A) in the biocontrol of blue rot in In Vivo trials on Golden delicious apples. The isolate resulted in a significant reduction in lesion diameter of almost 50%.

Zhao and Yin [119] reported the application of *P. guilliermondii* at a dose of 1 × 10^8 CFU mL^{-1} can effectively control the rots caused by *B. cinerea, P. expansum* and *C. gloeosporioides*, on "Red Fuji" apples while now good physical and chemical quality of the fruit.

Mokhtarnejad et al., [120] have developed two effective formulations of *P. guillemondii* with inorganic (talk, kaolin) and organic (Rice bran, wheat bran) carriers, and the viability of the yeast cells in formulations stored at 4°C and 24°C. Results showed that yeasts cells could survive at organic and inorganic carriers for more than 6 months. The storage at 4°C gave the highest number of viable cells for all formulations examined. The usefulness of powder formulations of *P. guilliermondii* with attention to biocontrol efficacy has been indicated.

Three other species of the genus *Pichia*, little reported in the literature, have also shown some efficacy in bicontrol of post-harvest fungal diseases of the apple. These are: *P. fermentans, P. angusta* and *P. kluyveri: P. angusta* was tested, and for the first time, by Fiori et al., [121], and eight isolates showed significant biological control activity against *B. cinerea* and *M. fructicola*, while the efficacy against *P. expansum* was low. Genus *P. kluyveri,* on the other hand, was tested, in a recent study [96] against several Fungi (*B. cinerea, C. acutatum* and *R. stolonifer*), and showed, In vitro, a 100% inhibition against the spore germination of all fungal species, In vivo, a 38 and 22% growth inhibition of *B. cinerea* and *R. stolonifer*, when it almost completely inhibited the growth of *C. acutatum*[96].

(3) Genus *Aureobasidium*

A. pullulans is the only speciess of this genus that has been reported in the literature, in the biocontrol of post-harvest apple fungal rots. But several strains of this speciess have been selected for their antagonistic power against the main agents of apple rot in storage. Leibinger et al., [28] have evaluated, in laboratory tests, on "Golden delicious" apples, the effectiveness of two strains of *A. pullulans* (CF10 and CF40) against *P. expansum*, *B. cinerea* and *Pezicula malicorticis* agents of post-harvest rots apples. Each of the two strains, applied at a dose of 10^7 CFU ml^{-1}) allowed a significant reduction in the size and number of lesions caused by the three pathogens, The effectiveness of these two strains of *A. pullulans* was more important on *B. cinerea* and *P. expansum*. In experiments conducted by Castoria et al., [79], another strain, *A. pullulans* LS-30 significantly reduced the infection caused by *B. cinerea* and *P. expansum* on apples by 76.5% and 88.5% respectively.

Achbani et al., [14] were able to isolate from the surface of the apples (Golden Delicious variety) two other strains of *A. pullulans*, Ach 1-1 and 1113-5, who showed a high antagonistic power (> 80%) at 25 ° C, against *P. expansum* and *B. cinerea*. El Hammouchi et al., [122] have developed molecular markers and a semi-selective

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medium (PDA medium supplemented with (0.5 mg L^{-1} euparen, 1mg L^{-1} sumico, 2.5 mg L^{-1} hygromycin B, 30 mg L^{-1} streptomycin sulfate and 1 mg L^{-1} cycloheximide), allowing the identification and the quantification of the two strains Ach 1-1 and 1113-5 of *A. pullulans*. Significant production of strain Ach 1-1 yeast biomass (10^6 g dry wt l^{-1}) was obtained in 48 h, in fed-batch fermentor, with a glucose solution [123] . The biomass produced was dried in a fluidized bed dryer with a final viability of 62%. After 7 months at 4°C, the viability was 28% of the initial value. The formulated yeast was also evaluated for its antagonistic activity againt *P. expansum* at pilot scale. A protection level of 89% was achieved with the biomass preparation at $1 \cdot 10^8$ c.f.u. ml^{-1}after 28 and 7 days for apples stored respectively at 5 and 25°C [123]. Another strain *A. pullulans* (GE1 7), has been reported recently for its ability to inhibit the mycelial growth of *P. expansum* by 83.4% [59].

Still within the genus *Aureobasidium*, Sukmawati et al., [13], Screened two strains of *Aureobasidium* sp. nov. (T 3 and T4) that had the capacity as biological control agents against the growth of *Aspergillus brasiliensis*. Based on in vivo antagonistic activity tests on damaged apples, the T4 isolate (50% rot incidence; 25% disease severity) has shown greater capacity as biological control agents for *A. Brasiliensis* compared to isolate T3 (incidence of decay 100%; severity of the disease 25%), while T3 were able to reduce decay symptoms in apples inoculated with anothers pathogen speciess *A. flavus*. The ability of the two isolates to reduce the growth of *A. Brasiliensis* was better than that of synthetic fungicide Dithane M-45 0.3% (incidence of caries 100%; severity of the disease 44%).

(4) Genus *Rhodotorula*

Two species of this genus have shown antimicrobial activity against the fungi responsible for post-harvest rot of the apple: *R. glutinis* and *R. mucilaginosa*. *R. glutinis* was also found to be effective against apple rots caused by *B. cinerea* and *P. expansum* [124]. Both pathogens were completely inhibited by *R. glutinis* applied at doses of 1×10^8 and 5×10^8 CFU ml^{-1}, respectively. The effectiveness of *R. glutinis* (CF35 strain) in the biocontrol of post-harvest rot of apples caused by *P. expansum*, *B. cinerea* and *Pezicula malicorticis*, was evaluated, in laboratory tests, on "Golden delicious" apples beforehand stored for more than 8 weeks at 2°C and 95 % relative humidity[28]. *R. glutinis*, at a dose of 10^7 CFU ml^{-1}, significantly reduced the size and number of lesions caused by all three pathogens, after storage for 4 weeks at 4°C. The reduction was more marked when the antagonist was applied. The efficacy of another strain of *R. glutinis* (strain HRB6) as a biological control agent for apple blue rot was also demonstrated under semi-commercial and commercial storage conditions [125]. Another strain of *R. glutinis* (LS-11) has been also reported to be very effective (80 % reduction in blue rot compared to the control) against blue mold in apples [126].

In addition to its antagonistic effect, *R. glutinis* is also known for its ability to reduce the accumulation of mycotoxins, including patulin, in infected fruit [107,124,127].

For *R. mucilaginosa*, Li et al., [128] have found that this strain has biological control efficacy against *P. expansum* in apples. Two strains (A1 and A7 strains), inhibited the growth of *P. expansum* in vitro from 31.5 to 89.1 % and caused significant damage reduction in apples stored at 5 °C and 20 °C [39].

Yang et al., [129] tested the biocontrol efficacy of a strain of *R. mucilaginosa* isolated from the surface of peach blossoms against *P. expansum* on apples of the "Fuji" and demonstrated that inoculation of apples with a suspension of *R. mucilaginosa* of (1×10^8 CFU ml^{-1}) caused a 50 % reduction in disease incidence and a 20 % reduction in fruit lesion diameter. Recently Sukmawati et al.,[13] screened strain of *R. mucilaginosa* T1, isolated from *Cerbera manghas* L., who exhibited in In vitro test the potentiel ability to act as biocontrola gents for two destructive molds in apples, *Aspergillus brasiliensis* and *Aspergillys flavus*. In vivo, T1 reduced the growth of pathogens, thereby reducing apple rot (decay incidence 25%; disease severity 6.25%). Compared to Dithane M-45 synthetic fungicide (0.3%) the potentiel ability of T1 was was much better.

(5) Genus *Cryptococcus*

Tree epecies of the genus *Cryptococcus* were repported in littérature for biocontrol of (PHFRA), they are, in order of importance *C. laurentii, C. albidus* and *C. infirmominiatus*. *C. laurentii*, has been shown to be effective in post-harvest biological control of apple rot [130] it allowed a significant decrease in the incidence of the disease to 41.6% compared to 100% in the Control. The diameter of lesions also decreased from 22.8 mm in the control to 12.4 mm. The efficacy of *C. laurentii* (strain LS-28) against several apple rot agents (*Penicillium, Rhizopus, Botrytis and Aspergillus*) was reported [126]. LS-28 reduced rot from 80 to 100 % compared to the control, on apples stored 4-6 days at 20°C, and reduced *Penicillium* rot at 95% compared to the control on apples stored for 60 days at 4°C[126]. Blum et al., [38] tested *C. laurentii* isolated against *P. expansum*, *Glomerella cingulata* and *P. malicorticis* and found an effectiveness in the biocontrol comparable to that of synthetic fungicides such as thiabendazole and iprodione. The same result was obtained by [131] Blum et al., using isolat *C. laurentii* (36) on "Fuji" and "Gala" apples stored

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in the laboratory conditions (15-20 ° C et 60-70 % RH). In cold storage (1 °C et 90-95 % RH), isolat (36) was as effective as several systemic fungicides against *P. expansum*.

C. albidus has also been shown to be effective against *P. expansum* rot [125]. Fan and Tian [132] proved the capacity of *C. albidus* at a concentration (1×10^8 CFU / ml), to completely inhibit the decay of *B. cinerea* and *P. expansum* on "Fuji" apples stored at 23 and 1 ° C, especially when it was applied after or simultaneously with the pathogens.

The efficacy of the species *C. infirmominiatus* (strain YY6) was demonstrated against apple blue rot under both semi-commercial and commercial storage conditions[125].

(6) Genus *Metschnikowia*

Two species have been reported in the biocontrol of (PHFRA). The first is *M. fructicola*, their efficacy, against apple rot caused by *P. expansum* has been reported [67,133]. Strain *M. fructicola* AL27, was as effective as chemical fungicide in the biocontrol of *P. expansum* on apples of different cultivars ("Golden Delicious", "Granny Smith", "Red Chief" and "Royal Gala") stored at (22 ± 1 °C for 7 days) and at (1 ± 1 °C for 56 days), but its control potential was higher on "Golden Delicious" apples. The second speciess is *M. pulcherrima*.The efficacy of two strains from this speciess (2.33 and 4.4), was tested against *B. cinerea* under different storage conditions. Both strains inhibited the growth and germination of *B. cinerea* spores, but the biocontrol effectiveness was strongly depended on the concentration of the antagonist and the time of its application [72]. The efficacy of other isolates (GS37, GS88, GA 102 and BIO126) of the yeast *M. pulcherrima* against *B. cinerea*, *P. expansum*, *Alternaria sp*., *And Monilia sp*., on apple fruits, has been demonstrated [84]. In this study, all four strains were able to completely inhibit *Monilia sp*. after storage at 23 °C, as well as *B. cinerea* and *P. expansum* after cold storage (at 4 °C). In another study, BIO126 strain proved to be very effective in controlling blue and gray molds (Reduction in lesion diameter of 56.6% and 97.2%, for *P. expansum* and *B. cinerea*, respectively) [167]. This strain has the added benefit of not growing at temperatures of 37 ° C or higher, which is important from a toxicological point of view [167]. Two other strains of *M. pulcherrima* MACH1 and a GS9 also showed efficacy in the biocontrol of *P. expansum* on apples of different cultivars ("Golden Delicious", "Granny Smith", "Red Chief" and "Royal Gala") stored at (22 ± 1 ° C for 7 days) and at (1 ± 1 ° C for 56 days) [133].

(7) Genus *Saccharomyces*

Only one speciess *S. cerevisiae* was implicated in biocontrol of (PHFRA). Scherm et al., [71] tested In Vivo the efficacy of starin *S. cerevisiae* M25 in the biocontrol of blue rot, on "Golden delicious" and "Fuji" apples and resulted in a significant reduction in lesion diameter by 100 %, but its capability was significantly inhibited with the addition of nitrates or sugars (maltose and raffinose).

Another train, *S. cerevisiae* (YE-7), was tested in the biocontrol of blue mould on "Golden delicious" apples and showed significant reduction of the incidence of desease and patulin accumulation in the tissues of rotten apples by 48% and 42.6%, respectively, compared to control. This, when yeast is applied as pre-treatment or simultaneous treatment. Late treatment of pathogen-infected apples with YE-7, did not reduce patulin accumulation in rotten tissue compared to other treatments [134].

(8) Genus *Rhodosporidium*

A marine yeast, *R. paludigenum*, previously considered effective in the biocontrol of various post-harvest fruit diseases [135-137]. This species showed ability to control blue mould in apples reducing apple rot by 80% after 5 days of incubation at 25 ° C and inhibiting mold infections at high concentrations (1×10^7 and 1×10^8 mL^{-1} cells) [62].

The effect of a strain *R. paludigenum* as also avaluated on post-harvest blue mold and patulin accumulation in apples stored at 23°C [138]. *R. paludigenum* was able to control post-harvest decay in apples and to remove patulin in vitro effectively, by both biological degradation and physical adsorption. However, the application of the yeast at a high concentration (10^8 cells per ml) enhanced patulin accumulation in fruit after 7 days of storage 24.2 times compared to the controls.

(9) Other Genera (*Leucosporidium, Debaryomyces, Wickerhamomyces*)

A strain of yeast speciess, *Leucosporidium scottii* At17, isolated from the soil, was found to be effective against blue and grey rot of apple caused by *P. expansum* and *B. cinerea* [32]. This strain was selected for its ability to form biofilms on the surface of apples and for its resistance to fungicides commonly used in post-harvest, which suggests its use in an integrated pest management combined with low doses of fungicides.

Recently, biocontrol potentiel of different strains of *Debaryomyces hansenii* speciess against *M. fructicola* was demonstrated both in In vitro and In vivo trials [60]. One strain (KI2a) showed a high in vitro biocontrol activity, inhibiting mycelium growth by 69.5%, as compared to control fungal cultures. KI2a and another, strain (AII4b) reduced significantly brown rot on apple fruits by 85.1% and 70%, respectively, in comparison to infected fruits, which did not receive any pre-treatment [60].

The speciess *Wickerhamomyces* also showed a high potential for biocontrol of anomalus (BS91) against brown rot, resulting in an inhibition of mycelium growth of

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66.08% compared to the control, and a reduction in brown rot of apple by 70.02%, compared to fruit treated only with the pathogen [60].

4.1.2 Filamentous Fungi

In addition to yeasts, antagonistic filamentous fungi are also considered to be very promising agents that can be involved in integrated post-harvest disease management strategies [139]. Their use in biological control against post-harvest diseases of apples however remains very limited, probably due among other things to: (1) Their biocontrol potential is much slower than that of chemical fungicides ; (2) their need for high humidity for their spore germination, development and sporulation ; (3) their sensitivity to UV radiation. Two genera have been reported for their biological control potential against apple fungal rots, *Trichoderma* and *Muscodor*

(1) Genus *Trichoderma*

Trichoderma harzianum is the only speciess of genus *Trichoderma* reported in the literature as biological control agents for (PHFRA). *T. harzianum* was used to control fungal diseases of apple, caused by *Alternaria alternata*, *P. expansum* (blue rot), *B. cinerea* (grey rot), *M. fructigena* (brown rot), weakness diseases caused by *Pythium* speciess and *Rhizoctonia* sp. [99,140-143].

Batta [89] attempted a formulation of *T. harzianum* conidia using an inverse emulsion (water-in-oil formulation) based on coconut and soybean. Treatment with conidia of *T. harzianum* formulated at a concentration of 6. 10^7 conidia ml^{-1}, significantly reduced the diameter of lesions on apples, inoculated *by P. expansum*, to 17,5 mm compared to 34 mm for controls (emulsion products, distilled water) and 26 mm for treatment with unformulated *T. harzianum* [89]. Dipping trials of injured apples in a suspension of T. harzianm conidia showed a significant preventive effect of *T. harzianum* conidia formulated against *B. cinerea* and *P. expansum* [89]. A biopesticide based on *T. harzianum* strain "TrichoPAL1" was produced with a suitable formulation of water-in-oil invert emulsions based on soybean oil (28.50%) coconut oil (19.50%) [144]. The efficacy test of this biopesticide against post-harvest mold (*B. cinerea* and *P. expansum*) on fresh apple fruit indicated a significant reduction in the diameter of the mold lesion on fruit stored at 20 ° C ± 1 °C and, an extended protection of two and a half months for apples stored under controlled and semi-commercial conditions. Cheng et al., [145] identified an amino acid oxidase (Th-1-AAO), produced by *T. harzianum* ETS 323, and showed that Th-1-AAO effectively inhibits, in vitro, the growth of hyphae of *B. cinerea*, causes cytosolic vacuolization in hyphae and led to a lysis of hyphae. Th-L-AAO treatment also showed direct disease control against *B. cinerea* in vivo. Apple fruit inoculated with *B. cinerea* and treated with Th-L-AAO showed significant inhibition of *B. cinerea*-induced lesions after 6 days. The untreated apples displayed a 12-fold increase in the lesion radius relative to the treated. More recently, it was demonstrated that the strain (TRIC8) of *T. harzianum* had potential as a biocontrol agent for the control of post-harvest decay of "Granny Smith" apple caused by *P. expansum* and *M. fructigena* by reducing lesion diameter of this fungi by 69.73% and 97.13% respectively [143]. Deng et al., [91] studied biocontrol activity of recombinant aspartic protease P6281 from *Trichoderma harzianum* (rP6281) , expressed in *Pichia pastoris*, against several pathogenic fungi, including *B. cinerea* and demonstrated rP6281 ability to control the grey mold rot on apples. Transmission electron microscopy revealed that rP6281 efficiently damages the cell wall of this pathogenic fungi.

(2) Genus *Muscodor*

The speciess *M. albus* is an endophytic fungus who produces a mixture of VOCs that are lethal to a wide variety of plant and human pathogenic fungi and bacteria [146].

It was reported that biofumigation for 24 h with a culture of *M. albus* grown on autoclaved rye grain completely controls blue (*P. expansum*) and grey mold (*B. cinerea*) of apple [147]. It was also reported that *M. albus* volatiles had a significant effect on the germination, growth and survival of *B. cinerea* and *P. expansum* [95]. Their results clearly showed that increasing the weight of *M. albus*-colonized grain from 0.25 to 1.25 $g \cdot L^{-1}$ had a significant effect on the ability of *M. albus* volatiles to inhibit spore germination of the two fungi.

4.2 Bacterial antagonists in control of (PHFRA)

In the biological control against post-harvest diseases of apples, bacterial antagonists hold second place in terms of importance after yeasts. Several bacteria have been identified as playing an important role as biological control agents against many phytopathogenic fungi [98,148-150]. Among the 125 researches included in our study 27 (20,8%) was devoted to bacterial antagonists (Figure 1). Nine speciess belonging to 6 different genera have been studied for their biocontrol power of post-harvest fungal diseases of apples (Figure 3). The speciess that have aroused the most interest from researchers are *Pseudomonas Syringae, P. fluorescence, Pantoea agglomerans, Baccillus amyliquefaciens* and *B. Subtilis* (Figure 3).

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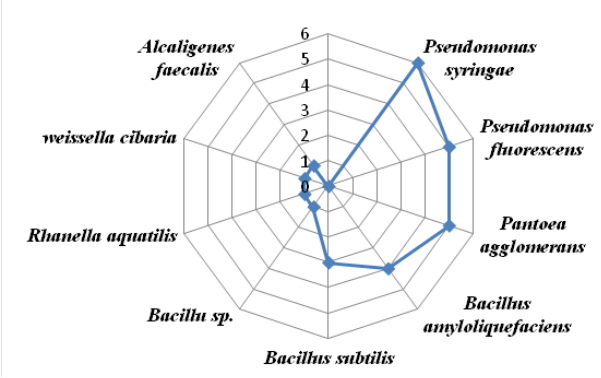

Figure 3. Biocontrol bacterial species of (PHFRA) and the number of related studies

Among the various bacteria for biocontrol of post-harvest diseases of the apple, *Pseudomonas* syringae seems to be the most used and the most reported in literature. The results obtained with regard to its effectiveness are indeed very promising. The strains of *P. syringae* van Hall have been marketed under the name of BioSave (sold by EcoScience) approved in the United States, provided biological control against blue rot (*P. expansum*), grey rot (*B. cinerea*) and Mucor's rot of pome fruits, including apples and pears [43,52,151]. A strain of *P. syringae* designated MA-4 originally isolated from the surface of apple leaves collected from eastern Ontario and which has been shown to be effective against *R. stolonifer* [152], was also effective against blue (*P. expansum*) and gray (*B. cinerea*) rots in post-harvest apples, especially when combined with cyprodinil, a commercial fungicide [153]. Another strain (*P. syringae* CPA-5), also showed great ability in the biological control of *P. expansum* and *B. cinerea* on cold-stored "Golden Delicious" apples, and a great capacity to colonize the surfaces of apples under cold storage conditions [154].

Another *Pseudomonas* speciess, *P. fluorescens*, has been reported to be effective in the biocontrol of grey rot caused by *Botrytis* sp. [155]. Furdermore, Etebarian et al., [156], evaluated one Isolate of *P. fluorescens* (1100-6), as a potential agent for the biocontrol of blue mold in apple caused by *P. expansum* or *P. solitum*. This isolate decreased the growth of *Penicillium* spp., and produced large areas of inhibition in double agar culture tests. The metabolites it produces decreased the colony area of *Penicillium* isolates from 17.3% to 78.5%. Similarly, applied 24 or 48 hours before inoculation with *Penicillium* spp., it significantly reduced the severity and incidence of apple rot, after 11 days at 20°C for *P. expansum*, and after 25 days at 5°C for both species (*P. expansum* and P. solitum). these researchers [156] also reported that isolate (A506), a microbial pesticide marketed for the control of fire blight agent *Erwinia amylovora* [157], could potentially be used as an effective biocontrol agent for post-harvest disease control in apples.

Pantoea agglomerans is also another well-reported species in the AFRS biocontrol (Figure 3). Several strains of *P. agglomerans* have been reported to be effective against apple rot caused by, *B. cinerea*, *R. stolonifer*, *P. expansum* [51,83,107,148,158].

In laboratory tests, a high level of control of those three fungi was also obtained with *P. agglomerans* strain CPA-2 [83]. In tests under semi-commercial conditions, this strain allowed the reduction of blue mold by 81and 100% on apples stored at 1 ° C in ambient air an in oxygen-poor atmosphere, respectivelly, and it was as effective as imazalil in controlling gray mold [83]. The efficacy of this same strain against blue mold has also been proven on "Golden Delivious" apples, stored at 1°C [107]. These researchers even showed the capacity of this strain to control the accumulation of patulin in the treated apples. Their work has, however, shown the ineffectiveness of this strain in controlling the disease and the production of patulin on apples previously stored in ambient atmosphere. Still against blue mold, another strain, *P. agglomerans* EPS125, was shown to be effective when tested by on apples, "Golden Delicious", stored under controlled atmosphere (0 ° C, 2% O2 and 2% CO2) and apples "Granny Smith" stored at 20°C [148]. This strain has, in fact, reduced rot from (12.5-14.1%) to (2.5-2.6%), on "Golden Delicious" apples and (80-73%) to (7-8%) on "Granny Smith" apples, with an efficiency of (80-81%) and (91-88%), respectively.

Two other interesting species of the genus *Bacillus* were reported for their biological control potential against post-harvest apple rot fungi, *B. subtilis* and B. amyloliquefaciens.

B. subtilis applied to injured apples has been reported to reduce fruit rot caused by *Botrytis cinerea, Alternaria alternata*, *P. expansum* and *Pezicola malicorticis* [28,158].

Several strains of *B. amyloliquefaciens* were reported for their high potentiel in AFRS biocontrol. Strain 9001 isolated from healthy apple from an infested orchard was assessed as having biological control activity against ring rot in apples in vitro and in vivo [159]. its application both in the field during the growing season and in post-harvest results in a significant reduction of disease incidence of within the storage period of 4 months at room temperature. Another strain of (*B. amyloliquefaciens* BUZ-14) has also been reported for its biological control potential against *P. expansum* in apples [160]. Preventive treatment with this strain reduced the incidence of *P. expansum* on apples from 100% to 20%. Effectiveness of the strain BUZ-14 against *P. expansum* was also demonstrated by Calvo et

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al. [90], and study revealed iturin as a key metabolite in the inhibitions with an In vivo MICs of 33.9 µg mL^{-1}. However, the study showed that this strain had no effect in controlling other rots on apples, including gray and brown rots.

Recently, biocontrol potentiel of the *B amyloliquefaciens* SF14 against *M. fructicola* was demonstrated both In vitro, In vivo and in Semi-commercial large-scale trials[161]. Results obtained in semi-commercial trial,were not significantly different from both commercial bacterial strains *B. subtilis* Y1336 and *P. agglomerans* P10c, but significantly lower than that of thiophanate-methyl fungicide.

The only species of the genus *Rahnella* reported in the literature in the biocontrol of (PHFRA) is *R. aquatilis*. This epiphytic bacteria was isolated from the surface of fruit and apple leaves, and tested for its antagonistic properties against *P.expansum* and *B. cinerea* In vitro and In vivo on "Red Delicious" apple. Bacteria inhibited, by ditrect contact, spore germination of the two pathogens and reduced incidence of the two diseases by almost 100 % on apples stored at 4°C and by only 60% and 0% for the diseases caused by and *B. cinerea* respectively on apples stored at 15°C [78].

Other bacterial species were also reported in littérature for biocontrol of (PHFRA) including *Weissella cibaria* [162] and *Alcaligenes faecalis* [161].

Weissella cibaria is a lactic acid bacteria. One isolat of this species, *W. cibaria* (TM128), was able to to decrease blue rot infection levels, on "Golden Delicious" apples, by 50% [162].

Use of *Alcaligenes* species for post-harvest management was reported for phytopathogen fungi on some stored fruits [161,163,164]. Strain *A. faecalis* ACBC1, selected from among the species highly effective for *M. fructigena*, agent of brown rot of fruit, has shown great potential for preventing brown rot both in vitro, by strongly inhibiting mycelial growth of than in vivo, by significantly reducing the severity of the disease. It has also been confirmed effective in a large-scale semi-commercial trial [161].

4.3 Actinomycetes used in the Biocontrol of Post-harvest Apple Rot

Actinomycetes, represent potential agents for the biocontrol of several plant diseases [165, 166, 167, 168]. However, few studies have investigated their antagonistic effect against phytopathogenic post-harvest apple fungi. We will cite two studies here. In the first, the antagonistic activity of more than 100 Actinomycetes against *C. gloeosporioides,* the causative agent of apple bitter rot was evaluated [40]. Their In vitro bioassays revealed that six of the isolates had significant inhibitory effects against the mycelial growth of the pathogen. In vivo post-harvest experiments indicated that the six antagonists inhibited significantly the rotting of apples, either by inhibiting the appearance of the disease on healthy fruit, or by preventing the spread of lesions on diseased fruit. Among the tested antagonists, one isolate who was the most effective in in vitro bioassays was identified as *Amycolatopsis* (Pseudonocardiaceae family). The second study [61] shows that treating apples with strain *Streptomyces rochei* A-1 induces their resistance to ring rot caused by *B. dothidea*, and reduces the area of lesion and the incidence of the disease by 65.4% and 27.1%, respectively, compared to the control, after storage at 25 ° C for 7 days. Treatment with *Streptomyces rochei* A-1 also improves significantly the activities of peroxidase, superoxide dismutase, catalase and phenylalanine ammonia-lyase, and strongly inhibits lipid peroxidation [61].

5. Improvement the Effectiveness of Microbial Biological Control of (PHFRA)

Over the past 30 years, new approaches have aimed at developing biocontrol systems of (PHFRA) in order to overcome existing limitations, such as the great variability in the efficacy of antagonists, reduced spectrum of activity of this antagonists and their use for preventive action only. In our study we will develop two main approaches: Combination of several biocontrol agents (Antagonistic mixtures) and Combination of microbial biological control with other control methods.

5.1 Combination of Several Biocontrol Agents

In most studies on biological control of post-harvest spoilage agents, the effect of each control agent is considered individually [169]. To improve the effectiveness of biocontrol of post-harvest diseases to acceptable levels, and to broaden the spectrum of action of antagonists, researchers have studied the combination of several control agents. Most of their works showed that the combined use of at least two antagonists exhibit more effective control of post-harvest rots on many fruits than antagonists applied alone [170, 171]. Over the past three decades several combinations of microbial biocontrol agents have been tested for their antagonistic potential against the main fungi responsible for apple rot after harvest. Some researchers have combined strains belonging to the same speciess [92], others have combined strains belonging to different species but of the same genus [125,172]. Several researchers have tested

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combinations between strains belonging to different genera but from the same group [28,59,170-173]. Finally, other researchers tested combinations between strains belonging to different groups such as bacteria combined with fungi [28,174]. Very recently, Zhimo et al., [175] explored a natural probiotic microbial consortium (commercial kefir grains), composed with 7 bacterial ans 4 yeast genera, in biocontrol of apple blue rot.

The results of all this research differ from one study to another and from one combination to another but they were, generally, very encouraging (table 3)

Table 3. Biocontrol of (PHFRA) by microbial antagonistc mixtures

Pathogen	Combined Biocontrol agents	Results achieved	Reference
P. expansum	*P. syringae* + Sporobolomyces roseus	bicontrol by the mixture (M) was more effective than by each antagonist individually	Janisiewicz et Bors [174]
P. expansum	*C. laurentii* + *C. infirmo-miniatus*	Biocontrol as effective as chemical treatment with thiabendazole at a high dose of 528 µg / ml.	Chand-Goyal and Spotts [125]
Pezicula malicorticis ***P. expansum*** ***B. cinerea***	*M1: A. pullulans* (CF10 and CF40) and one strain of *R. glutinis* (CF35) *M2: B. subtilis* (AG704 and HG77) and *A. pullulan*s strain CF10	M1completely suppressed gray and blue rot to the same extent as Euparen ; M2 completely eliminated brown rot and significantly reduced blue rot ; M1 and M2 improved biocontrol againste *P. malicorticis*	Leibinger et al. [28]
P.expansum ***B. cinerea***	*M1:R. glutinis* SL 1 + *C. albidus* SL 43 *M2: R. glutinis* SL 30 + *C. albidus* SL 43 *M3: R. glutinis* SL 1 + *C. laurentii* SL62	M1 and M2 (synergistic power against *P. expansum*) ; M3 (Synersistic power *B. cinerea*) ; none of the (M)s was effective against both molds at the same time.	Calvo et al. [170]
P. expansum	*M. pulcherrima, + C. laurentii*	The two antagonists were more effective when mixed.	Conway et al. [173] ; Janisiewicz et al. [171]
P. expansum ***B. cinerea***	*M1: R. aquatilis-R. glutinis ; M2: R. aquatilis-C. laurentii* (106 cell / ml)	M1 was more effective (inhibited the two mold and reduced diseases incidence to zero) *R. aquatilis* was strongly stimulated by the presence of *R. glutinis.*	Calvo et al. [172]
P. expansum	*C. oleophila* (L06 + L07 smooth + L07 rough)	(M) was as effective as the chemical treatment with (fludioxonil + ciprodinil) at a dose of 1g L-1.	Guerrero et al. [92]
P. expansum	*Meyerozyma guilliermondii* KL3 and *A. pullulan*s GE17 (108 cells ml-1)	(M) inhibited spore germination of pathogen from 86% versus 82% for GE17 alone ; KL3 was ineffective against blue mold	Agirman and Erten [59]
P. expansum	commercial kefir grains (fresh and milk-activated forms) 7 bacterial + 4 yeast genera	Effective inhibition of the *P. expansum*	Zhimo et al. [175]

5.2 Combination of Microbial Biological Control with Other Control Methods

In the literature, several alternative methods have been combined with biocontrol in order to improve the fight against post-harvest diseases of apples. Some researchers have combined biocontrol with a physical treatment such as heat treatment of apples [119,176,177], or their storage under modified or controlled atmosphere [106,178-180], others have combined biocontrol by antagonists with treatments of a chemical nature, using: (1) GRAS substances including calcium chloride CaCl2 [39,132,178, 180-182]; sodium bicarbonate (BCS) [176,180,183] or ethanol [176,184] or (2) Host defense elicitors or substances delaying the senescence of apples, such as: glycine betaine [118,185]; phytic acid [117,129] ; ascorbic acid [186,187]; cytokinin N6-benzyladenine [188]; indole acetic acid (AIA) [189,190]; glycolchitosan [191]; salicylic acid [130] ; iprodione [132] ; L-serine and L-aspartic acid [157] ; or fungicides at low rates [192,193] , including cyprodinyl [153] and thiabendazole [125].

The results of scientific researches cited in our study (Table 4), showed that combined treatments improve the effectiveness of antagonists against the main post-harvest agents of apples, which is often comparable to that of conventional fungicide treatments.

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Table 4. Biocontrol of (PHFRA) by combining microbial antagonists with other control methods

Pathogen	Biocontrol agents and combined treatments	Results achieved	Reference
Biocontrol combined with physical treatment.			
B. cinerea, M. piriformis P. Expansum	*P. fluorescens* (Isolats 1-112, 2-28 et 4-6) + AC (1,5% CO2 + 1,2% O2; 1°C)	Under AC the 3 isolates were as effective as BioSave® against gray mold and blue mold on "Ambrosia" apples	Wallace et al. [180]
P. expansum B. cinerea et C. gloesporidoides	*P. guillermondii* + hot air treated apple (38°C during 96 h)	Total inhibition of infection of apples by the three pathogens	Zhao et Lin [119]
P. expansum	M. fructicola pretreated by mild heat shock (HS) (30 min at 40°C)	-Greater biocontrol activity -Faster growth rate in apple wounds stored at 25 °C -Better tolerance of to oxidative stress	Liu et al. [67]
P. expansum	*Cryptococcus laurentii and chitosan*	combination of chitosan and *C. laurentii* resulted in a synergistic inhibition of the blue mold rot, being the most effective at 0.1% of chitosan with low viscosity (12 cP).	Yu et al. [191]
P. expansum	*M. pulcherrima* BIO126 + dipping Golden delicious apples in deionised water at 50°C for 3 and 10 minutes	BIO126 combined with hot water provided a good control of the pathogen at 23°C (29.2% of reduction) and 4°C (38.2%).	Spadaro et al. [176]
C. acutatum P. expansum	*M. pulcherrima* ST1-D9 and FMB-24H-2+4 day at 38% (Pommes Golden delicious)	100 % apple protection.	Conway et al. [177]
P. expansum	*C. saitoana* + *Chitosan*	Additive effects compared with the two treatments used alone. No evidence of synergistic effect	de Capdeville et al. [65]
P. expansum B. cinerea	Trichosporon sp. and C. albidus + AC (3% O2 et 3% CO2) or (3% O2 et 8% CO2) at 1°C	Significant improvement in biocontrol against the two pathogens	Tian et al. [179]
P. expansum	C. sake CPA-1 + storage under AC (3% O2 et 3% CO2) "Golden delicious" apples	Reduction of the incidence of the disease by 97% (storage under AC against 40% (Storage in air).	Usal et al. [106]
P.expansum	*P. syringae* + 4 day at 38°C "Gala" apples	Reduction in the incidence of decay by 70% to 25% for biocontrolalone	Conway et al. [178]
biocontrol combined with a chemical treatment (inorganic salts and plant extracts)			
P. expansum	*Meyerozyma guilliermondii YS-1, Meyerozyma caribbica YS-3, C. albidus* YS-4 or *Cryptococcus sp*. YS-5 + CaCl2 (2%) on "Golden delicious" apple	Decays on yeast + CaCl2-treatement were substantially smaller (74-77%) and (49%-73%) lower than those on apples treated with pathogen alone after 1 and 2 weeks of incubation, respectively.	Tournas and Katsoudas [194]
B. cinerea, M. piriformis P. expansum	*Pseudomonas fluorescens* (Isolat 4-6) + CaCl2 or + BCS or + Salicylic acid (SA),	In vitro, antagonist + chemical additives has not improved its effectiveness against pathogens. On "Ambrosia" apples, *isolate 4-6* + BCS had efficacy against the three pathogens comparable to that of the fungicide Scholar®, after 15 weeks in cold storage at 1 ° C.	Wallace et al. [180]
P. expansum	*Sporidiobolus pararoseus* Y16 + glycine betaine (GB)	S. pararoseus amended with 1 mMGB reduces the diameter of blue rot lesions on apples, reduces spore germination and germ tube length of *P. expansum.* But affects the flavonoid content and the pH of apples.	Abdelhai et al. [185]
P. expansum	*Candida oleophila* combined with CaCl2	The combined treatment significantly improves biocontroland significantly induces the activities of chitinase and β-1,3-glucanase.	Cai et al. [181]

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P. expansum	*P. caribbica* treated with Glycine Betaine (GB)	GB decreased the incidence of the disease from 48.8% (untreated yeast) to 32.1% and improved growth and stress tolerance of the yeast	Zhang et al. [118]
P. expansum	*P. caribbica* + phytic acid (0,2% v/v)	Significant improvement in phytic acid control	Mahunu et al. [117]
P. expansum	*R.mucilaginosa* + phytic acid (4 µmol)	Reduction of the incidence of the disease from 86.1% to 62.5% (apples treated with phytic acid)	Yang et al. [129]
P. expansum	*P. caribbica* treated with ascorbic acid	Improvement of the yeast biocontrolactivity of its growth and its tolerance to oxidative stress	Li et al. [186]
P. expansum	*Rhodotorula mucilaginosa* (A1) at 20°C + CaCl2 at different concentrations	Reduction of the lesion area to (185.1 -1738.1) mm2, depending on the concentration of CaCl2, against 2452.84 mm2 (control)	Gholamnejad et al. [39]
P.expansum	*C. guilliermondii* and *P.membranefaciens* + calcium solution to 2% (*In vitro* and *In vivo*).	Decreased spore germination rate and cell growth of the pathogen ; reduction in the incidence and severity of the disease.	Gholamnejad and Ebabarien [182]
B. cinerea *P. expansum*	*C. laurentii* treated with glycochitosan.	100% reduction in the incidence of gray and blue rot (treated yeast) compared to 23% and 25%, respectively (untreated yeast).	Yu et al. [191]
P. expansum	*M. pulcherrima* or *C. laurentii* + (SBC)	The SBC improved the effectiveness of *M pulcherrima* but not *C. laurentii.*	Conway et al. [173]
P.expansum	*P. syringae+ Cyprodinil (Fungicide) at different concentrations.*	A combined traitment was more effective than either P syringae or cyprodinil alone	Errampalli and Brubacher [192]
P. expansum *B. cinerea*	*M. pulcherrima (BIO126)* + ethanol (20%) or SBC (5%)	Significant improvement in biocontrol against *P. expansum* on Golden delicious apples stored at 23°C.	Spadaro et al. [176]
P. expansum	*C. guillermondii* (3C-1b and F1) + CaCl2, (11 g l-1), ammonium molibdate (6.17 g l-1), Na2CO3, (20 g l-1) or 2-deoxy-D-glucose	Enhancement of biocontrol of *the two strains* efficacy by all GRAS substances on “Fujji” ans “Golden delicious” apples	Scherm et al. [71]
P. expansum	*P. syringae* (MA-4) + Cyprodinyl in different doses (from 2,5 to 20 µg/ml)	Increased repression of the disease to 35% to 91% compared to 11% with the MA-4 strain alone.	Zhou et al. [153]
P. expansum *B. cinerea*	*C. albicans + iprodione at 50 ppm a.i. on Fuji apples*	Better control with the addition of iprodione	Fan et Tian [132]
P. expansum	*P. syringae* + CaCl2 (2%).	Reduction in incidence to almost 89% compared to 25% with the antagonist alone.	Conway et al. [178]
biocontrol combined with several treatments of different nature.			
P. expansum	*M. pulcherrima* + *C. laurantii* + several combinations of SBC	Improved biocontrol on Golden delicious apples.	Janisiewicz et al. [171]
P. expansum	*M. pulcherrima* +*C. laurentii* +BCS + AC (1,4 kPa O2 et 3 kPa CO2 during 2 or 4 month at 1 ° C)	The combination of all of these treatments completely eliminated the rot of P. expansum on “Golden Delicious” apples.	Conway et al. [173]
P. expansum ; Colletotrichum acutatum	*M. pulcherrima* T5-A2 +1-méthylcyclopropene (1-MCP) Air heated to 38 ° C for 4 days + AC (1,1% O2, 1,8% CO2) “Golden Delicious” apples	1-MCP increases bitter and blue rot but the combination (Antagonist + hot air + AC) effectively controls the two rots on apples even on those treated with 1-MCP.	Janisiewicz et al. [195]
B. cinerea	*P. anomala* (K)(105 ufc/ml)+ 1,3-glucane (2g/l)+ CaCl2(20g/l)	Significant improvement in the percentage of protection of Golden delicious apples against *B. cinerea* (up to 100%)	Jijakli et al. [113]
P. expansum	*P. syringae +4 days à 38°C +CaCl2 at 2%*	91% reduction in decay compared to 25% with the antagonist alone	Conway et al. [178]

6. Discussion and Conclusion

Post-harvest rots are the main constraint that affects the profitability of the apple industry worldwide. The losses they cause are considerables and can reach up to 30% of the harvested fruit. Although several approaches have been proposed for the management of these diseases after harvest, chemical control applied before or post-harvest, remains the most used method. However, growing concerns about fungicide residues in fruits, the development of resistant strains among pathogens, as well as the environmental risks associated with their continued use, have spurred the search for safe and effective alternative strategies. Among these strategies, biological control based on the use of beneficial microorganisms or the metabolites they produce to control the development of diseases has been the most studied

During the last three decades, a considerable research effort has been devoted to the isolation and identification of bacteria, yeasts and other fungi which effectively control the main post-harvest pathogens fungi of apples. Currently, considerable information is available with respect to their efficacy, their application under storage conditions, their mixture with safe substances, or according to the formulation.

In all of the research studies included in our project that exceed 190 studies, microbial antagonists have often demonstrated their effectiveness against the main post-harvest rot agents in apples by expressing fungicidal or fungistatic activity against them. Under certain conditions, some of these antagonists have even enabled total inhibition of the growth of one or more pathogenic fungi.

The experience accumulated over all these years of research has therefore been crowned with great success. However, there are still some limitations that hinder the development of microbial biological control of postharvest fungal rot in apples.

(1) Most of the research conducted was carried out under laboratory conditions:

In most works, the researchers isolated strains from different sources, then examined their antagonistic power against generally a single pathogen of apple rot in conservation, rarely against several. Note here that the species *P. expansum* and *B. cinerea* were the most targeted by researchers, certainly because of the enormous damage they cause worldwide to apples in conservation.

The study of the antagonistic power of the isolated strains is done first by in vitro tests where the isolates are confronted with the pathogen in Petri dishes containing a culture medium. Following these tests, the most efficient isolates are selected (first screening). In others, these potential antagonists are also tested in vivo on artificially injured apples. It is important to note here that little research has studied the potential effect of the type of injury (size, depth, etc.) on the biological control power of the antagonist agent. In addition, these in vivo tests rarely targeted multiple varieties of apples at the same time.

However, many efforts are made to determine the optimal dose and the appropriate time to apply the biocontrol agent and also to study the action of certain external factors such as temperature and humidity, on the effectiveness of the biocontrol. Most of the published research has not gone beyond this stage. The screening of strains via in vitro and in vivo tests is certainly an essential step in the development of antagonists, but this type of tests is, in fact, only the first link in a very long chain leading to the development of biopesticides in appropriate formulations, safe for human health and the environment, and can be marketed.

(2) The effectiveness of control agents (PHFRA) is not very high:

In all the studies carried out on the biological control of post-harvest diseases of apple, the efficiency rarely reaches 100% and the efficacy of the same biocontrol agent is not stable and often varies from one test to another, certainly due to various biotic (host species, pathogenic species) and abiotic (temperature, humidity, relative humidity) factors.

The discovery and development of these biocontrol agents have been based on the paradigm of isolating a single antagonist that is effective against several different postharvest pathogens and was expected to be effective on different commodities that vary in their genetic background, physiology, pathogen susceptibility, and pre- and postharvest management practices [175]. This paradigm has resulted in several limitations, including inconsistent efficacy and a narrow range of biocontrol activity on specific hosts or pathogens These shortcomings constitutes one of the serious limitations which hinders their commercial success [31,35].

Several approaches have been suggested for improving the biocontrol efficacy of postharvest biocontrol agents. Attempts to enhance efficacy have included the use of mixture of different fungal antagonists in combination. Many researchers have reported that such mixtures has been shown to be more effective in controlling the post-harvest rot of apple and many other fruits, than any antagonist applied alone [170-173]. One approach to enhance the biocontrol efficacy that provides a new outlook to postharvest biocontrol is to broaden the spectrum of action of biological control agents by utilizing compatible microbial consortia instead of single antagonists which

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could comprise natural or synthetic mixtures of interacting microbial populations that thrive in many diverse environmental niches [175].

Attempts to enhance efficacy have included also the combinations of biological control with a variety of other alternative treatments, such as combining a biological control agents treatment with physical means (heat, hot water brushing), natural and food-grade chemicals, and different packaging techniques [196-198]. Those alternative treatments result in an additive or even synergistic effect to improve the control of fruit decomposition [196]. An appropriate combination of alternative control measures can provide long-term commercially acceptable control of post-harvest disease in apples and may help reduce dependence on fungicides [173]. Some of them have direct effects on pathogens, while others can act indirectly by increasing the resistance of fruits to pathogens or by delaying their senescence [196]. The results of scientific researches cited in our study (Table 4), showed that combined treatments improve the effectiveness of antagonists against the main post-harvest agents of apples, which is often comparable to that of conventional fungicide treatments.

Other attempt to enhance efficacy of bio-control agents was to extend their activity under pre-harvest conditions [199]. Researchers propose, to improve the effectiveness of post-harvest biocontrol agents for apples, to start treatment at the pre-harvest stage to guarantee colonization of wounds by the biocontrol agent before infection by the pathogen [104,113]. Such pre-harvest application would have numerous benefits, such as decreasing the level of damages, which can occur during the post-harvest treatment. However, the development of a formulations allowing the use of antagonistic microorganisms, both before and after harvesting, is an area which has been less widely explored.

(3) There is little knowledge about the durability of a control methods for apple protection:

The prolonged and massive use of antagonists in particular, those which have a mode of action by antibiosis, can lead to the development of resistance in phytopathogenic fungi to natural fungitoxic molecules [200]. However, there are few studies on the long-term effect of biological control agents of (PHFRA). The persistence of the efficacy of a control method in space and time is an important factor in the success of biocontrol It is therefore necessary to develop knowledge concerning the possible erosion of this effectiveness, which will result in identifying types of biological control agents with lower risk of efficacy loss, i.e., modes of action of biological control agents that does not favor the selection of resistant isolates in natural populations of plant pathogens [201].

(4) The modes of action of biological control agents of (PHFRA) are not yet well elucidated:

In a large number of studies carried out in the field of biocontrol of (PHFRA), mechanism involved in the antagonistic action of the selected strains against the pathogens tested is not identified, and when it is, each mechanism is generally examined separately. However, as already mentioned in paragraph 3, it is rare that only one mechanism is involved in the suppression of a disease. Biological control agents have, in fact, a very high specificity vis-à-vis the target disease, which must certainly require the combination of several agents for the biological control of post-harvest diseases of the apple.

In addition, the laboratory tests, which are the only tests used in the majority of studies carried out on the biocontrol of (PHFRA), lead to the selection of certain modes of action only, such as antibiotics or direct parasitism. Strains with other modes of action may not be selected. Laboratory tests, in fact, do not allow the expression of all the mechanisms that an agent can involve in its pathogenic action.

Understanding the mode of action of microbial control agents is essential to achieve optimum disease control. Also understanding the mode of action is important to be able to characterize possible risks for humans or the environment and risks for resistance development against the antagoniste [202].

Although many studies have been conducted on the mode of action of post-harvest microbial antagonists, our understanding is still very incomplete, and further investigations are still required.

Advanced microbiological, microscopic, biochemical and molecular techniques are currently available and can be used effectively to improve our knowledge of the mechanisms of action of microbial antagonists [11].

(5) Biocontrol research of (PHFRA) does not take into account all the interactions that the biological control agent can have:

Most of the research that has been done in bio-control of (PHFRA) does not support all of the interactions that the biocontrol agent can have, namely the interaction with host tissue, with the pathogen, the Microbial communities on plant surfaces and the environment, which are essential for developing the biological control system. Until now, scientific approaches have focused on the different components of these interactions but separately.

Special attention has been paid in recent years to the importance of the microbial communities (microbiome) present in and on plant tissues and which plays an essential role in the health and physiology of fruit after it is harvested [203].

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Spadaro and Droby, mentioned in their literature review [27] the importance of epiphytic microflora in impacting disease control through their interactions with host plants, pathogens, and biological control agent, in a quadritrophic interaction system, and recommended to take into consideration all the components of this quadrithrophic relationship when studying mechanisms of action in posthavest biocontrol. Droby and Wisniewski, [203] proposed using plant improvement or genetic modification of plants to intentionally modulate the composition of the microbiome and its function, by recruiting disease antagonists and plant growth promoters which improve the plant health and the quality of harvested products, and thus try to develop natural or synthetic consortia that can be used to prevent post-harvest diseases and reduce physiological disorders in harvested products.

To conclude, the biological control against post-harvest fungal diseases of apples is very promising, the results obtained so far are very encouraging. Compared to the use of fungicides causing ecological and health hazards in food chain, use of bio-control agents will be useful not only in minimizing the loss to farmers but also in reducing the fungicidal residue in apple fruits used for human consumption. The application of new technologies such as meta-omic technologies will open up new research opportunities which will certainly improve the understanding of post-harvest biological control and will make it possible to overcome the weaknesses which still hamper the development of biological control of (PHFRA).

But whatever progress may be made, biological control of (PHFRA) can only be important in the context of a modern integrated pest management strategy where it is essential to reconcile and coordinate the use of biological control agents with other means of control, including chemical control.

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