PRESENT SITUATION AND FUTURE PERSPECTIVES OF BIOFERTILIZER FOR ENVIRONMENTALLY-FRIENDLY AGRICULTURE

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ABSTRACT

Global warming and climate change have resulted in unexpected drought, stormy rainfalls, extremely high temperature, cold damage and hurricanes in many places around the world where such disastrous tragedy had never occurred in the past decades. Establishing an environmental friendly co-existing mechanism on earth is of vital importance. In recent years, agrochemicals are extensively applied to obtain higher yield. Intensive application of agrochemicals leads to several agricultural problems and poor cropping systems. Farmers use more chemical fertilizers than the recommended levels for many crops. Excessive use of chemical nitrogen fertilizer not only accelerates soil acidification but also risks contaminating groundwater and the atmosphere. Biofertilizers and organic fertilizers offer a safer option for reducing agrochemical inputs. They are being developed in several laboratories in Taiwan over the years. Microorganisms including rhizobium, phosphate-solubilizing bacteria and arbuscular-mycorrhiza (AM) fungi have been isolated from various ecosystems, and their performances in laboratory and field are continuously assessed. Experiments in greenhouses and in the fields revealed that many crops responded positively to microbial inoculations which not only improved the growth and quality of crops but also significantly reduced (1/3~1/2) the usage of chemical or organic fertilizers. Biofertilizers gained higher popularity in an increasingly eco-conscious world, since they are environmental friendly and can generally be used to promote healthy crops. In order to improve and maintain the productivity of agricultural lands, an integrated approach to screen out more favorable biofertilizers is urgently.

Key words: Biofertilizer, arbuscular-mycorrhizal fungi, phosphate solubilizing bacteria, rhizobium, environmentally-friendly

INTRODUCTION

Global warming and climate change have resulted in unexpected drought, stormy rainfalls, extremely high temperature, cold damage, hurricanes and tornadoes in many places around the world where such disastrous tragedy had never occurred in the past decades. Heavy casualties and agricultural losses in these areas therefore significantly affected the lives and security of residents and consequently, the regional economy. Establishing an environmental friendly co-existing mechanism on earth is of vital importance. For agricultural practices, effective strategy must be conducted to reach the goal of a friendly environment.

In recent years, chemical pesticides and fertilizers were extensively applied to maintain high crop yield. Overusing agrochemicals led to several agricultural problems and poor cropping systems. The excessive application of chemical nitrogen fertilizer not only accelerated soil acidification but also risked contaminating groundwater and the atmosphere, and weakened the roots of plants that made them easy prey on unwanted diseases.
Biofertilizers are microorganisms that assist plants to grow by increasing the quantity of nutrients. The living microorganisms that co-exist with the plants will promote the supply of important nutrients and, consequently are crucial for the overall productivity of the soil. Using biofertilizers offers a better option in reducing agrochemical inputs, and helps maintain soil fertility and strength.

An increasing number of farmers and agriculturists are choosing biofertilizers over their chemical counterparts as they are found to be gentler on the soil. The value of biofertilizers has further increased in an increasingly eco-conscious world. In addition, soil quality is also improved through the uptake of these environmental friendly fertilizers. Biofertilizers also contributes in reducing the negative impact of global warming.

**MICORRHIZAL FUNGI**

Mycorrhizas are highly evolved, mutualistic associations between soil fungi and plant roots. The host plant receives mineral nutrients from mycorrhizal fungi, while the fungus is given photosynthetic products from the host plant (Jakobsen 2002). Arbuscular mycorrhizal fungi (AMF) are the most common type of mycorrhizae, which can be found in approximately 80% of the plants on earth (Hildebrandt et al., 2002). The external mycelia of AMF, extending several centimeters from the roots, plays an important role in the water uptake of plants, particularly in arid and semi-arid habitats. It is also well established that AMF can improve phosphate intake for the plants when phosphate availability is limited; and hence, beneficial to plant growth. AMF inoculation can not only improve the tolerance of water stress and pathogens (Kothari et al. 1990), but it also helps in the survival percentage of transplants, and overall growth of plants. These qualities can be attributed to the higher rate of photosynthesis, or/ and accumulation of carbohydrates and proteins. Auge (1987) demonstrated that chlorophyll and leaf starch levels were higher in AMF inoculated rose under water stress than the control. Mycorrhizae are thus considered an important microbial symbiont that can benefit plant growth.

In my laboratory, we have been evaluating effects of AMF inoculation on growth, yields, and disease tolerance of papaya (Carica papaya L.), onion (Allium cepa L.), adzuki bean (Phaseolus angularis Wight), bitter gourd (Momordica charantia L.), cucumber (Cucumis Sativus L.), scarlet sage (Salvia splendens Ker-Gawl.), petunia (Petunia x hybrida Hort, ex Vilm), Calla lily (Zantedeschia aethiopica), and tropical fruit trees such as atemoya (Annona atemoya), Guava (Psidium guajava L.), Indian jujube (Zizyphus mauritiana Lam) and coffee (Coffee Arabica L.) etc. The results showed that these crops were colonized by AMF and their growth and yield were enhanced. For example, the neck diameter, fresh weight, and leaf number of onion seedlings were significantly improved by AMF inoculation. The plant height, stem diameter, leaf number, shoot fresh weight, root fresh weight and leaf area of the inoculated papaya seedlings were greater than those of the non-inoculated samples. After transplantation, the stand establishment percentage of papaya seedlings increased from 84% to 92%, and the flower number per plant raised up from 15% to 20% as well. Inoculation of *Glomus* spp. not only shortened the time required for flowering, but also increased fruit production. Calla lily inoculated with *Glomus* spp. showed better bulb quality and yields, while reducing the time for bulb production and improving disease tolerance against *Rhizoctonia solani* and *Erwinia carotovora* subsp. carotovora. In addition, we examined in the green house whether *Glomus* spp. inoculated in Adzuki bean seedlings would increase drought tolerance. The results showed that the plant dry mass and pod dry mass in well-watered mycorrhizal inoculated plants were significantly higher than those of non-mycorrhizal and well-watered counterpart plants, while even the plant dry mass and pod dry mass in drought stressed mycorrhizal inoculated samples were significantly higher than those of non- mycorrhizal ones. From the measurements of water absorption, net photosynthetic rate, chlorophyll content, and leaf water potential etc., we learned that the beneficial effect of mycorrhizal infection on plant growth and yield was attributed to better water absorption, higher net photosynthetic capacity, higher leaf water potential restored capacity, and higher chlorophyll content in plants infected by AMF (Wang 1989,1998).

**PRODUCTION OF ARBUSCULAR MYCORRHIZAL FUNGI INOCULUM BY AEROPONIC CULTURE**

Aeroponic culture of AMF is a biotechnology that allows both efficient production of AM inoculum and soil-free investigation of mycorrhizae. It is a much higher aerated environment than traditional hydroponics and has proven to be an efficient system for growing VAM inoculum without a physical substrate (Hung and Sylvia 1988). Furthermore,
the lack of a physical substrate in aeroponic culture makes it an ideal system for studying the biology of these obligate biotrophs.

The production of AMF inocula via aeroponic culture enhances the multiplication of spores, hyphae, and roots. In addition, the roots may be sheared to produce extra inocula, which are easy to handle and they are also of high propagule density (Sylvia and Jarstfer 1992). Periodic changes in nutrient solution may serve to reduce the population of deleterious organisms and the concentration of toxins that may occur in the nutrient solution. Periodic root pruning reduces interplant root contact and should aid in sanitation. A variety of pump designs were used to produce a spray or mist from misting or micro-nozzles.

Several significant benefits may be obtained by using aeroponics to produce inocula of AMF. The soilless culture technique avoids soil-associated plant pests. Absence of soil mineral matter allows roots to be sheared to higher propagule densities and thus, provide more effective inoculations. Small-scale on-site inoculum production using aeroponic chambers could allow optimal timing of inoculations and higher propagule densities from fresh inocula. Small batches of selected isolates of VAM fungi should result in fewer loss and better growth when colonized plants are transplanted into nutrient-poor and water-stressed environments.

**RHIZOBIAL INOCULANTS**

Nitrogen is one of the major plant nutrients, which are referred to as the master key elements in crop production. It can be made available through chemical or biological processes, though chemical nitrogen fertilizers are relatively expensive (Zilli et al., 2004). Symbiotic nitrogen fixer and phosphate solubilizing microorganisms play an important role in supplementing nitrogen and phosphorus to the plant, allowing a sustainable use of nitrogen and phosphate fertilizers. The use of these microbes as fertilizers in the field has been reported as beneficial to crop yield. This is especially important in the developing countries where farming will continue to be in the hands of small farmers (Rao, 1999).

Study on the selection of efficient rhizobial strains for inoculation in Taiwan started in 1958. Collection, isolation and subsequent selection of effective rhizobial strains have yielded fruitful results in agriculture. Nevertheless, marked variations were observed among rhizobial strains (Young and Chao 1983). Wu (1958) selected a number of pure rhizobial strains from lupin, alfalfa, peanut, crotalaria and soybean, and conducted wide range of field experiments to screen the effective inoculants. Yield was significantly increased when lupin, alfalfa, peanut and soybean were inoculated with selected rhizobial strains compared to those from non-inoculated plants.

Fast and slow-growing soybean rhizobial strains were isolated and selected from Taiwan soils for inoculation (Young et al. 1982; Young and Chao 1983) in 1980s. Several effective isolates were deposited in the Culture Collection and Research Center (CCRC) of the Food Industry Research and Development Institute in Taiwan (CCRC 1991). A few field experiments were conducted to determine the effects of single and mixed inoculations with rhizobium and Arbuscular-Mycorrhiza (AM) in six different tropical soils in Taiwan (Young et al. 1988b). The results indicated that inoculation with rhizobial strains alone increased N2 fixation and soybean yield in three out of six fields. Inoculations with rhizobial strain singly, or in combination with AM, without any N2 fertilizer applications, significantly increased soybean yield from 5% to 134% in the field experiments. The results from other experimental sites also showed that a mixed inoculum of rhizobium and AM can be an efficient biological fertilizer that maximizes soybean yields. The combined effect of the mixed inoculum was a striking achievement in the field of bio-fertilization. AM might have provided the essential phosphorus for the growth of soybean plants.

**PHOSPHATE SOLUBILIZING BACTERIA**

Phosphorus is also one of the master key elements in crop production. However, a great part of soil phosphorus (approximately 95-99%) is in an insoluble form; dominant in alkaline soil and unable to be utilized by the plants (Lee, 2005). Phosphate solubilizing microorganisms are capable of solubilizing calcium, aluminium and iron phosphates as well as rock phosphates and mineralizing organic phosphorus, making the phosphorus in the soil available to the crops (Tambekar, 1998). The bound phosphate made itself available for soil microorganisms like bacteria and fungi (Jisha and Ashok, 2006). To ensure the availability of phosphorus for plants, large amounts of fertilizers are usually used on a regular basis. Unfortunately, a large portion of fertilizer phosphorus was quickly transferred to the insoluble state. After applications, only very little (20-25%) of the applied amount are useful (Goldstein, 1986).
Phosphate-solubilizing bacteria (PSBs) were isolated from various tropical soils in Taiwan. Aliquots of soil diluted in sterile water (1:10 soil/water) were plated on calcium phosphate medium (modified from Subba Rao, 1982) for the isolation of P-solubilizing bacteria. The basic research on P-solubilizing biofertilizers was successfully established during 1990s in Taiwan (Young, 1990; Chang and Young, 1992; Young et al., 1998a, b; Young and Chen, 1999. Chang and Young, 1999, Young et al., 2000a & b; Liu and Young, 2001; Young, et al., 2003). Crop plants such as peanuts, various horticultural plants and vegetables were successfully inoculated with P-solubilizing biofertilizers to obtain higher yields. Several field experiments concluded that P-solubilizing biofertilizers not only improved the growth and quality of crops, but also significantly reduced (1/3~1/2) the usage of chemical or organic fertilizers.

CONCLUSION

To improve and maintain the productivity of agricultural lands, the integrated approach to determine the most favorable plant-microorganism interaction is vital. The current trend of low input chemicals in sustainable agricultural systems will contribute to the goal. We have successfully shown the use of AM fungal inoculum in the field for many crops. Current inoculum development technologies are capable of producing sufficient quantities of effective inoculum with applausible shelf life for economical use in many horticultural systems (Safir 1994).

Council of Agriculture (COA) in Taiwan (or any organization) held various seminars as well as workshops on the application of biofertilizers, so that farmers would have the opportunity to understand the effects of biofertilizers and are willing to use them. Farmers were invited to inspect the growth of AMF, Rhizobial or PSB inoculated crops in the fields and were encouraged to participate in workshops after viewing the successful outcomes of using biofertilizers. Consequently, the total land area which was found to be applied with the biofertilizers has reached 12,000 hectares yearly. The application of inorganic chemical fertilizers was thus significantly reduced to 30-50%. This helps in the realization of environmental friendly and sustainable agriculture.

RECOMMENDATION

Over the past decades, biofertilizer inoculants have been produced by the Agricultural Research Institute of COA, National Chung-Hsing University, National Pingtung University of Science and Technology and some other agricultural biotechnological companies in Taiwan. Several issues need to be taken care of when producing bio-fertilizers:

1. Application methods of biofertilizer
   Very convenient method
   Well mixing with or spraying on seeds, roots, or seedlings

2. How to produce a marked effect of biofertilizer
   (1) Quality requirement for the biofertilizer
   (2) Mix with organic liquid fertilizer (such as humic acid, amino acids, see weed concentrates etc.)
   (3)Fitness with right crops

3. Safekeeping and focusing on biofertilizers
   (1)Keeping in cool places
   (2) Avoiding light, heat, or high temperature
   (3) Avoiding the mix with pesticide

4. Quality requirement for biofertlizers
   (1) High population in the content
   (2) High activity of microorganisms
   (3) High adaptation for any crop
   (4) Low impurities and contamination

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