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MYCORRHIZA ENRICHED BIOFERTILIZERS FOR ENHANCING PLANT GROWTH

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Biofertilizers and mycorrhizae are very important to any revegetation effort, as they help to rebuild the living soil that can get damaged by any earthwork. Most desirable species will have a very difficult time out competing weeds without mycorrhizae, or the slowly released nutrients provided by biofertilizers.

Mycorrhizal Fungi

Mycorrhizal fungi form a bridge between the roots and the soil, gathering nutrients from the soil and giving them to the roots. There are two major types of mycorrhizae: Ectomycorrhizal Fungi (EM) and Endomycorrhizal Fungi (AM). While both types penetrate the plant roots, ectomycorrhizae spread their hyphae between root cells, while endomycorrhizae hyphae penetrate root cells. Ectomycorrhizae hosts include members of the Pine, Oak and Beech families, as well as few others in scattered families. Endomycorrhizae are the most common, and are found in grasses, shrubs, some trees, and many other plants.

EM fungi are usually specific to a certain host species, but most species of endomycorrhizae will form relationships with almost any AM host plant, and is therefore much easier to specify. There are four major plant families that usually do not form mycorrhizae: Amaranthaceae (Pigweed family), Brassicaceae (Mustard family), Chenopodiaceae (Goosefoot family) and Zygophyllaceae (Caltrop family). These plant families are well known as weeds. Therefore, if you do not ensure an adequate supply of mycorrhizae, you may inadvertently inhibit growth of desirable species and allow for rapid growth of undesirable species.

Relationships between Biofertilizers and Mycorrhizal Fungi

Plant roots secrete "food" for bacteria and fungi, which attracts nematodes (worms) to the roots, because nematodes eat bacteria and fungi, and excrete Nitrogen, Sulphur and Phosphorus in a form that the plants can use. The nematodes only keep 1/6 of the nitrogen that they process – 5/6 is excreted to the plant. Once the nematodes have excreted the nutrients, the hyphae of the mycorrhizal fungi pick them up and transfer them into the plant. Because of this symbiotic relationship, the least-leachable form of Nitrogen you can apply is bacteria and fungi, and bacteria are the most Nitrogen-rich organisms on earth.

AM hyphae pick up more nutrients than just those excreted by nematodes, however. One of the most beneficial properties of AM mycorrhizae is its ability to "mine" the soil great distances from the roots for nutrients, especially those, such as phosphorus, that are poorly mobile in the soil. AM Mycorrhizae also assist in picking up water further away from the roots, and block pest access to roots. Mycorrhizae also benefit plants indirectly by enhancing the structure of the soil. AM hyphae excrete gluey, sugar-based compounds called Glomalin, which helps to bind soil particles, and make stable soil aggregates. This gives the soil structure, and improves air and water infiltration, as well as enhancing carbon and nutrient storage.

Most natural, undisturbed soils have an adequate supply of mycorrhizae for plant benefits; however, the following practices can reduce mycorrhizae populations to inadequate levels.

Effects of Biofertilizers containing Arbuscular Mycorrhiza fungi on plant growth

The majority of plants growing under natural conditions are associated with mycorrhizae. Mycorrhizal colonization of roots results in an increase in root surface area for nutrient acquisition. The extrametrical fungal hyphae can extend several centimetres into the soil and absorb large amounts of nutrients for the host root (Khan et al., 2000). There is well-documented evidence that Arbuscular Mycorrhizal Fungi (AMF) contribute to increasing availability and uptake of P and micronutrients. The mycorrhizal symbiosis, by linking the biotic and geochemical portions of the ecosystem, can also be regarded as a bridge connecting the root with the surrounding soil microhabitats.

The utilization of microbial products has several advantages over conventional chemicals for agricultural purposes: (i) microbial products are considered safer than many of the chemicals now in use; (ii) neither toxic substances nor microbes themselves will be accumulated in the food chain; (iii) self-replication of microbes circumvents the need for repeated application; (iv) target organisms seldom develop resistance as is the case when chemical agents are used to eliminate the pests harmful to plant growth; and (v) properly developed biocontrol agents are not considered harmful to ecological processes or the environment. Biofertilizers are products containing living cells of different types of microorganisms, which have an ability to convert nutritionally important elements from unavailable to available form through biological processes (Vessey, 2003).

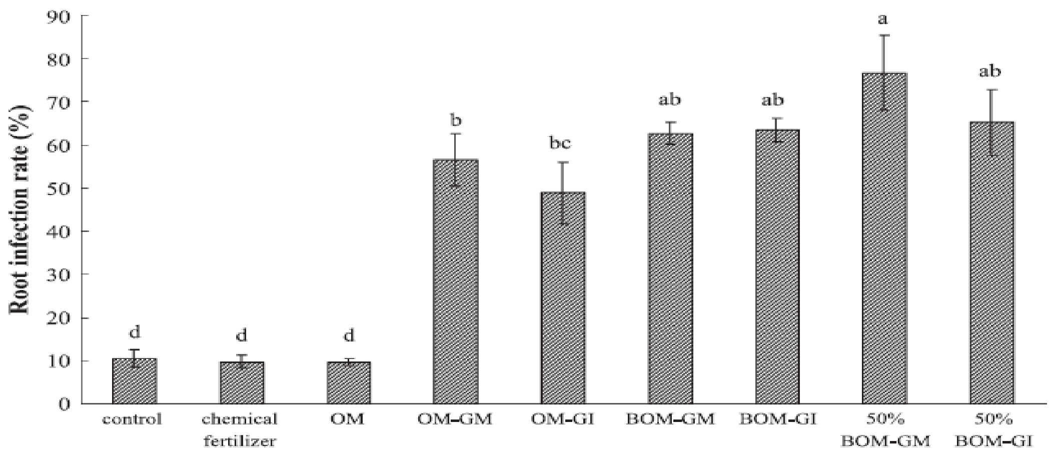
In recent years, biofertilizers have emerged as an important component of the integrated nutrient supply system and hold a great promise to improve crop yields through environmentally better nutrient supplies. However, the application of microbial fertilizers in practice, somehow, has not achieved constant effects. The mechanisms and interactions among these microbes still are not well understood, especially in real applications. Wu et al. (2005) conducted an experiment to see the effect of mycorrhiza fungi on the growth of maize and they found that the mycorrhizal inoculum significantly increased the extent of AMF colonization of the root system compared to the uninoculated control treatments.

The inoculation with beneficial bacteria and low fertilization level increased root colonization by both G. mosseae and G. intraradices. It has already been noted that the rhizobacteria can act as dmycorrhization helper bacteria T, which improve the ability of mycorrhizal fungi to colonize plant roots. The mechanisms by which these bacteria stimulate AM colonization are still poorly understood. Specialized bacterial activities such as the production of vitamins, amino acids, and hormones may be involved in these interactions. The presence of rhizobacterial inoculation might have assisted in the germination of a large number of spores thus leading to a higher infection percentage. Some PGPR endophytic species are known to have cellulose and pectinase (Verma et al., 2001) and these activities could not doubt aid in mycorrhizal infection.

The present results demonstrated that the population size of the inoculated rhizobacteria varied in accordance with the levels of fertilization and AMF colonization in the rhizosphere (Fig. 1). The low level of fertilization (50% BOM+GM and 50% BOM+GI) resulted in a higher level of mycorrhizal root infection and a larger community of A. chroococcum in the rhizosphere, compared to the treatments with a high level of fertilization

(BOM+GM and BOM+GI). According to our preliminary results (in preparation the propagation of A. chroococcum was seriously inhibited when the Ammonium N concentration exceeded 200 mg kg-1. However, a sharp decline of the number of P and K solubilizers (with a decrease rate of 42.3% and 25.5%, respectively) was observed with the increase of G. mosseae colonization level.

It implies that maize is likely more dependent on the symbiosis with G. mosseae than P solubilizers under the condition of insufficient nutrient supply, e.g. when P deficiency occurs. Toro et al. (1997) reported a drop of density in the introduced P-solubilizing bacteria from 106 to 103 cfu g-1 soil in the rhizosphere with the increase of root mycorrhizal colonization. By contrast, the colonization with G. intraradices showed a strong stimulating



effect on the propagation of these two solubilizers. Mycorrhizal colonization with G. mosseae allowed the introduced populations of beneficial soil microorganisms like Azotobacter, Azospirillum and

Figure 1: Plant root infection rate by arbuscular mycorrhiza fungi in different treatments

phosphate-solubilizing bacteria to maintain a higher abundance than non-mycorrhizal plants and thereby exerted a synergistic effect on plant growth.

In practice, good indigenous species are preferable to be selected as inocula in order to ensure a successful colonization of mycorrhizae since the possible competition may occur between introduced and autochthonous populations in unsterilized field soil conditions.

Enhancement of alkalinity tolerance in two cucumber genotypes inoculated with an arbuscular mycorrhizal biofertilizer containing Glomus intraradices

Yossef et al. (2010) conducted an experiment and suggested that to avoid or reduce losses in production caused by alkalinity in high yielding genotypes would be to inoculate them with arbuscular mycorrhizal fungi capable of reducing the detrimental effect of external pH on crop performance. Arbuscular mycorrhizal fungi are the most widespread root fungal symbionts and are associated with the vast majority of higher plants (Sensoy et al., 2007). Arbuscular mycorrhizal fungi have been shown to improve soil structure, enhance plant nutrient acquisition (P, N, Zn, Cu, and Fe), overcome the detrimental effect of salinity, improve drought tolerance, suppresses root knot nematode (Zhang et al., 2008), and alleviate cultural and environmental stresses through greater effective root area and penetration of substrate (s) and activation and excretion of various enzymes by infected arbuscular mycorrhizal fungi roots and/or hyphae.

However, the benefits of arbuscular mycorrhizal fungi inoculation depend on the specific host–fungus combinations and also on the types of the inoculums used. Enhancing the tolerance of plants to alkalinity when inoculated with AM fungi has been studied and proposed until now for ornamental crop production such as vinca and roses, whereas no published data is available on the effect of arbuscular mycorrhizal fungi inoculation under high pH conditions on the agronomical and physiological responses of an important vegetable crop widely grown in the Mediterranean region, such as cucumber. Differences in mycorrhizal responsiveness between different crops and between different genotypes within the same crop have also been demonstrated. In wheat, growth of modern cultivars tended to benefit less from mycorrhizal inoculation than older cultivars. Similarly, improved soybean cultivars showed a lower growth response to colonization than older cultivars.

No consistent trend has been observed among corn cultivars. Cultivated tomatoes and oats, however, are more responsive to mycorrhizal colonization than closely related wild species. We hypothesize that arbuscular mycorrhizal inoculation with a biofertilizer containing Glomus intraradices would give an advantage to overcome alkalinity problems. To verify this hypothesis, two cucumber genotypes (hybrid or open-pollinated variety) were inoculated and their yield, growth, fruit quality, net photosynthesis, electrolyte leakage, and mineral composition were compared with those of noninoculated plants when both were grown in sand culture at two pH values in the nutrient solution (6.0 or 8.1). Under alkaline conditions, yield and biomass production were higher in inoculated than noninoculated plants.

Alkalinity tolerance of cucumber plants inoculated with G. intraradices may be due to the better uptake and translocation of mineral elements in particular P, Mg, Fe, Zn, and Mn to the shoot. Arbuscular mycorrhizae can mitigate growth reduction by alkalinity (Cartmill et al., 2007) but the mechanism involved remains unresolved. Cartmill et al. (2007) concluded that the alkaline tolerance of Rosa multiflora is primarily related to the enhanced nutrient uptake (N, P, K, Ca, Fe, Zn, Al, and B), leaf chlorophyll concentration, low Fe reductase activity, and low soluble alkaline phosphatase activity. Similarly, Cartmill et al. (2008) stated that the tolerance of vinca plants inoculated with a mix (ZAC-19) of Glomus species isolate (Glomus albidum, Glomus claroideum, and Glomus diaphanum) to alkalinity was associated with increased P uptake due to a higher soluble phosphatase activity at moderate HCO_3^- concentrations, with the maintenance of other leaf nutrients when HCO_3^- increased up to 10 mM, and with an increase of antioxidant activity, and thus detoxifying activity, probably as a result of their enhanced micronutrient status.

In many plant species, the high alkalinity induces Fe deficiency due to reduced Fe availability in soil or uptake. Bicarbonate can also precipitate Fe internally making this nutrient less available in roots (Römheld 2000). We have shown that the increase in the concentration of $NaHCO_3$ from 0 to 10 mM in the nutrient solution significantly decreased the leaf Fe concentration by 39%, but inoculated plants accumulated on average 14% more Fe than noninoculated plants. This suggests that arbuscular mycorrhizal inoculation with a biofertilizer containing G. intraradices enhance the uptake and translocation of Fe toward the shoot. The higher uptake and accumulation of Fe in inoculated than noninoculated cucumber plants was the main mechanism reducing the detrimental effect of alkalinity (Fe deficiency) on yield and plant growth. Iron deficiency can cause various physiological changes in leaves, as iron is an important cofactor of many enzymes, and interferes with several aspects of plant biochemistry, including photosynthesis and pigment synthesis.

Influence of inoculation with arbuscular mycorrhizal fungi on the growth and mycorrhizal infection of transplanted onion

There is increasing interest in using arbuscular mycorrhizal (AM) fungi in agricultural and horticultural crop production. Recently, the possible use of mycorrhizal inoculation in sustainable agriculture was discussed. The benefits of symbiosis are particularly enhanced uptake of immobile nutrients, improvement of drought tolerance and disease resistance. Some studies revealed a stimulatory effect of mycorrhizal fungi on the growth and nutrient uptake of onion in pot and field experiments. The growth of plants may be considerably enhanced by inoculation with suitable AM fungi, particularly if levels of available soil phosphorus are low (Powel, 1984). In commercial practice, however, the crop is often over-fertilised, because the cost of P fertiliser is trivial relative to the large profit margin from this crop.

Nevertheless, as was shown by Nelsen and Safir (1982) the vegetative growth of onion mycorrhizal seedlings was less affected by drought than that of nonmycorrhizal seedlings. This effect is of potential importance in the field because growth of onions could be markedly reduced by lack of water. The number of field studies using the mycorrhizal inoculation in non-sterilised soils is limited. Where field soils are fumigated and most of the indigenous mycorrhizal fungi are killed, mycorrhizal inoculation is often successful. However, the inoculation in non-sterilised soils could also be beneficial because of the addition of a more efficient strain of the fungus or to more rapid infection rates. The transplants can be inoculated with pure strains of highly effective AM fungi, giving the introduced strains a competitive advantage over strains of indigenous fungal population in the field soils. Inoculation of plants seedlings before transplanting may increase crop uniformity, reduce transplant mortality and improve growth.

Miroslav Voshtka (1995) conducted five field experiments and suggested that the stimulatory effect of mycorrhizal inoculation was found in almost all experimental treatments. This effect is more apparent in previously sterilised soil where competition with native indigenous endophytes is reduced or eliminated. The best way to eliminate the indigenous population of fungi seems to be by steam sterilisation rather than by fumigation with Basamid. Nevertheless, when experiments 1, 2 and 3 are compared (although they were conducted in different years) it seems that steam sterilisation could probably inhibit later mycorrhizal infection of later transplanted inoculated seedlings. Comparing the infection of control and Glomus intraradices inoculated plants from all three experiments higher values were found in non-sterilised than fumigated soil and the lowest in the steam sterilised soil. These differences might be caused by some climatic variability between years.

The experiments were conducted on the same field and also the growth of plants was comparable, except in the third experiment when the plants grew a little better. Therefore, the degree of sterilisation was probably the prevailing factor affecting the performance of indigenous and introduced fungal symbionts of transplanted plants. The findings of Snellgrove and Stribley (1986) about growth inhibition or substantial mycorrhiza reduction or elimination from Basamid fumigation were not found. The extent of positive growth response in all experiments is in agreement with results reported by others. Furlan and Bernier-Cardou (1989) found a 41% increase following inoculation by Gigasporacalospora. Snellgrove and Stribley (1986) reported bulb yield increases of up to two times after inoculation by Glomus mosseae and Powel (1981) observed a 92% increase of onion shoot biomass as a result of mycorrhizal inoculation.

The most effective symbionts over all experiments were Glomus intraradices and Glomus etunicatum. The latter has been used previously in pot trials with onion, but did not increase the growth of plants. The results correspond with findings of Snellgrove and Stribley (1986) who found a positive effect of pre-inoculation on the production of bulbs with diameter higher than 20 mm. Negative correlations between higher soil-available phosphorus content and mycorrhizal growth response has been reported. In the fifth experiment, fertilisation increased soil P content and irrigation most probably enhanced P availability because of better diffusion in moist soil.

Both these treatments decreased the positive mycorrhizal growth response. This decrease was more apparent for shoots than bulb yield. Mycorrhizal plants might allocate more biomass to the bulbs where the nutrient requirement is probably higher. These results are similar to those reported for pot-grown onions but are in contrast to the findings of Snellgrove and Stribley (1986) who found a positive correlation between irrigation and mycorrhizal growth response. When the plants are not fertilised and irrigated, the soil nutrient diffusion is low and some nutrients, particularly phosphorus, become the limiting growth factor.

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