STUDY ON MYCORRHIZAL STATUS OF CITRUS IN BAITADI

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ABSTRACT

Root and soil samples of citrus (Citrus reticulate Blanco) were collected from Baitadi area. Twenty nine samples were processed to find out the number and types of mycorrhizal spores and root samples were processed to find out percent root colonized by mycorrhizae. The percent root colonized by VAM fungi ranged from 27 to 92. The higher percentage colonized roots indicated healthier roots. It also indicated that the soil condition for the symbiotic relation was good. The percentage of root colonized by VAM was less on the sample indicated as diseased one. There was positive correlation between number of spores and percentage root colonized by VAM fungi. The diseased root, in general had lower root colonization. Number of spores in 200 grams of soil ranged from 165 to 6089. Two soil samples had very high spore population, 27 and 30 spores per gram soil. Predominantly Glomus spp. was found in most of the soil sample. Other genus found were Gigaspora, Scutellospora, Sclerocystis and Acaulospora.

INTRODUCTION

The term mycorrhiza was coined by Frank, way back in 1885. The literal meaning of mycorrhiza is fungus root (Myco= fungus, rhiza=root). In other world, we can say that the fungus and plant root together act as root system for the development of plants. Mycorrhiza may be defined as mutualistic, symbiotic relationship formed between fungi and living roots of plants. One of the seven mycorrhizae known is vesicular-arbuscular mycorrhiza. The name vesicular-arbuscular refers to the formation of typical morphological structures called vesicles and arbuscles in the cortex region of the roots. The VA mycorrhizal association is found in most crop plants, including citrus.VA mycorrhizae are geographically ubiquitous and occur in plants growing in arctic, temperate and tropical region. The mycorrhizae occur over abroad ecologicxal range from aquatic to desert environment.

In this mutualistic symbiotic relationship between two partners both partners are benefited from the relationship unlike in the pathogenic relationship where one partner is benefited at the cost of other. In this relationship, it is believed that there is exchange of carbon and phosphorus nutrition between two symbionts for mutual benefit. The philosophy behind the symbiosis is that it is more economical interms of energy for plants to take fungal help at the expense of its carbon rather than to form its own root system.

The benefit of mycorrhiza to the crops is well documented. Higher plants growth and yield response has been reported in many crops such as citrus, barley, soybean, mungbean etc. Mycorrhiza improves the nutrient status of plants, especially phosphorus (P). Mycorrhizae increase water efficiency of the crops, alleviate micronutrient deficiency, alleviate heavy metal toxicity, and increase salt tolerant of the crops and rduce disease of plants. The primary benefit seems to be more efficient supply of P to the plants.

Mycorrhizal Dependency. Some plants are highly mycorrhizal dependent and others are less dependent. While some other do not form mycorrhizae at all. The crops like onion, citrus, and cassava, strawberry are highly mycrhizal dependent. The crops like wheat, oat, potato are less dependent. It is hypothesized that the plant species with magnoloid root system (root with less fine root hairs) are more mycrrhizal dependent than those with graminoid (root with more fine root hairs). Citrus is considered highly mycorrhizal dependent plants. Therefore in developed countries like USA and Europe all citrus seedlings are inoculated with efficient strains of VAM fungi.

Citrus and Mycorrhiza.There are many reports that VAM fungi stimulate growth of citrus plant by promoting P absorption and by other mechanisms. Y.H. Shrestha and others (1995 and 1996) reported that citrus trees inoculated with *Gigaspora ramisporophora* grew larger and had better fruit quality as compared with non VAM check trees. The fruits of inoculated trees were larger, had higher sugar content in the juice, and better peel color. They also observed that under high air temperature stress condition, the

photosynthesis and transpiration rate of VAM inoculated trees were faster than non VAM ones. The inoculated trees had larger leaf area and higher P concentration and the tree growth was more vigorous than that of non inoculated ones. VAM trees had three times more photosassimilates per tree than uninoculated ones because inoculated trees had a leaf area 3 times greater than uninoculated one and grew more vigorously.

In Nepal, citrus is grown in the mid-hill without much use of chemical fertilizers and pesticides. In such condition, the indigenous VAM fungi in the soil play important role. This preliminary study was done to find out the mycorrhizal status of citrus in Baitadi.

MATERIALS AND METHODS

Root and soil samples were provided by ECARDS- Nepal. The roots were washed in tapwater and placed in glass vials and covered with a 10% potassium hydroxide (KOH) solution. The KOH solution in the vials did not exceed 75 % of the volume of the vial to prevent boiling over in the autoclave. The roots were autoclaved at 15 psi for 15 minutes. The KOH solution cleared the root cytoplasm and nuclei and allowed stain penetration. The KOH solution was drained off and the vials were rinsed using at least three change of tap water until no brown color appeared in the rinsed water. The root samples were covered in the vials with alkaline hydrogen peroxide (H_2O_2) for 20 minutes until the roots were bleached. The roots were rinsed with tapwater to remove H_2O_2. The roots were covered with 1 % hydrochloric acid for 4-5 minutes to acidify the roots. The acid solution was poured off. The roots were then covered with lactic acid solution containg Trypan blue solution (0.1 % w/v) and autoclaved for 10 minutes. After removing from the solution the roots were placed in petri plates for destaining. And, the percent of colonization by VAM fungi was determined by Gridline Intersect Method.

The soil samples obtained were used to determined type and number of spores. Sucrose centrifugation method was used to separate spores from the soil. Two hundred grams soil was placed on a 20-mesh screen and washed with water to remove large debris. The sieved soil was collected in a bucket. The sieved soil was mixed by stirring and allowed to stand for half minute. The soil in the water was decanted through 325-mesh sieve and the residue from the sieve was collected in a beaker. The collected residue was transferred into 50 ml- cetrifuge tube and was centrifuged for 4-5 minutes at 1750 revolutions per minute (rpm) in a horizontal rotor. The supernatant liquid was carefully decanted and the pellet was re-suspended in a sucrose solution (450 gram cane sugar in 1 liter water). Again the solution was centrifuges for 0.5 –1.0 minute at the above speed. The supernatant was poured onto a 325 sieve and was rinsed with water several times to remove the sugar. The spores captured in the sieve were transferred to petri plates and spores were observed and counted on dissecting microscope (20 X). Some selected spores were mounted in the glass slides and identified at genus level.

RESULTS AND DISCUSSION

Percent Root Colonized by VAM fungi: The percentage of root length colonized is expressed as the number of intersections with root colonization out of 100 total intersections counted. The counting was made on three replicated petri plates to minimize error. The percent root colonized by VAM fungi ranged from 27 to 92. Percent root colonized by AMF in diseased, mild diseased and healthy was 50.8, 63.2 and 77.0 respectively. The higher percentage colonized roots indicated healthier roots. It also indicated that the soil condition for the symbiotic relation was good. The percentage of root colonized by VAM was less on the sample indicated as diseased one. There was positive correlation between number of spores and percentage root colonized by VAM fungi. The diseased root, in general had lower root colonization.

Spores in soil: VAM fungi are obligate in nature. They infect and live with living roots only. They do not have sexual cycle. They produce asexual spores in soil. The spores can withstand adverse condition and serve as propagules for next season. Some fungal propagules inside the roots and soil may serve as infecting units. Some of the spores can remain in soil without germination for many years. The shape, size and makeup of the cell wall are the characters used for the identification of VAM fungi. It is difficult to identify the spores at species level. Only specialized taxonomist can identify the fungi at species level. The fungi produce spores when adverse condition starts. In annual crops, the spores are produced at the time of

flowering. In case of perennial crops, it is believed that the spores are produced after flowering. Some species produces too many spores while others produce a few spores. Therefore, higher number of spores in the soil can not be taken as good sign of mycorrhizal response. The number of spores from soil taken from diseased trees was less. Some soil samples from diseased trees had too many nematodes. Because of more or less same density, nematodes come with the spores of VAM fungi. The nematodes could be pathogenic. It could not be clear if less number of spore was due to presence of nematodes. Number of spores in 200 grams of soil ranged from 165 to 6089. Two soil samples had very high spore population, 27 and 30 spores per gram soil. Average number of spores per gram soil in diseases, mild diseased and healthy was 2.67, 7.53 and 2.75 respectively. Many types of VAM fungal spores were identified. The spores could be identified only at Genus level. Predominantly *Glomus* spp. were found in most of the soil sample. Other genus found were *Gigaspora ro Scutellospora*, *Sclerocystis* and *Acaulospora*. In a few samples black spores, that seemed to be dead were observed. After some days in the petri plates, mycelia come out of the spores. The mycelia are different from AM type. They seem to have parasitized the spores of VAM fungi. Some spores had holes, might be punctured by nematodes. Number of spores is not a good indicator of AM fungi. At this stage, due to lack of replicated samples; it is hard to come to some conclusion. However, there are clear indications that indigenous VAM fungi have positive role to play in the citrus crop in Baitadi.

RECOMMENDATION

Further research and development work to be done.

Research:

1. Determine mycorrhizal status from healthy and diseased trees in different agro-ecological zones,
2. To find cultural practices adopted by different farmers having healthy and diseased orchards
3. Isolate and multiply VAM fungi in nurse annual host like corn for testing and mass production.
4. Test efficiency of some selected VAM fungi in nurseries at different locations.
5. To study effect of chemical fertilizer and pesticides on mycorrhizae in citrus

Development:

1. Develop nursery practices using VAM fungal inoculation
2. Educate farmers about the importance of mycorrhiza in citrus and other crops.
3. Encourage farmers to use well decomposed organic fertilizers.
4. .Teach farmers to use sod culture system of Bahai grass (*Paspalum notatum*) or other grasses to increase VAM population.

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