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Am Fungi Mediated Effect on Plant Survival in A Coastal Saline Soil with Relation to other Amendments

KEYWORDS: Saline-alkali soil, arbuscular mycorrhizal (AM) fungi, PGPR, degraded ecosystems

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ABSTRACT *Screening of AM fungi on plants growing on saline-alkali soil of south-west coastal of Kumta (Karnataka) was undertaken. Field experiments were conducted to know the species in this region after supplying various reclamative amendments. Results revealed that plant survival rate got improved by the amendments lead to decrease the saline- alkali soil stress condition like pH and soil concentration. The significant effect of amendments with inoculation of different AM fungi was determined on selected plants. The inoculation of efficient species like Glomus macrocarpum in plants enhanced their survival rates. Maximum plant survival was observed when Glomus species with PGPR and gypsum amendments were grown in saline-alkaline soil. It can be concluded that Arbuscular mycorrhizal fungi with different phosphorous and potassium with PGPR on legume plants could bring reclamation of coastal sand dune soil on saline- alkaline affected soils. Therefore microbial component could play important role in revegetation of such ecosystems.*

INTRODUCTION

Coastal sands are heterogeneous due to frequent changes in physiochemical conditions, shoreline regression salt spray, low nutrients availability and high temperature. This area has inadequate nitrogen, phosphorus and organic matter adversely affect the flora and well fauna of coastal sand dunes (Koske and Gemma, 1997; Lakshman, 1998). The establishment of a suitable plant cover is known to improve the chemical, physical and biological properties of soil (Cabello, 2001) but the abiotic stress conditions of saline-alkali (SA) soil make it unsuitable for plant growth. Plants differ in their capacity to tolerate salts. The degree of salt tolerance depends on concentration and nature of salts as well as physiological stage of growth at which plant is exposed to salinity (Krishnamurthy *et al.*, 1987 and Amazallag, 1996).

Arbuscular mycorrhizal fungi, which are key soil microbial component, are known to play an important role in reclamation and revegetation of such degraded ecosystems (Gould *et al.*, 1996; Lakshman, 1999). They are known to increase drought resistance of young seedlings (Lakshman, 1996). They also detoxify certain soil toxins there by enable seedlings to withstand extreme acidity or alkalinity and also increase nutrient absorption capacity of plants (Richa Raghawanshi and Upadhyay, 2005). The present work was undertaken to study the diversity of arbuscular mycorrhizal fungi (AMF) and its mediated effect on five selected plants, survival in a coastal sand dune soil in relation to other amendments being used to reclaims such soils.

MATERIAL AND METHODS

This study was conducted at a coastal saline site located in Kumta of North Canara district in Karnataka. Kumta is situated at 17^1- 43^1 to 0^0 North latitude and 74^1-10^1 to 60^0 East longitude and 3m above the sea level in South Western parts of India. This region has a mixture of dry/ moist, sub humid climate dominated by tropical mansoonic character. The coastal saline soil had a pH of 5.56, electric conductivity 3.84 m mho/cm, organic matter 0.07%, organic carbon 0.27%, 68.5mg/ g available Sodium, 84.4mg/ g available Phosphorous and 8.67 mg/ g available potassium. The performance of the selected nitrogen fixing tree species; *Acacia melanoxylon* R. Br., *A. nilotica* Willd. *Albizia lebbeck* Benth. and *Dalbergia sissoo* Roxb. and non-nitrogen fixing tree species *Azardirachta indica* Juss. was monitored in coastal saline soil amended with other conventional and non-conventional amendments. The conventional amendments including nitrogen (125kg of N/Ha as urea), phosphorus (50 kg of P/ha as single Super Phosphate), Potassium (50 kg K/ha as nitrite of potash), farmyard manure (FYM; 200 tones/ acre) and gypsum. The gypsum requirement of the costal saline soil was calculated by the modified procedure proposed by Abrol *et al.*, (1975). Accordingly gypsum was added on the autoclaved coastal saline soil surface four weeks before transplanting the plants to allow its proper decomposition and mixing in the soil.

Mycorrhiza inoculation:

Screening of arbuscular mycorrhizal fungal spores on five plants was undertaken in saline soil. Spores of indigenous AM fungi were isolated following the procedure of Gerdemann and Nicolson (1963). The AM fungal spores were identified on the basis of spore size, shape, subtending hyphal attachment, colour, ornamentation and thickness of wall layers, reaction to Melzer's solution. As it was described by (Blackwell *et al.*, 1985) and with the help of manual written by (Schenck and Perez, 1991). Inoculum preparation of AM fungi was adapted following the procedure of (Ferguson and Woodhead, 1982). The selected experimental plants were inoculated with *Glomus macrocarpum*, *Acaulospora laevis* and plant growth promoting Rhizobacteria (PGPR) and it was monitored in saline alkaline soil. All the experimental pots were completely randomized design with three replications for each treatment. Per cent of mycorrhizal colonization was done following the procedure of Phil-

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lips and Haymann, (1970).

Isolation and quantification of spore:
Fifty grams of freshly collected soil sample was put into one or two liters of plastic beakers. Soil is suspended with about 500 ml to 1litres of tap water. Soil macro-aggregates had been crushed with glass rod. After 30 minutes of settling down of soil particles, the upper layer of soil suspension is poured into the sieve (600µm, 500 µm, 300 µm, 250 µm, 150 µm, 75 µm and 45 µm) to retain the spore of 45-250mm size. The procedure was repeated until the upper layer of soil suspension is transparent. The retained material on the sieve was decanted into a beaker with a stream of water and estimation of spores was carried out by modified method of Gour and Adholeya (1994). Later single spore or sporocarps were easily picked up from the sample with the help of syringe or fine point brush and mounted on a glass slide with a drop of polyvinyl lactophenol (PVL) and a cover slip was placed. Subsequently, recovered spores were identified with the help of manual and different taxonomic keys proposed by different workers (Schenck and Perez, 1990; Frank and Mortan, 1994).

Evaluation of AM fungal colonization:
Arbuscular mycorrhiza (AM) fungal structure is usually not observed without appropriate staining. Freshly collected root sample should be washed gently and be free from soil particles. Ultrasonic treatment is effective to disperse soil particles closely adhered to roots. Roots were treated with 10% KOH solution for 30 minutes to 1-2 hours in a hot bath, depending on thickness of root structure. Acidified root samples are stained with 0.05% tryphan blue in lactic acid for 10-15 minutes in a hot bath or for a few hours without heating. The roots are destained with lactic acid or lacto-glycerol and are now ready for microscopic observation. The stained roots be observed first under a dissecting microscope with transmitted illumination and then observed first under a compound microscope. Fungal structures are stained and can easily recognized (Phillips and Haymann, 1970). The following formula was used to calculate the root colonization (Giovanetti and Mosse, 1980).

$$\text{Per cent Root colonization} = \frac{\text{Number of colonized segments}}{\text{Total number of segments examined}} \times 100$$

Seed bacterization:
A plant growth promoting Rhizobacteria (PGPR) strain *Pseudomonas fluorescence* was obtained from the University of Agricultural Sciences Dharwad. Seeds of the test plants were surface sterilized by 2.4% (v/v) in Sodium hypochlorite solution for 2-3 minutes, rinsed in sterile air steam. Seed bacterization was done by the method of (Weller and Cook, 1983). Bacterized seeds contained approximately $4x10^8$ colony forming unit of the bacteria per seed.

The matured seeds of selected species of the locally grown variety were soaked overnight in water. These seeds were then grown in pots which already filled with sterilized garden soil. This was done to present the seedlings from salinity shock at germination stage. After 4 weeks, individual plantlets were transferred to earthen pots (30cm x 30cm) containing 8 Kg of mycorrhizal free saline- alkaline soil. Soil free from indigenous mycorrhizal fungi was prepared by mixing sieved (2mm mesh size). SA soil suspension of 200g soil/ liter water containing indigenous AM fungi. AM fungal spores per 200g soil containing indigenous AM fungi were given to autoclaved SA soil of each pot. Bacterized seeds were used for sets, which were to receive PGPR inoculation. The control sets of plants were transferred to SA soil without any amendments. The survival rate of different plant species under other amendments was noted down after three months for a period of one year.

RESULTS AND DISCUSSION
The rhizospheric soil sample of five different plants of coastal saline soil was screened to understand the distribution of AM fungi. Different species of AM fungi such as *Glomus*, *Acaulospora*, *Gigaspora*, *Sclerecystis* and *Enterophospora* were found to be dominated indigenous AM fungal spores in all samples. The dominant 10 AM fungal species were recorded from all experimental plants, where the *Glomus* species was found predominantly in all plants (Fig. 1). In *Acacia melanoxylon* R. Br., *A. nilotica* Willd and *Azardirachta indica* Juss. *Glomus mossae* and *Glomus macrocarpum* species were predominatedly recorded where as *Acaulospora*, *Gigaspora*, *Sclerecystis* and *Enterophospora* were noted in the rhizosphere of *Albizia lebbeck* Benth. and *Dalbergia sissoo* Roxb. AM fungal spores *Acaulospora laevis* were more in number compared to other AM species. Spore numbers of *Entrophospora infrequens*, *Sclerocystis dussi* and *Gigaspora margarita* were found less in rhizosphere of all the experimental plants. And thus the dominant AMF species such as *Glomus macrocarpum* and *Acaulospora laevis* were selected for further experimental studies.

Different bioinoculants (AMF) treatments were given to the experimental plants in order to know the effect on plants survival in a coastal saline soil in relation to other amendments. Less than forty-four per cent survivals of plantlets of all the selected plant species was noted over the control sets after they were transferred from garden soil to SA soil. This indicated that the

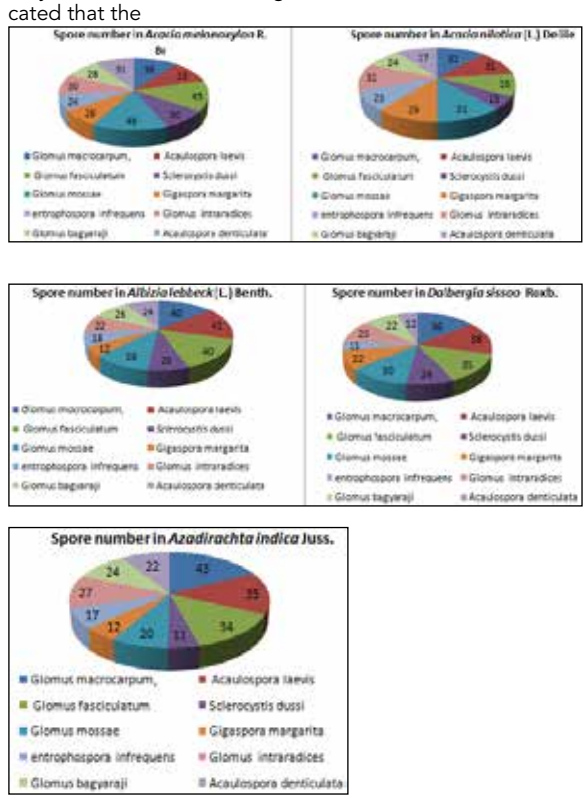

Fig. 1 AM fungal spore diversity in twenty five rhizospere soil samples of coastal saline alkali soil of five selected plants.

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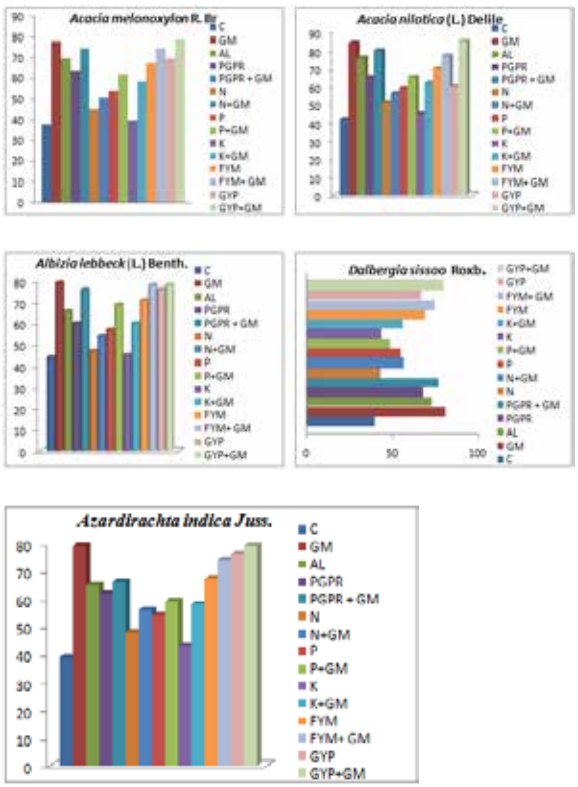
Fig.2. Effect of various amendments on survival of different plants species grown in Kumata coastal saline alkaline soil of Karnataka



(C-Control, GM-*Glomus macrocarpum*, AL-*Acaulospora laevis*, PGPR- Plant Growth Promoting *Rhizobacteria*, N-Nitrogen, P-Phosphorus, K- Potassium, FYM-Farm Yard Manure, GYP-Gypsum)

survival of plants was highly effected by the salinity and alkalinity higher concentration (Fig. 1). The survival of all plant species reached up to a maximum of 45% in K amendment soil. Application of N, P and seed bacterization improved the survival of the plantlets up to fifty to sixty five percent in case of all the plants with AMF inoculation and it is less than 55% without AMF in all plants except *Acacia nilotica* (59%). Application of PGPR alone raised sixty to sixty eighty percent in all plants, where with AMF showed seventy five to eighty percentages, it is less than seventy percent in *Azardirachta indica* (66%). Application of FYM, gypsum or mycorrhizal fungi boosted the percent survival of all plants species (survival recorded between sixty to eighty five per cent). As compare to *Acaulospora laevis*, *Glomus macrocarpum* was more effective in improving plants survival rate in most of the plants (Fig 2). The effect of amendments on survival percentage of plants increased when they were pre-inoculated by *Glomus* species. Amendment of gypsum or FYM in *Glomus* species inoculated plants enhanced the survival of the plants up to seventy seven and eighty five per cent in case of *Acacia nilotica*.

Potassium application did not alter the per cent survival of plants as compared to other treatments, although high level of K in young seedlings with expanding tissue is associated with salt tolerance in many plant species. Since Na^+ can be substituted for K^+ for uptake and it is believed that similar mechanism of uptake may operate for both ions (Malibari *et al*, 1990), it is possible that excess of Na^+ ions present in the soil must have masked the effect of K. Thus further addition of K might have not improved the soil conditions much in the coastal saline soil.

Nitrogen application was able to improve the survival rate of the plants up to fifty seven percent. Since organic matter content of alkali soils are extremely low, the major fraction of nutrients N required for plant growth in these soils comes from applied nitrogenous fertilizers, which might have been one of the reasons for improved survival of plant upon N amendment. However, the efficiency of N fertilizer used in SA soil remains very low because considerable fraction of N in ammonium form, applied as fertilizer or formed biologically from urea and decomposition of organic matter, is lost through volatilization and denitrification. However as seedlings of higher plants can take up the NH^+ form of nitrogen (Richa Raghuwanshi and Upadayaa, 2005). Application of N in plants during early seedling stages has shown positive results. Phosphorous was able to improve the survival of the plantlets because it is mainly required during early stages of growth and root development and is released very slowly in SA soil. Phosphorus was added in the SA soil at the time of transplantation near the root zone to reduce its fixation on clay surface and by calcium carbonate and the released P can easily be absorbed by the active root zone, thereby showing encouraging results. Increased seedling survival recorded in seeds bacteriazed by *P. flurescences* could be due to production of stimulatory growth substances (Hayman 1986) and siderophores (Loper, 1998), which improve plant growth and their establishment. Therefore the present investigation supports the earlier contribution of Abe *et al*., (1994).

Arbuscular mycorrhizal fungi from the main component of soil micro biota in most agro-systems. Hence, AM fungi have been shown strong influence on plant diversity. Since these fungi are obligate symbionts, their population and diversity are determined by plant species, human activities also affect these fungi. These modify the structure and functioning of plant communities in a complex and unpredictable way and therefore, mycorrhizas are essential for plant survival in coastal sand dunes which are chracterized by their low phosphorous content (Juniper and Abott, 1993). Association of AM fungi in coastal saline soils dependent on the availability of organic matter (John *et al*., 1973). Significant difference was seen in edaphic features (moisture, pH, phosphate, sodium, potassium and nitrogen) between naturally vegetated and non-vegetated Coastal saline soils of the southwest India can be related to the availability of organic matter and AM fungal colonization (Beena *et al*., 2000). Organic matter supplies nitrogen and phosphorous to dune vegetation and alkaline phosphatase activity of AM fungi decreased in soil devoid of organic matter. Besides fungal hyphae, polysaccharides produced by the hyphae firmly bind the microaggregates (Tisdall, 1991).

CONCLUSION

It may be concluded to derive maximum benefits from the coastal saline soils, it is necessary to conserve the below-ground biota including AM fungi. Elimination of mycorrhizal fungi decreases the plant equilibrium as well as productivity, which results in destabilization. The study concludes that gypsum or FYM amendment in plants inoculated with indigenous mycorrhizal fungi was the best combination. Future studies should concentrate on the importance of mycorrhizae on coastal saline soils stabilization and application of stress-tolerant mycorrhizae in agriculture.

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