1. INTRODUCTION

Nitrogen, a major and essential nutrient for plant growth, often limits crop production. Especially in modern agriculture, nitrogen is probably the most important input for increasing the yield potential of nitrogen-responsive new crop varieties. Also, under intensive cropping substantial loss of nitrogen can occur from the soil through processes such as volatilization and erosion. Although it is a major nutrient, limited water resources, low crop productivity in arid and semi-arid regions and increasing costs of fertilizers have discouraged the widespread use of nitrogenous (N) fertilizers. Hence the need for intensification of studies concerning nitrogen fixation especially biological nitrogen fixation. Even though molecular nitrogen is abundant in the atmosphere, it is generally deficient in soils resulting in decreased yield of the crops. As a result of its vital role and its low supply, the management of nitrogen resources is an important aspect in agricultural productivity.

Although increasing nitrogen demand of crops is mainly satisfied by the application of mineral fertilizers, biological nitrogen fixation, a process involving the reduction of atmospheric nitrogen to ammonia by microorganisms, which accounts for nearly 60% of the earth's newly fixed nitrogen (Postgate, 1982), has assumed great importance in maintaining soil fertility status. If the molecular nitrogen is not converted into suitable form (ammonia), the plants are unable to utilise it. This activity of reduction of atmospheric nitrogen to ammonia, known as biological nitrogen fixation, is confined to microorganisms and in particular to some procaryotes. The well known diazotrophic bacteria belonging to the genera like
Acetobacter, Azotobacter, Azospirillum, Azoarcus, Burkholderia, Enterobacter, Herbaspirillum, Kelbsiella, Pseudomonas and Rhizobium are able to exert positive effects on plants by producing and secreting plant growth regulators (PGRs) and/or by supplying biologically fixed nitrogen (Bazzicalupo and Okon, 2000). Besides the above genera, Rahnella aquatilis, a new diazotrophic bacterium, was isolated for the first time from the rhizosphere of wheat and maize using spermosphere model (Berge et al., 1991).

The nitrogen gain through non-symbiotic systems is low compared to symbiotic systems. However, the contribution by heterotrophic nitrogen fixers can be of considerable importance under conditions of high organic matter and moisture availability. Further, some of the investigations emphasize the significant contribution of diazotrophic associative bacteria like *Azospirillum* inoculated to foxtail millet (Cohen et al., 1980; Kapulnik et al., 1981b).

Despite several studies on nitrogen fixation, information on the diazotrophs associated with the rhizosphere of foxtail millet is rather limited (Lakshmi Kumari et al., 1976; Lakshmi et al., 1977; Cohen et al., 1980; Nur et al., 1980a; Kapulnik et al., 1981b). Five varieties of foxtail millet viz., Lepakshi, Prasad, Chitra, Krishnadevaraya and Narasimharaya were selected for the present study on nitrogen fixation. Isolation of diazotrophic bacteria from soil and rhizosphere using nitrogen-free media with a particular carbon source encourages the growth of specific bacteria. For example, use of glucose or mannitol leads to the frequent isolation of diazotrophs of Azotobacteriaceae while use of malate leads to the isolation of *Azospirillum* sp. In
order to avoid bias based on a single carbon, a medium with four carbon sources (Omar et al., 1989) was used for the isolation of diazotrophs.

Inoculation of plants with beneficial bacteria can be traced back to centuries. From experience farmers knew that when they mixed soil taken from a previous legume crop with soil in which non-legumes were to be grown, yields often improved. By the end of the 19th century the practice of mixing 'naturally inoculated' soil with seeds became a recommended method of legume inoculation in the USA (Smith, 1992).

For almost 100 years, *Rhizobium* inoculants have been produced around the world primarily by small companies (Bashan, 1998). Apart from soybean inoculation, which has made a major agricultural impact in the USA, Brazil and Argentina, significant contributions to the production of other legumes were made in Australia, North America, Eastern Europe, Egypt, Israel, South Africa, New Zealand and to a lesser extent in developed countries in Asia, Africa and Central and South America. Inoculant technology has had no input on productivity of the family farm because inoculants are not used or are of poor quality (Eaglesham, 1988).

Inoculation with non-symbiotic and associative rhizosphere bacteria like *Azotobacter* were used on a large scale in Russia in the 1930's and 1940's. The practice had inconclusive results and was later abandoned (Rubenchik, 1963). Interest in *Azotobacter* as an inoculant for agriculture has only recently been revived. An attempt to use *Bacillus megaterium* for phosphate solubilization in the 1930's on large scale in Eastern Europe apparently failed (Macdonald, 1989).
Two major achievements in plant inoculation technology occurred in the late 1970's: (i) *Azospirillum* was found to enhance non-legume plant growth (Dobereiner and Day, 1976) (ii) biocontrol agents, mainly of *Pseudomonas florescens* and *P. putida* groups, began to be intensively investigated (Kloepper and Schroth, 1981; Defago *et al.*, 1992; Glick, 1995; Glick and Bashan, 1997). In recent years, various other genera of bacteria such as *Bacillus, Flavobacterium, Acetobacter* and several *Azospirillum*-related microorganisms have also been evaluated as inoculants (Kloepper, 1994; Tang, 1994; Tang and Yang, 1997).

Small millets, which are produced mainly by subsistence farmers as rainfed crops, continue to play an important role in the diets of people living in interior rural and tribal areas in the semi-arid tropics (Pushpamma, 1986). Small millets may be defined as millets cultivated for their small grains which are borne on short and slender grass plants. They are also called as minor millets but they are not unimportant. Small millets have a wide adaptation. They can withstand a certain degree of soil acidity and alkalinity, stress due to moisture and temperature, and variations in soils from heavy to sandy infertile soils. Small millets are grown from the extreme southern tip of India at sea level to the temperate north Himalayan areas up to an altitude of 3,000 meters with consequent variation in photoperiod from short to long days (Sampath *et al.*, 1986).

Foxtail millet is one of the minor millets. It is one of the ancient crops probably domesticated in eastern Asia and known to Chinese as early as 2,700 B.C. Jars filled with husks of foxtail millet were found at Ban-po in Shanxi province dating
from the Yang-sha period (Chang, 1973). Foxtail millet cultivation was also reported in early agricultural sites from Switzerland and Austria dating back some 3,000 years. The species became widespread as a cereal in Europe during Bronze age (de Wet, 1986). In India it is mainly grown in Madhya Pradesh, Andhra Pradesh, Tamilnadu, Karnataka, Orissa, Gujarat, Maharashtra and Uttar Pradesh.

Anantapur district of Andhra Pradesh receives a poor annual rainfall of 154 mm and a temperature of $31^\circ \pm 9^\circ$C resulting in frequent droughts. Under these conditions, foxtail millet, one of the short duration crops (75 to 90 days), is cultivated as a minor millet mixed crop. Foxtail millet is chosen for the present study because it is one of the staple food crops of this area which is nutritious (125 mg protein g$^{-1}$) and contains all the essential amino acids. Further it not only resists drought conditions but also withstands delayed monsoon and is suitable for light black and red soils of Anantapur district. The present study is aimed at:

- Isolation and enumeration of bacterial populations (including *Azospirillum* spp.) from the rhizosphere of foxtail millet
- Purification of the isolates
- Screening of the isolates for diazotrophs
- Characterization of the isolates by standard biochemical tests
- Inoculation studies in pot culture using three selected strains of *Azospirillum* either alone or in combination with N fertilizer
- Response of inoculation and fertilization on growth and yield parameters of two varieties of foxtail millet during two seasons