123

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SCHOOL OF GRADUATE STUDIES

EFFECT OF TWO *AZOSPIRILLUM* ISOLATES ON THE GROWTH AND NITROGEN CONTENT OF TEF (*ERAGROSTIS TEF* (ZUCC.) TROTTER)

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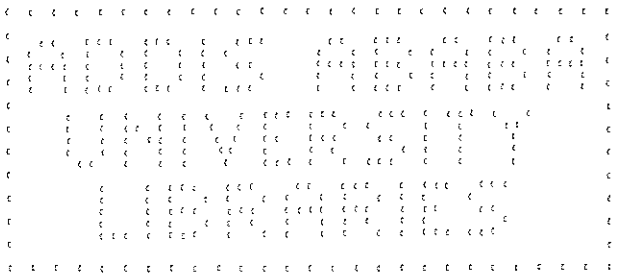

TABLE OF CONTENTS Pages

LIST OF TABLES **Pages**

ABSTRACT

Two diazotrophic bacteria, coded as A31 and A32, were isolated from roots of the *tef* varieties DZ-01-354 and DZ-01-196, respectively grown in pots on loam soil from Debrezeit. These isolates were characterized to the generic level and further comparison with the description of the type cultures *Azospirillum lipoferum* (ATCC 29707) and *Azospirillum brasilense* (ATCC 29145). These comparisons showed that the two isolates closely resemble A. lipoferum . However, on the basis of some differences in biochemical characteristics, it was suspected that these isolates may be different strains of *A. lipoferum.*

In an attempt to investigate the effect of these isolates on the yield and nitrogen content of tef, inoculation studies on pot grown *tef* plants was conducted. Root inoculation of seedlings of the two tef varieties with 1ml sterile water suspension (10^7-10^8 colony forming units (cfu) /ml) of these isolates produced significant ($p<0.05$) increases in height, grain yield, total shoot and root weight, root shoot ratio, total grain nitrogen and protein content of grains. Grain yield increases up to 11.6% and total nitrogen and protein content of grains up to 5.7% over and above un inoculated controls were observed.

The bacterial isolate A32 has performed better than A31 in promoting growth and yield of both varieties. Differential plant responses due to different bacterial treatments was found to be significant ($p<0.05$) for parameters as height, root shoot ratio and total nitrogen and protein content of grains on both varieties. Of the two *tef* varieties Dz-01-196 responded better than DZ-01-354 to inoculation with either of the isolates as demonstrated in a higher percent increases over the respective controls for most growth parameters The existence of a possible specific interaction between host cultivar and bacterial isolate which governs plant yield responses was suspected. Finally, the potential role of *Azospirillum* inoculation of tef seedlings on reducing fertilizer requirements was discussed.

1.INTRODUCTION

Tef (*Eragrostis tef* (zucc.) Trotter) is a cereal food crop of Ethiopia. It is said to be originated in Ethiopia and has been under cultivation since ancient times (Constanza *et al.*, 1979). Ethiopia is the only country in the world wherein the grain is used to prepare *injera* a soft, thin, and sour tasting pancake like local bread). When compared to wheat or barley, *tef* has a high nutrient content: protein (11%), carbohydrate (73%), fat (2.6%) and minerals (mainly iron and calcium (3%) (IAR,1987). Being a highly preferred food mainly among the majority of the urban population, it accounts for about two third of the daily protein intake in the diet of the population (Ethiopian Nutrition Survey, 1959 cited in Seyfu, 1993).

The crop is adapted to a wide range of environments and is presently cultivated under diverse agroclimatic conditions. It can be grown from 300 M to 2800 M above sea level under various rain fall, temperature and soil regimes (Seyfu, 1987; Hailu *et al.*, 1992). It performs better than any other cereal, not only in the moisture stressed and semi arid regions, but also in water logged soils of Ethiopia (Hailu *et al.*, 1990). Despite its agronomic versatility, suitability to marginal conditions, and high market price, low grain yield per unit area is one of the major limitations of *tef* (Seyfu, 1993). According to the reports of IAR (1996), the national average yield of this cereal is about 10 quintal ha^{-1} and the low grain yield level is attributed to various biotic and abiotic stresses and socioeconomic factors. The most frequently reported production problems are lack of improved varieties, lodging and lack of agricultural inputs such as fertilizers.

The application of chemical fertilizers to impoverished soils is one way of improving soil fertility. While the nutrient status of soils can actually be improved by the addition of chemical fertilizers, these inputs are very expensive for subsistence farming systems in most developing countries and this is reflected in its low consumption in Africa (Darmwal and Gaur, 1988; Rao, 1995). Moreover, the problems associated with the application of chemical fertilizers are not only less expensive but also ecological. When these inputs are persistently applied they are proved to be environmentally unsafe (Taylor, 1979; Mertens *and* Hess, 1984; Mengel, 1992). Consequently, there arose a need to look for less expensive and environmentally friendly agricultural technologies.

The use of root associated or rhizospheric microorganisms to improve the soil nutrient status and/or influence the yield of food crops is one of these alternatives. Different microbial inoculants have been developed and popularized since the introduction of *Rhizobium* inoculants (Rao, 1995). Inoculant preparations of different nitrogen fixing microorganisms such as *Rhizobium, Azotobacter*, and certain Cyanobacteria have been shown to improve the N status of soil and increase the yield of different crops to various degrees (Mengel, 1992; Rao, 1995). Other beneficial rhizosphere microorganism such as phosphate solubilizing bacteria (Richards, 1987; Rao, 1995) and the vesicular arbuscular mycorrhizal (VAM) fungus (Graw, 1970; Hayman, 1980; Buwalda *et al.*, 1983) are also shown to improve the availability of phosphorus. Thus, the use of such microbial inoculants not only increases the yield but also reduce the need for costly inorganic fertilizers in agricultural systems.

On the other hand, agronomists have been looking for an effective plant-microbe associations that could some how alleviate the fertilizer need of non leguminous food crops (Boddey *and*

Doberiener, 1988; Quispel, 1991). The search for less costly and effective plant root-microbe system in cereal agriculture has led to the discovery of a number of such beneficial associations (Boddey and Doberiener, 1988; Boonjawat *et al.*, 1991). Of all these systems the diazotrophic association between bacteria of the genus *Azopirillum* and roots of various grasses and cereals has been given special attention for its potential contribution in yield improvement and/or fertilizer reduction in cereal agriculture (Reynders and Vlassak, 1982b).

Inoculation studies conducted in the field and in the greenhouse during the past 20 years using *Azospirillum* (*A. lipoferum* and *A. brasiliense*) have proved the yield promoting effect of the organism in different soils and climatic regions (Fallik *and* Okon, 1996). Significant yield increases have been reported on several crops such as foxtail millet, sorghum, maize (Kapulnik *et al.*, 1981a, Cohen *et al.*, 1981; Sarig *et al.*,1984, Fallik *and* Okon, 1996), rice, wheat, barley (Reynders and Vlassak, 1982a, b; Boddey *et al.*, 1986; Bahattarai *and* Hess, 1993) and a number of other cereals from diverse geographic regions. Positive inoculation responses with respect to increases in dry matter yield (Sarig *et al.*, 1984; Pacovesky *et al.*, 1985; Bahattarai *and* Hess, 1993 Fallik *and* Okon, 1996), total nitrogen content of grains (Kapulnik *et al.*, 1983; Mertens *and* Hess, 1984; Okon *and* Kapulnik, 1986; Bahattarai *and* Hess,1993), and improved water status (Sarig *et al.*, 1984) and nutritive quality of the crops (Pacovesky *et al.*, 1985) have been well recorded.

Ecological studies on the geographic distribution of the bacteria have revealed that the bacteria have a wide range of distribution covering diverse geographic regions (Doberiener *et al.*, 1976 cited in Rao, 1995) with much higher frequency in tropical than temperate regions (Reynders and Vlassak, 1982b). Bacterial counts of tropical soils yielded numbers as high as

10^4-10^7 cfu/g dry weight of roots (Zimmer *and* Bothe, 1988) as compared to 10^2-10^4 cfu/g dry weight of roots from temperate regions (de Conick *et al.*, 1988). Moreover, much more successful positive inoculation responses of cereals have been found to be more consistent in tropical and subtropical countries than in temperate regions (Kapulnik *et al.*, 1981a, b; Rai *and* Gaur, 1982; Millet *and* Feldman, 1984; Boddey *and* Doberiener, 1988). Despite the tropical abundance and intimate association of the diazotroph with tropical grasses and cereals, there has been very little or no research attention given to exploit the agronomic benefits of this association in most tropical countries particularly in tropical Africa.

Ethiopia is a tropical country whose economy mainly depends on agriculture. The different agroecological zones of the country allow the cultivation of various food crops including wheat, maize, sorghum, *tef,* barley etc. Of this, *tef* is the most important one to which 1.4 million hectare of land is devoted to its production in the country (Seyfu, 1987; Hailu *et al.*, 1992). Despite its popularity and outstanding agronomic qualities, *tef* yield is generally low at farm level for several reasons mentioned earlier. Attempts to improve the yield of *tef* through selection went back to the early 1960's. Following the establishment of techniques for its conventional crossing of in the mid 1970's, breeding and/ or mutation experiments for the production of varieties with high grain yield, high protein content and early maturity progressively produced promising results (IAR,1981).

Consequently, improved varieties labeled as DZ-01-354, DZ-01-196, DZ-01-99, DZ-01-787 and others have been released and some of these varieties are domesticated by farmers through the years. These varieties are found to be superior to the local varieties with respect to agronomic characters such as yield and maturity period. The Variety DZ-01-196 (very white

seed color commonly known as"magna" *tef*) is able to give a total yield of 14-16kg/ha on farmer's plot despite its narrow geographic adaptation. The most popular variety, DZ-01-354 (pale white seed color and with a wider adaptation) is found to give 17-22kg/ha on farmer's plot (DZARC, 1983; Seyfu, 1987). However, such yield improvements are obtained when the improved varieties were treated with a recommended dose of fertilizers. Hence, the need for costly inputs remain an economic constraint in *tef* production.

Despite the rising price of chemical fertilizers, there is an increasing trend in the use of these inputs in *tef* production across the country. According to the reports of the Central Statistics Authority, agricultural sample survey (1996/97) about 41.33% of the total (2, 501, 000.98 ha.) fertilized land under cereal cultivation is devoted to *tef* production. With the aim of assessing the difference in production costs and effect of fertilizers on the yield of improved and local varieties of tef, Abate (1993) compared three improved (DZ-01-196, DZ-01-99 and DZ-01-354) and one local variety in five "woredas" of east Shewa. The results showed that while fertilizer application could appreciably improve the yield of all varieties tested, improved varieties did not significantly differ in yield performance from local variety either in the presence or absence of fertilizers. The author further suggested that low selling price for the products and high cost of inputs as the primary reasons why farmers do not apply new technology at recommended rates.

Azospirillum inoculation of various cereals has shown to reduce the fertilizer requirement in cereal agriculture. Lin *et al.*(1983) reported a reduction of 42 and 39kg/ha of nitrogen fertilizer due to inoculation for pearl millet and guinea grass, respectively. Rai and Gaur (1982) and Fayez *et al.* (1985) obtained a yield response of wheat due to inoculation, which is

equivalent to 40kg N/ha without inoculation. However, successful inoculation responses requires the isolation of effective *azospirilla* from roots of homologues hosts (Suikiman and New, 1990) and the use of these isolates on the same host from which it was isolated. Moreover, perhaps for reasons owing to different ecological factors, field performances of *Azospirillum* isolates effective in one geographical area failed to exhibit a similar effect on a similar host at a different geographical site (Millet *et al.*, 1984). Therefore, the domestication of *azospirilla* inoculants into agricultural systems presupposes knowledge of the indigenous *azospirilla* population in terms of effectiveness and competitive ability (Boddey and Doberiener, 1988). This suggests the importance of isolating effective isolates from homologous hosts for use in trials on similar hosts in suitable ecological conditions.

Although a lot has been done towards the improvement of *tef* yield and other desirable agronomic traits through breeding and/or genetic manipulation, alternative techniques for the utilization of rhizosphere microorganisms for growth promotion had been highly neglected in the country. The fact that chemical fertilizers are becoming increasingly costly for farmers in the developing nations like Ethiopia necessitates the need for an investigation of less-costly agricultural technologies. Screening of rhizosphere microflora for the selection and proper utilization of their beneficial effects have been made on several cereals and grasses (Bilal and Malik, 1987; Boddey and Doberiner, 1988; Berge *et al.*, 1991; Fages and Arsac, 1991). Meanwhile, Tekalign, 1984 cited in Tekalign (1987) investigated the effect of VAM fungi (*Glomus fasiculatum*) on the mineral nutrition of tef while Asfaw (1993) examined the effect of a phophate solubilizing fungus on the growth and yield of *tef*. Except these works no information pertaining to this issue is available in the country with respect to this unique cereal, *tef*. It is therefore essential to screen indigenous *Azospirilla* population in the

rhizosphere of *tef* and investigate their effect on its growth. Subsequently the information generated may help in the endeavour of preparing *Azospirilla* inoculants for use in small scale tef production in the country.

1.1 OBJECTIVE

General objectives

•To evaluate the effect of indigenous *Azospirillum* isolates on the yield and nitrogen content of *tef*

SPECIFIC OBJECTIVES

•To isolate, characterize and identify the indigenous *Azospirilla* population from the rhizosphere of *tef* .

•To evaluate the effect of these isolates on the yield performance and nitrogen content of two *tef* genotypes grown in pots in the glasshouse .

1.2. Description of the bacteria

The bacterium was first isolated by Bjerinick from a diluvial nitrogen-poor sandy soil in the Netherlands (Becking, 1982). He described the organism in 1923 as *Azotobacter spirillum* and later renamed it as *Spirillum lipoferum* (Neyra and Dobereiner, 1977). Dinitrogen fixation was initially claimed by Bjerinick (1921), However, Schroder (1932) failed to find dinitrogen fixation and growth in a nitrogen- free medium using a single cell culture. In the same year Becking re-isolated the organism from several sources and showed with the aid of N^{15} that dinitrogen fixation occurred in pure culture (Becking, 1982) .

Except for a few reports, relatively little attention was given to this organism until it was isolated in 1974 from tropical grasses of *Digitaria spp in* Brazil (Dobereiner and Day, 1976 cited in Tarrand *et al.*, 1978; Rao, 1995). This isolate was identified as *S. lipoferum* following the descriptions in Bergey's Manual (1957) and the original descriptions by Bjerinick (Neyra and Dobereiner, 1977) . Since 1974 *S. lipoferum* has been isolated from the roots of a variety of forage grasses, legumes, grain crops and soils with the help of effective isolation procedures (Doberiener, 1988).

S.lipoferum is characterized as gram negative, motile, short rod or slightly curved to spiral in shape and contains polyhydroxybutyrate (PHB)granules (Okon *et al.*,1976). *S .lipoferum* colonies exhibit different colors as pink, deep pink, red, white or yellow (Dalton, 1980). When growing in N-free semi solid media it forms a characteristic (often white, thin, and undulating) subsurface pellicle indicating a preference for micro-aerophilic conditions for Nitrogen fixation ((Neyra and Doberiener, 1977).

Okon *et al.* (1976) reported that unlike most diazotrophs, the organism has a very poor oxygen protection mechanism under nitrogen fixation conditions and can not grow in air using N_2 as a nitrogen source. However, when supplied with fixed nitrogen (NH_4^+) it grows vigorously as an aerobe. According to Neyra and Dobereiner (1977), temperature requirement of the species is generally high. The fastest growth range for most of the isolates was observed between 32^0 C and 38^0 C and nitrogenase activity was maximal between 33^0 C and 40^0 C . On the other hand, according to Dalton (1980) the pH requirement for maximal nitrogenase activity in pure culture is generally between 6.8 and 7.8 and its occurrence in soil is found to be highly pH dependent Neyra *et al.* (1977) found that *S. lipoferum* is the only organism known to bring about both N_2 fixation and dissimilation of NO_3 to gas. *S. lipoferum* is favored by sugar-poor substrates (Okon, 1976) and its nitrogen fixation ability is best supported by organic acids such as malate, succinate and lactate, pyruvate (Dalton, 1980).

The accumlation of many physiological, morphological and genetic data on this diazotroph and other related strains from different geographical origins necessitated the change in the taxonomic status of the *S. lipoferum* group into a genus level, *Azospirillum* (azote-nitrogen, spirillum-small spiral) (Krieg and Doberiener, 1978 in Bergey's Manual (1986) under which two new species namely *A. lipoferum* and *A. brasilense* were proposed. In the succeeding years three more species have been isolated from the rhizosphere of various species of grasses (Bhattarai and Hess , 1993). One of these isolates was recovered from roots of many grasses in Amazonia in 1983 and named *A. amazonese.* While a second one found principally with roots of kallar grass (*Leptochloa fusca)* from saline soils of Pakistan in 1987 and later identified as *A. halopraeferans.* In 1989 the third isolate *A. irakense* was isolated in association with rice roots in Iraq (Reihnold-Hurek and Gills, 1994).

This bacterium was known to influence the growth and yield of various grasses and cereals. Several mechanisms including biological nitrogen fixation (Boddey *and* Doberiener, 1988; Boddey *et al.*, 1991), production of phythormones and subsequent alteration of root morphology and increased nutrient uptake (Lin *et al.*, 1983; Zimmer *and* Bothe, 1988; Fallik *and* Okon, 1996) and altering root membrane activity (Bashan *and* Levanony, 1991) have been proposed. Consequently, interest in this organism has grown considerably for its possible agronomic significance. Several inoculation experiments have been conducted to evaluate its potential in yield improvement and/ or reducing fertilizer requirement of major food crops (Mertens and Hess, 1984; Millet *et al.*, 1985; Pacovsky *et al.*,1985; Fallik and Okon, 1996). These trials proved that inoculation of *Azospirillum spp* could enhance the yield of various cereals. Today, the potential of *Azospirillum spp.* as biofertilizer for yield promotion of various non-leguminous food crops is getting good recognition at least in some parts of the world (such as India and Israel) as being economical and an ecologically safe agricultural technology .

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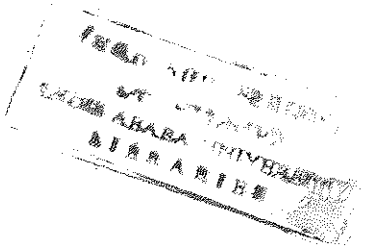


2 MATERIALS AND METHODS

2.1 Sampling sites

Samples in this study were collected from two sites. One sample is from Holetta (45 Km west and the second from Debrezeit, 50 Km south east of Addis, respectively). The specific sampling sites in the two sampling areas had been preferably chosen to be the regional research centers, the Institute of Agricultural Research (IAR) at Holetta and Alemaya University of Agriculture (Research center) at Debrezeit. The sampling plots in both sites were those on which *tef* had been continuously harvested for the last seven years.

2.2 Collection of samples

Soil samples were collected from Debrezeit in two occasions and only once from Holetta. The first sampling from both sites was used for *Azospirillum* establishment (section 2.3 below) while the second sampling (i.e from Debrezeit) was used for pot experiments (section 2.7 and 2.8). Soil samples were collected from *tef* rhizosphere after removing debris and the top 6-10 cm soil. About 4- 6 Kg of rhizosphere soil (from each sampling site) was collected in plastic bags for use in section 2.3 and a bulk sample 70kg soil for use a in pot experiments. The *tef* seed samples consist of two varieties that had been cultivated in the corresponding sampling sites in the previous season. A *tef* variety called DZ-01-354 was collected from Debrezeit and DZ-01-196 from Holetta. Both the soil and seed samples were transported to the laboratory and were used in glasshouse experiments the following morning.

2.3. *Azospirillum* establishment in the glasshouse

A mixture of 1.5 Kg of soil and 0.5 Kg of washed and autoclaved sand was filled into each of six 3-liter synthetic pots (Addis Foam and Plastic Industry Products, Addis Ababa). Two pots were labeled as Holetta fertilized black soil, the other two Debrezeit unfertilized light loam soil, the last two with Debrezeit fertilized black soil. *Tef* seeds from the respective areas were surface sterilized with 0.5% (W/V) $HgCl_2$ for two minutes at room temperature, thoroughly washed with sterile water (Sriskandarajah *et al.*, 1993). Six to ten treated seeds from the respective areas were sown in the corresponding pots. The pots were then kept in the glass house with regular watering (twice a day). Thinning to five plants/pot was made after seedling emergence and the plants were allowed to grow for about one month .

2.4 Media preparation , Isolation and Culture conditions

Two seedlings/ pot were randomly dug out. The seedlings were then cut at the soil line with a sterile razor blade. The root part from each seedling was then transferred to sterile petri plates and taken to the laboratory. Whole roots were then washed thoroughly with sterile distilled water. Washed roots were finally cut into 0.5 cm pieces with sterile razor blades . One root piece was aseptically inoculated into 9 ml small test tubes containing 4ml semisolid nitrogen free basic (Nfb)medium (Bahattari and Hess,1993).

One liter of Nfb medium was prepared with(g/l):

Semi solid nitrogen free basic medium

(After Bahattarai and Hess, 1993)

Malic acid, 4

Glucose, 1

$Na_2MO_4.2H_2O$, 0.02

KOH, 0.7

yeast extract, 0.21

$MgSO_4.7H_2O$, 0.2

Nacl, 0.2

Agar, 1.75 (Semisolid) pH-6.9

$CaCl_2$, 0.02

$MnSO_4.H_2O$, 0.1

$FeSO_4.7H_2O$, 0.002

$K_2HPO_4.3H_2O$, 0.5

Nitrogen Free Bromothymol blue medium (NFB)

(After Lakishami *et al.*, 1982 cited in Rao,1995)

K_2HPO_4, 8g

KH_2PO4, 4

Agar, 1.75g

$MgSO_4$, 0.2g

NaCl, 0.1g

$CaCl_2$, 0.02g

Malic acid, 4g

NaOH, 3g In 1 liter of distilled water. pH was adjusted to 7.0

Yeast extract, 0.05g

$Na_2MoO_4.2H_2O$, 0.002g

MnSO4, 0.001g

H_3BO_3, 0.0014g

$ZnSO_4$, 0.004g

$FeCl_3$, 0.0002g

Bromothymol blue, 2ml

A total of 12 tubes were incubated at 35^0 C for 3-4 days. At the end of incubation period tubes with typical subsurface pellicle were selected (Bilal *et al.*, 1990; Suikiman and New, 1990) and a loopful of the pellicle was streaked on to nutrient agar plates. Representative colonies from each plate were purified by successive streaking on nutrient agar plates. Pure isolates were maintained on Tryptone Soya Broth (TSB) agar slants and kept at 4^0C. These slants were used as stock cultures with occasional sub culturing every month.

2.5 Characterization and identification of isolates

The morphological characteristics of each pure isolate were then examined by gram reaction and wet mount procedure. Each pure isolate was re inoculated into Nfb medium and incubated at 35°C for 3 -4 days (Bahattarai and Hess, 1993) so as to confirm nitrogen fixation (i.e ability to grow in Nfb) and pellicle formation in pure culture. Tubes with characteristic pellicle were then chosen and pellicle formation in these isolates were checked three to four times by inoculating and re inoculating a loop ful of the pellicle into a fresh Nfb medium. These isolates were further tested for the production of alkali in Nitrogen Free Bromothymol media (NFB) (the constitution of which is shown above), which changed the color of the media from green to deep blue (Bilal *et al.*, 1990; Suikiman and New, 1990). This was followed by standard biochemical tests meant for the identification of *Azospirillum* sp.

2.5.1 Biochemical and physiological tests

2.5.1.1 Aesculin hydrolysis :- To 100ml of peptone water 0.1g aesculin and 0.05g ferric citrate were added. The solution was then boiled, poured into tubes and autoclaved at 110c for 10min. Tubes were finally inoculated with a loopful of culture of the isolates and incubated at 37^0C overnight. Blackening of the medium was considered as a positive test.(Collins and Lyne, 1976).

2.5.1.2 Presence of catalase:- Two to three drops of 3% Hydrogen peroxide solution was placed on cultures grown on nutrient agar plates and effervescence of oxygen was taken as a positive test (Collins and Lyne, 1976).

2.5.1.3 Presence of Cytochrome oxidase:- A small piece of Whatmann filter paper was soaked with 1% aqueous tetramethyl-p-phenelyne diamine dihydrochloride solution. And a loop ful of 24 hr old culture was scrapped and rubbed on the filter paper. The appearance of a blue color in 10 to 20 sec. was considered as a positive oxidase test (Collins and Lyne, 1976).

2.5.1.4 Acid production from glucose aerobically:- This test was made on three different media namely yeast extract based-glucose broth (**M1**), a similar media with a phosphate concentration twice that of m1 (**M2**) and peptone based glucose broth (**M3**).A loopful of culture was inoculated to M1 tubes having the following composition (g/l): glucose, 10.0; $MgSO_4.7H_2O$, 1.0; KH_2PO_4, 0.4; $MnSO_4.H_2O$, 0.002; $(NH_4)_2SO_4$,, 1.0; yeast extract, 2.0; bromothymol blue (aqueous), 0.025. The pH was adjusted to 7.1 with KOH. Inoculation was carried out in **M2** whose composition is identical with **M1** except for phosphate concentration.

Similarly inoculation was also made on a peptone based glucose-broth of the following composition (g/l): peptone, 2.0; $MgSO_4.7H_2O$,1.0; $(NH_4)_2SO_4$, 1.0; glucose, 10.0; $FeCl_3.6H_2O$, 0.002; $MnSO_4.H_2O$, 0.002; bromothymol blue (aqueous), 0.025. The pH was adjusted to 7.1 with KOH. All tubes were incubated at 37^0 C for 48 hrs (Tarrand *et al.*, 1978).

2.5.1.5 Acid production from glucose and fructose media anaerobically:- This test was made in;-

A) Yeast extract based-glucose broth:- composition as in M1 above.

B) Yeast extract based-fructose broth:- composition as in M1 above except that glucose was replaced with fructose.

C) Peptone based-glucose broth:- composition as in M3 above

D) Peptone based-fructose broth:- composition as in M3 above except that glucose was replaced with fructose.

After inoculation, all tubes were placed into anaerobic jar. A lighted candle was placed inside the jar and the lid is closed so as to remove the oxygen in the jar consequently create anaerobic condition. The jar was then incubated at 37° C for 48hrs (Collins and Lyne, 1976; Tarrand *et al.*, 1978).

2.5.1.6 Acid production from different sugars:- the following basal medium was used for testing different sugars (Glucose, Mannitol, Maltose, Xylose, Lactose and Galactose). The composition of which is (g/l): yeast extract, 0.05; K_2HPO_4, 0.25; $FeSO_4.7H_2O$, 0.026; $Na_2MoO_4.2H_2O$, 0.001; $MnSO_4.H_2O$, 0.2; NaCl, 0.1; $CaCl_2.2H_2O$, 0.026; $(NH_4)_2SO_4$, 1.0; biotin, 0.0001; bromothymol blue (aqueous), 0.0375; agar (Difco), 2.0.The pH was adjusted to 7.1 with KOH. The sugars were added to the basal medium after having been filter

sterilized to give a final concentration of 1%. Tubes were then inoculated with cultures and incubated at 37^{0}C for 72h (Tarrand et al., 1978)..

2.5.1.7 Sole carbon source utilization

The ability to utilize different sugars as sole carbon source for growth in nitrogen free media was tested by using the nitrogen free semisolid medium of Day and Doberiener (1976)with some modification. The composition of which is (g/l): sugar, 10; KH_2PO_4, 0.4; K_2HPO_4, 0.1; $MgSO_4.7H_2O$, 0.2; NaCl, 0.1; $CaCl_2$, 0.002; $FeCl_3$, 0.01; $Na_2MoO_4.2H_2O$, 0.002; biotin, 0.0001. The medium was inoculated directly from cultures grown in semisolid malate medium (with 0.2% agar) and the growth response at 37^{0}C is observed in 3 days (Tarrand *et al.*, 1978).

2.5.1.8 Indole production:- Cells were cultured for 48hrs in 1.0% tryptone broth (Difco) containing 0.1% tryptophan. Indole production was determined by adding drops of Kovac's reagent (dimethyl aminobenzaldehyde) into the culture tubes. A pink color confirms a positive test (Collins and Lyne, 1976).

2.5.1.9 Hydrogen sulfide production:- Tubes with sterile SIM (sulfur indole motility) medium were inoculated with cultures and incubated for 48hrs. at 37^0 C. Blackening of the medium at the end of incubation period was considered a positive test (Collins and Lyne, 1976).

2.5.1.10 Starch hydrolysis:- cultures were streaked on starch agar (10% solution of soluble starch in water, steamed for 1hr. and 20ml. of this solution was added to 100ml. of melted nutrient agar). This agar was then poured on plates and the isolates were streaked on it and

incubated at 37^0 C for 3-5 days. Each plate was then flooded with dilute iodine solution. The formation of a clear zone around the colony was considered as a positive starch hydrolysis test (Collins *and* Layne,1976).

2.5.1.11. Presence of urease:-2.4g of urea agar base was suspended in 95ml of distilled water which was then boiled and sterilized by autoclaving at 115^0C for 20min. The mixture was subsequently cooled to 50^0C and 5ml of a pre sterilized 40% urea solution was aseptically introduced. It was then mixed and distributed in 10ml amounts into sterile test tubes. The tubes were finally set in the slope position. These urea agar slants were inoculated with isolates and incubated at 37^0 C for 3-12hrs. Pink coloration of the medium shows positive reaction for the test (Collins and Lyne, 1976).

2.5.1.12 Nitratase test:-

Reagents:- Solution 1:- 0.8g sulphanilic acid was dissolved in 100ml 5N acetic acid with gentle heating. Solution 2:- 0.5g %-naphthylamine was dissolved in 100ml 5N acetic acid with gentle heating.

A heavy suspension of the inoculum of the isolates was prepared in 1ml peptone water and heated to 37^0C for 15min in a water bath. This was followed by addition of 3 drops of solution **1** mixing, and then 3 drops of solution **2**. It was shaken and allowed to stand for 2min. The appearance of a pink color at this stage was considered a positive reaction (i.e nitrate reduction +ve or dissimilation of NO_3 to NO_2). And in case no color change occurred zinc dust was sprinkled into these tubes. The development of a red color confirms a negative reaction (i.e nitrates present) while no color change was considered a positive reaction where

nitrates were converted to nitrite and further reduced to molecular nitrogen(Collins and Lyne, 1976)

2.5.1.13 Biotin requirement :- Media of the following composition was prepared with (0.0001g/l) or with out biotin (g/l): succinic acid, 5.0; K_2HPO_4, 0.5; $FeSO_4.7H_2O$, 0.01; NaCl, 0.1; $MgSO_4.7H_2O$, 0.2; $Na_2MoO_4.2H_2O$, 0.002; $CaCl_2.2H_2O$, 0.026; $(NH_4)_2SO_4$, 1.0 and pH was adjusted to 7.0 with KOH. Cultures of the isolates were inoculated into duplicate tubes (with biotin and with out biotin) and incubated at 37°C for 48hr. Growth of the isolates in the presence and absence of biotin was confirmed by the turbidity observed in either of the duplicate tubes (Neyra *et al.*, 1977).

2.5.1.14.Growth in 3% NaCl:- The carbon source in the media for this test can be glucose or malate. Since *A. lipoferum* can utilize glucose as sole carbon source while *A. brasilense* does not. The presence of either of these organisms can be detected using duplicate tubes where the carbon source in one is glucose and malate in the other. A nitrogen deficient semisolid medium of the following composition was prepared in three portions and mixed after autoclaving.(g/l) **1** NaCl, 28 ; $MgSO_4.7H_2O$, 5; $CaCl_2.2H_2O$, 0.01; $Na_2MoO_4.2H_20$, 0.01; Tris, 50mmol; Yeast extract, 0.001 ; dissolved in 800ml of distilled water. **2**.0.5g of KH_2PO_4 dissolved in 100ml of distilled water. **3** $FeCl_3$. $6H_2O$, 0 015; glucose or malate, 5; agar, 1.75. The pH in **1** was first adjusted and the three of them were sterilized separately and then mixed. This medium was aseptically transferred to duplicate tubes and each of these tubes were inoculated with a loopful of the bacterial isolates. The tubes were incubated at 37° C for 48hr. and the development of pellicle or any other growth form was examined (Wong *et al.*, 1980).

2.6 Soil and seed sample preparation

A soil sample of 70 Kg was collected from an unfertilized *tef* test plot (section 2.2) at Debrezeit (AUA, Debrezeit Research Center). The bulk soil sample had a light color and loamy texture with pH of about 7.3. Bulk sand sample was collected from the campus compound (Addis Ababa University, Science Faculty). The sand was then washed thoroughly to remove soil or dust particles and dried in an oven. The soil sample and the washed sand was then mixed in 3:1 (soil: sand) proportion and the mixture sterilized in an autoclave. Thirty six 3-litre plastic pots (8 cm diameter and 19 cm deep, Addis foam and plastic factory products) were surface sterilized by rinsing the interior of the pot with 95 % ethanol. The autoclaved mixture was then filled into each pot and the mouth of each pot immediately wrapped with UV treated plastic bags. A handful of two *tef* varieties, namely, DZ-01-196 and DZ-01-354 were surface sterilized as in section 2.3. Six to eight treated seeds of DZ-01-196 were then sown into each of the 18 pots and a similar DZ- 01- 354 seeds into the remaining 18 pots. Six of the pots from the first group (DZ-01-196) and another six from the second were labeled as control groups for the respective treatments.

2.7 Glasshouse pot experiment

The pot experiment was carried out under glasshouse conditions at the Science Faculty campus between Nov. 1997 and Feb. 1998. Mean minimum and maximum temperatures inside the glass house during the study period were 15.03 and 33.7^{0}c, respectively. Relative humidity ranged from 66% to 75%.The thirty six pots were placed in the glass house and their arrangement were randomized based on a draw from a lot. The pots were watered regularly with a sterile distilled water to keep the soil moisture at field capacity. The number of seedlings in each pot was thinned down to four after ten days of emergence.

2.8. Preparation of inoculum

Tryptone Soya Broth (TSB) slants of *Azospirillum* isolates maintained at 4^0C were used as stock cultures for inoculant preparation. A loop full of the stock culture was inoculated into nutrient broth tubes and incubated at 37^0C for 24 hr. A loop full of the liquid culture was then streaked onto nutrient agar plates to check purity. An isolated colony was then inoculated into nutrient broth tubes and a 24 hr culture from these tubes was streaked on TSBA plates and incubated at 37^0 C for 24 hr. The culture on a TSB agar was chopped into pieces and inoculated into a 250-ml Erlenmeyer flasks containing TSB and incubated with gentle shaking at 37^0C. The bacterium was harvested by centrifugation (10min 3000 x g) at early stationary phase (36 hr.) and washed three times in sterile saline solution (0.85% NaCl). It was then resuspended in sterile distilled water and adjusted to the required cell concentration (10^7-10^8 cells/ml) at 600nm using a spectrophotometer for inoculation of plants (Kapulnik, *et al.*, 1985b; Suikiman and New, 1990).

2.9 Inoculation of seedlings with *Azospirillum* isolates.

Fifteen and twenty days after planting 1.0 ml of bacterial suspension was injected near the root system of each plant with a sterile disposable 1ml syringe (Kucey, 1988). Control pots were inoculated similarly with equal amount of sterile distilled water. Cross-contamination was minimized by having a distance of 20 cm between pots of different treatments.

2.10 Growth parameters of standing crop

A difference in Plant height (i.e. from the base of primary tiller to the tip of the panicle at maturity) was recorded for each plant in each pot. And the number of productive tillers were counted for all plants in each pot.

2.11.Measurement of *tef* yield

Following harvest (seventy four days after sowing), the root mass of four plants in each pot was carefully dug out from the soil and adhering soil particles removed by hand and thoroughly washed with water by keeping it in bowl filled with water for 20 to 30 min. Fine root pieces were retrieved by passing the water through 0.5mm sieve (Kirchmann, 1988). The shoot part of each sample was hand threshed and grain yield (g) per plant was recorded before the shoot was oven dried. Fresh weight of shoot and root was recorded using Mettler E2000 sensitive balance. Dry matter yield was also recorded after drying the shoot and root samples at 60^0C for 48 hr in an oven.

2.12 Determination of nitrogen content of *tef* grains

Dried grain samples from each pot were milled into powder using a coffee grinder, passed through 0.5mm sieve, and 0.3g sub sample weighed on analytical balance. Each sub sample was then subjected to Kjeldahl digestion for the determination of total nitrogen in samples.

Total nitrogen was determined following the macro kjeldahl procedure as follows. The method involves three processes: digestion, distillation and titration. During digestion, organic

nitrogen is converted to ammonium-nitrogen with the help of potassium sulfate which raises the temperature when cupric sulfate is used as a catalyst.

Organic nitrogen + H_2SO_4..K_2SO_4.....$(NH_4)_2SO_4$ + H_2O

then the amount of nitrogen is estimated from the amount of ammonia liberated by distilling the digest with alkali (NaOH).

$2(NH_4)2SO_4 + 4NaOH$..... $2Na_2SO_4 + 4NH_3 + 4H_2O$

The ammonia liberated is trapped by boric acid and titrated with HCl

$NH_3 + H_3BO_3$.....$NH_4H_2BO_3$

$NH_4H_2BO_3 + HCl$.....$NH_4Cl + H_3BO_3$

2.12.1.Reagent preparation

A) Sulfuric acid-Selenium mixture: Selenium powder (3.5g) was dissolved in one liter of sulfuric acid by heating to about 330^0 C, while covering with watch glass until color changes to light yellow (in about 3 to 4 hr.)

B) Digestion mixture: Salicylic acid (7.2g) was dissolved in 100 ml of the sulfuric acid-selenium mixture.

C) Mixed indicator:-0.5g of bromocresol green plus 0.1g methyl red was dissolved in 100ml of 95% ethanol and the pH adjusted to 4.5.

D) Receiver solution:- A 4% boric acid receiver solution was prepared by dissolving 40g boric acid in about 600 ml of very hot deionized water. The solution was then mixed and cooled to room temperature. This was followed by addition of 10ml of bromocresol green solution

(100mg in 100ml methanol) and 7ml of methyl red solution (100mg in 100ml of methanol). The whole mixture was then diluted to one liter with hot deionized water and gently mixed. Twenty five milliliter of this solution was transferred to a 250ml Erlenmeyer flask to which 100ml distilled water was added. Based on the color of this mixture, two to three drops of 0.1M HCl or 0.1M NaOH was added to the stock solution. This was followed by the transfer of 25ml portion of the solution and mixing it with water until color change appeared. This procedure was repeated successively until the 25ml boric acid and 100ml water mixture assumed a neutral grey color. This color was used as a reference end point for titration of ammonia with acid in the determination of ammonia in samples.

2.12.2 Kjeldahl Digestion procedure

A finely grounded grain sub sample (0.3g) was transferred to a digestion tube to which 2.5ml of the digestion mixture (B) added. The mixture was carefully swirled to moisten the plant material and allowed to stand for at least 2h The tubes were then placed in a heating block and heated at 100°C for at least 2h after which time they were removed, cooled, and three 1ml 30% H_2O_2 solution successively added while mixing it after each addition. The tubes were again placed on the preheated block and heated at 330° C until the digest turned to colorless or light yellow (in about 2hr). After removing the tubes from the block, it was cooled at room temperature and 48.3ml of distilled water added to the digest and mixed. This mixture was allowed to stand over night. Total nitrogen in plant samples was determined by distillation of the aliquot (50ml) from the digest with 40% sodium hydroxide, which was received in 25ml of 4% boric acid (in a kjeltec 1002 distilling unit). It was then titrated with 0.1N HCl to the end point of mixed acid indicators. A Blank tube with out sample was treated similarly as the experimental tubes.

Total nitrogen in the sample was calculated using the formula:

$$\%N = \frac{(T-B) \times N \times 14.007 \times 100}{\text{weight of the sample in mg}}$$

Where, T-titration volume for the sample

B-titration volume for the blank

N-normality of the acid

The total nitrogen in grains was determined as mean grain nitrogen of four plants per pot for each treatment.

2.13.Determination of crude protein in grain

Total grain protein was calculated by multiplying the total grain nitrogen with a conversion factor of 5.7. This conversion factor was recommended by Tkachuk *and* Trivna (1969) and Tkachuk (1977) cited in Alemayehu (1995).

2.14.Determination of total nitrogen in soil samples

The initial and final nitrogen content of soil used in pot experiment was determined by the Kjeldahl procedure. A one gram finely grounded sub samples from the original bulk soil (before sowing *tef* seeds) and from each of the thirty six pots (after harvest) were all subjected to the procedure as follows:

One-gram soil sub sample was mixed with 0.8g of $CuSO_4.5H_2O$ and 7g of K_2SO_4 into a digestion tube. And 12ml of concentrated H_2SO_4 was added to the mixture. These tubes were

placed on the digestion block, which was heated to 420° C. The digestion was run for 2hr at this temperature. The tubes were then removed from the block and allowed to cool for 10-20min. 75ml of deionized water was added to the digest in each tube and allowed to stand over night. A blank digest with out a soil sample was similarly treated as in experimental tubes. Total nitrogen content of samples was calculated as in section 2.13.2

2.15.Statstical Analysis

For each data mean values per treatment were calculated. Data were subjected to one way analysis of variance and comparison of means at 5% level was tested by LSD. Statistical analysis was done using a statistical program packet; STATSTICA.

3 RESULT

3.1 Characterization and identification of bacterial isolates

Preliminary screening of twelve isolates in semi-solid Nitrogen free (Nfb) media yielded six microaerophilic diazotrophs (i.e. capable of growing in Nfb and forming typical subsurface pellicle). Further morphological and cultural characterization of pure isolates reduced the possible candidates to five. These isolates were then subjected to physiological and biochemical tests and two of them identified as bacteria belonging to the genus *Azospirillum*. Both isolates were recovered from Debrezeit light soil. No *Azospirillum* isolate was discovered from tef roots grown on soil from Holetta. Isolate A31 was recovered from the tef variety DZ-01-354, whereas isolate A32 from the tef variety DZ-01-196.

The two isolates were motile, gram negative, slightly curved short rods that can grow in Nfb medium forming a subsurface pellicle (Table 1). The growth in Nitrogen Free Bromothymol blue (NFB) medium was always accompanied by alkali production. Microscopic examination of wet mount of the actively growing cultures showed slightly curved short rods which were actively motile. Physiological and biochemical characteristics (Table.2) of the two isolates were used to identify the genera. Further confirmation with regard to their identity was obtained by comparing these characteristics with those of the type cultures described by Tarrand *et al.*(1978) and Wong *et al.*(1980).

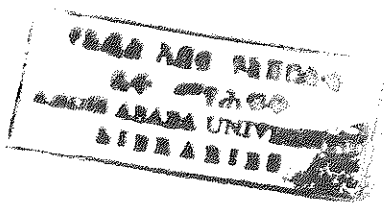


Table.1 Morphological and cultural characteristics of the two bacterial isolates

Isolate code	Colony characteristics					morphological characteristics			growth characteristics in Nfb medium.		Alkalinization of Nitrogen Free Bromothymol blue media (NFB)
	size	color	form	margn	elevatin	gram rxn	shape	motility	pellicle	D. F. S•	
A31	1.5mm	white	circular	entire	raised	-	s.c.rod*	+	present	2-3mm	+
A32	2.0mm	pink	circular	entire	raised	-	s.c.rod*	+	present	1mm	+

* s.c rod- slightly curved rods

• D. F. S-distance from the surface

Table 2. Physiological and biochemical characteristics of isolate A31 and A32 compared to *Azospirillum* type cultures (*A. lipoferum* ATCC 29707 and *A. brasilense* ATCC 29145)

	CHARACTERSTICS	Isolate A31	Isolate A32	*A. lipoferum*	*A. brasilense*
1	Cytochrome oxidase	+(strong)	+(moderate)	+	+
2	Catalase	+(strong)	+(moderate)	+	+
3	Esculin hydrolysis	+	+	+	+
4	Acidification of glucose media				
	4.1 yeast extract based broth	+	+	+	-
	4.2 yeast extract based broth(with 2x phosphate)	+	+	+	-
	4.3 peptone based broth	+	+	+	-
5	Acidification from glucose and fructose anaerobically				
	5.1 glucose(yeast extract)	+	+	+	-
	5.2 glucose(peptone)	+	+	+	-
	5.3 fructose(yeast extract)	+	+	+	-
	5.4 fructose(peptone)	+	+	+	-
6	Sole carbon source				
	6.1 glucose	+	+	+	-
	6.2 mannitol	+	+	+	+
	6.3 fructose	+	+	+	-
	6.4 malate	+	+	+	-
7	Indole production	-	-	-	-
8	Hydrogen sulfide production	-	-	-	-
9	Starch hydrolysis	-	-	-	-
10	Acidification of sugars				
	10.1glucose	+	+	+	-
	10.2 mannitol	+	+	+	-
	10.3 maltose	-	-	-	-
	10.4 xylose	+	+	+	-
	10.5 fructose	+	+	+	+
	10.6 lactose	-	-	-	-
	10.7 galactose	+	+	+	+
11	Urease	+	+	+	+
12	Denitrification	+	-	-	-
13	Dissimilation of NO_3 to NO_2	+	+	+	+
14	Growth in 3% NaCl	-	-	-	-
15	Biotin requirement	+	+	+	-

+- positive for the test _ negative for the test

3.2.Tillering of two *tef* varieties as influenced by inoculation

Inoculation of *tef* seedlings with *Azospirillum* isolates increased the mean fertile tiller number of both varieties (Table.3.). But none of these differences were significant (at $p<0.05$). A mean tiller number increase of 10.3% and 6.9% was obtained due to inoculation of DZ-01-196 seedlings with the bacterial isolate A32 and A31, respectively. And a mean increase of 10.8% and 10.5% was obtained upon inoculation of DZ-01-354 seedlings with isolate A32 and A31, respectively. Although isolate A32 produced a higher increment in tillering than A31 on both varieties the difference between bacterial treatments was not significant ($p< 0.05$) for both varieties.

Table 3 Effect of *Azospirillum* isolates on height and tillering ratio of two *tef* varieties.

Treatment	Height(cm)	Tiller
Dis-196	111.600 ± 1.511^{a}	3.625 ± 0.875^{a}
A31-196	117.882 ± 1.30^{b}	3.87 ± 0.612^{a}
A32-196	120.970 ± 1.240^{c}	4.00 ± 0.780^{a}

Treatment	Height(cm)	Tiller
Dis-354	102.400 ± 1.83^{a}	3.875 ± 0.850^{b}
A31-354	105.020 ± 1.321^{b}	4.283 ± 0.588^{b}
A32-354	106.950 ± 1.350^{c}	4.292 ± 0.624^{b}

Numbers are means and s.d of six replicates (4 plants in each pot)

Numbers in the same column followed by the same letter do not differ significantly at $p< 0.05$.

In tables 3-8 Dis-196 is to mean DZ-01-196 variety inoculated with sterile distilled water, similarly A31-196 mean DZ-01-196 variety inoculated with isolate A31, and A32-196 mean Dz-01-196 inoculated with A32. Accordingly the same holds true for the tef variety DZ-01-354.

3.3.Height of two *tef* varieties as influenced by inoculation

At harvest it was found that inoculation of *tef* with either of the bacterial isolates significantly increased the height of *tef* plants irrespective of the *tef* variety considered. As illustrated in Table 3 a marked difference existed between inoculated groups of the two varieties and their respective controls. Both varieties responded better when inoculated with bacterial isolate A32

than isolate A31. Moreover, a significant difference in mean height between different bacterial treatments was observed in both varieties.

3 4.Dry matter yields of two *tef* varieties as influenced by inoculation

The shoot and root dry matter yields of the two *tef* varieties is presented in Table 4. The mean shoot dry matter yield of both varieties was increased by inoculation with *Azospirillum* isolates. Both bacterial isolates produced a significantly higher dry matter (shoot and root) yield of DZ-01-196 variety. But a higher percent increase was obtained by inoculation of these varieties with isolate A32 (5.2% and 25.8% for shoot and root, respectively) than A31 (4.9% and 15.3% for shoot and root, respectively). In general, there is no significant difference ($p< 0.05$) in dry matter yields between different bacterial treatments of DZ-01-196 variety.

On the other hand, both bacterial isolates produced a higher (5.2% and 4.9% for A32 and A31, respectively) but non significant increase in shoot dry matter yield of DZ-01-354 variety. Root dry matter yield was also increased by inoculation with both bacterial isolates. However, a significant increase (21.9%) was obtained only upon inoculation of this variety with isolate A32. In general, in contrast to shoot dry matter yield, there is a significant root dry matter yield difference between different bacterial treatments of DZ-01-354 variety.

Table 4 Effect of *Azospirillum* isolates on shoot and root dry matter accumulation of two *tef* varieties

TEATMENT	SHOOT DWT(g)	ROOT DWT(g)	TREATMENT	SHOOT DWT(g)	ROOT DWT(g)
Dis-196	4.155 ± 0.267^{a}	0.382 ± 0.066^{b}	Dis-354	4.741 ± 0.410^{a}	0.427 ± 0.068^{e}
A31-196	4.360 ± 0.312^{b}	0.440 ± 0.087^{a}	A31-354	4.810 ± 0.245^{a}	0.467 ± 0.082^{d}
A32-196	4.370 ± 0.282b	0.480 ± 0.103^{a}	A32-354	4.836 ± 0.445^{a}	0.520 ± 0.062^{e}

Numbers are means and s.d of six replicates (4 plants in each pot)

Numbers in the same column followed by the same letter do not differ significantly at $p< 0.05$.; DWT- dry weight

Table 5 Effect of *Azospirillum* isolates on shoot and root fresh weight of two *tef* varieties.

Treatment	Shoot FWT(g)	Root FWT(g)	Treatment	Shoot FWT(g)	Root FWT(g)
Dis-196	9.280 ± 0.560^{b}	2.422 ± 0.083^{a}	Dis-354	10.448 ± 0.498^{a}	2.748 ± 0.099^{a}
A31-196	9.657 ± 0.490^{a}	2.738 ± 0.087^{b}	A31-354	10.627 ± 0.427^{a}	2.917 ± 0.078^{c}
A32-196	9.707 ± 0.496^{a}	2.968 ± 0.079^{c}	A32-354	10.668 ± 0.498^{a}	3.055 ± 0.080^{d}

Numbers are means and s.d of six replicates (4 plants in each pot)

Numbers in the same column followed by the same letter do not differ significantly at $p< 0.05$.

FWT- fresh weight

3.5.The influence of *Azospirillum* isolates on grain yield of two *tef* varieties

As shown in Table.5, inoculation of *tef* seedlings with both *Azospirilla* isolates produced a significant ($P<0.05$) grain yield increases. As in tillering, isolate A32 produced a higher percentage increase on both varieties than A31. Inoculation of DZ-01-196 seedlings with isolate A32 gave 11.7% grain yield increase over un inoculated control. While inoculation of DZ-01-354 seedlings with a similar isolate produced a 4.4% grain yield increase over the

respective control. Grain yield differences between different bacterial treatments was significant ($p<.05$) for both varieties.

3.6. The effect of *Azospirillum* isolates on grain nitrogen content of two *tef* varieties

The mean grain nitrogen content of inoculated and uninoculated plants is shown in Table 6. Inoculation of these varieties with *Azospirillum* isolates A31 and A32 significantly ($p<0.05$) increased the mean grain nitrogen when compared to uninoculated controls. Bacterial isolate A32 caused a significantly higher grain nitrogen accumulation on both varieties than isolate A31. There is no significant difference in mean grain nitrogen between inoculated groups of DZ-01-354 variety. But this difference is significant between inoculated plants of DZ-01-196 variety.

Table 6. Effect of *Azospirillum* inoculation on total nitrogen and grain yield per plant of two *tef* varieties.

Treatment	Grain yield(g)	% N in grain	Treatment	Grain yield(g)	%N in grain
Dis-196	1.248 ± 0.068^{b}	2.010 ± 0.063^{a}	Dis-354	1.632 ± 0.074^{d}	2.020 ± 0.077^{a}
A31-196	1.382 ± 0.062^{a}	2.090 ± 0.063^{b}	A31-354	1.690 ± 0.062^{e}	2.088 ± 0.054^{b}
A32-196	1.394 ± 0.069^{a}	2.126 ± 0.069^{c}	A32-354	1.704 ± 0.056^{e}	2.096 ± 0.040^{b}

Numbers are means and s.d of six replicates (4 plants in each pot)

Numbers in the same column followed by the same letter do not differ significantly at $p< 0.05$.

3.7.Total grain protein content of two *tef* varieties as influenced by inoculation.

The mean total grain protein content of inoculated and un inoculated *tef* varieties is presented in Table 7. The general trend and the differences between treatments are the same as in Table 6. Hence, DZ-01-196 seedlings inoculated with isolate A32 have the highest total grain protein among inoculated groups. The percentage increase in inoculated groups over their respective controls ranged from 3.4-5.8%. In general, the inoculation of the two *tef* varieties has improved the mean total grain protein.

Table 7. Effect of *Azospirillum* inoculation on total protein content of grains of two *tef* varieties.

Treatment	Total grain protein (%)	Treatment	Total grain protein (%)
A31-196	11.913 ± 0.431^{a}	A31-354	11.901 ± 0.290^{a}
A32-196	12.118 ± 0.268^{b}	A32-354	11.947 ± 0.216^{a}
Dis-196	11.457 ± 0.337^{c}	Dis-354	11.514 ± 0.435^{b}

Numbers are means and s.d of six replicates (4 plants in each pot)

Numbers in the same column followed by the same letter do not differ significantly at $p < 0.05$.

3.8.Root shoot ratio of two *tef* varieties as influenced by inoculation.

Table 8 shows root shoot ratio on fresh weight and dry weight basis of inoculated and uninoculated groups. Inoculation with either of the isolates produced a significantly ($p < 0.05$) higher percentage increase in root shoot ratio of both varieties on fresh weight basis. The bacterial isolate A32 induced the highest root shoot ratio on both varieties on fresh and dry

weight basis. Significant ($p < 0.05$) differences in root shoot ratio in fresh and dry weight basis were also observed for different bacterial treatments of both varieties. The percent increase over the respective control group is higher for the *tef* variety DZ-01-196.

Table 8 Effect of *Azospirillum* inoculation on root shoot ratio of two *tef* varieties.

Treatment	DWT-RSR	FWT-RSR	Treatment	DWT-RSR	FWT-RSR
Dis-196	0.091 ± 0.015^{a}	0.261 ± 0.014^{c}	Dis-354	0.090 ± 0.012^{a}	0.262 ± 0.010^{c}
A31-196	0.100 ± 0.017^{a}	0.284 ± 0.012^{a}	A31-354	0.096 ± 0.016^{a}	0.274 ± 0.010^{a}
A32-196	0.109 ± 0.023^{b}	0.306 ± 0.014^{b}	A32-354	0.107 ± 0.012^{b}	0.286 ± 0.012^{b}

Numbers are means and s.d of six replicates (4 plants in each pot)

Numbers in the same column followed by the same letter do not differ significantly at $p < 0.05$.

DWT RSR- root shoot ratio on dry weight basis FWT RSR- root shoot ratio on fresh weight basis.

4.DISCUSSION

4.1.Azospirillum isolates from *tef* rhizoplane

The rhizosphere of plants is associated with intense microbial activity (Alexander,1977; Hartman,1988). Nitrogen fixing microorganisms (Diazotrophs) are generally enriched in the rhizosphere soil compared with non-rhizosphere soil (Reinhold, 1986). Several Diazotrophic genera including *Azospirillum* are mostly present in the root environment of different cereals and grasses (Reinhold, 1986; Boddey and Doberiener, 1988; Boonjawat *et al.*,1991) *Azospirillum* spp. have been isolated from the roots of various graminae like wheat (Rai and Gaur, 1982; Chermisov and Kina, 1984; Bahattarai and Hess, 1993), maize (Fages and Arsac, 1991), sorgum (Berge *et al.*, 1991), corn, barley, rye and other grasses (de Coninck, 1988).

In the present study two bacterial isolates, which belong to the genus *Azospirillum*, were isolated from the root rhizoplane of *tef* (*Eragrostis tef*) at their vegetative growth stage. The characteristics of these isolates closely resemble that of the type culture ATCC 29707 (*A.lipoferum*). Neither of these isolates behaved differentially except in two cases. Firstly, isolate A31 gave a strong positive oxidase and catalase reactions whereas isolate A32 was found to be moderate for both tests. Secondly, while A32 reduced nitrate to nitrite only, A31 further reduced nitrite to molecular nitrogen, suggesting the denitrifying nature of the latter.

The slight deviations observed in oxidase and catalase tests may be attributed to the inoculum size or the amount of reagent used for the test or to errors in visual assessment of the reactions. As to the nitrate reduction property, *Azospirillum* spp. are distinguished by its versatility with respect to nitrogen metabolism (Dannberg *et al.*,1986). They have the ability

to fix atmospheric nitrogen in common but most of them have the denitrifying character (Hartmann and Zimmer, 1994). Rai and Gaur (1982) reported a variation in nitrate metabolism among three *Azospirillum lipoferum* isolates of which one was capable of denitrification while the other two were non-denitrifiers.

4.2.Effect of inoculation on root weight and root shoot ratio of two *tef* varieties.

Alteration of root morphology has been shown to be one of the few direct effects of *Azospirillum* on different plants (Okon and Kapulnik, 1986). *Azospirillum* inoculation on different plant roots was demonstrated to affect different morphological characteristics of roots. Inoculation increased the number of root hairs, root length and surface area (Kapulnik *et al.*, 1985a,b; Zimmer and Bothe, 1988) in hydroponic systems. Similar increases in fresh and dry weight of roots has been obtained for inoculated plants grown in pots on a soil substrate(Kapulnik *et al.*,1985a; Lee *et al.*, 1989).

The findings in the present study showed that mean fresh and dry weight of roots of both *tef* varieties was increased by inoculation with *Azospirillum* isolate A31 and A32 (Table 4 and 5). Root fresh weight of *tef* plants was increased significantly by 13.1% and 22.6% in DZ-01-196, or 6.13%and 11.2 and in DZ-01-354 varieties when inoculated with isolate A31 and A32, respectively. A corresponding root dry weight increment of 15.3% and 25.8%for DZ-01-354 varieties was obtained upon inoculation with isolate A31 and A32,respectively. These increases confirm the observations of Kapulnik *et al.* (1981a) on wheat, sorghum and panicum of Lee *et al.* (1989) on corn and of Schank *et al.* (1981) on digit grass (Digitaria spp.) although the percent increases in each observation varied.

In the present pot experiment due to a limited space in the pots, the root parts of the plants in each pot were entangled together forming a thick mesh of root hairs at the base of the pots and it was difficult to separate individual root systems. As a result, it became impossible to measure the root length or any other morphological characteristics of these plants. Therefore, differences in root development between inoculated and uninoculated controls were compared based on root weight measurements. However, the difference in root bulk between inoculated and uninoculated plants was obvious from visual observation.

The *Azospirillum* mode of enhancing plant growth is an open question (Bashan *and* Levanony, 1991; Bahattarai *and* Hess, 1993). Among the several mechanisms proposed so far, increased mineral uptake by the plant as a result of root proliferation and development due to inoculation has been suggested to play an essential role (Lin *et al.*, 1983; Kapulnik *et al.*, 1985a,b; Sarig *et al.*, 1988; Fallik and Okon, 1996). The effect of the bacteria on root development has been frequently related to the production of phytohormones (Tien *et al.*, 1979 cited in Harari *et al.*, 1988, Bottoni *et al.*, 1989; Sriskandarajah *et al.*, 1993) and to vitamins (Rodelas *et al.*,1993) by the colonizing bacteria. A higher level of Indolo-3-acetic acid has been observed in *Azospirillum* inoculated roots of maize plants as compared to uninoculated controls (Bellone, 1982; Fallik *et al.*, 1989). Moreover, alteration of root morphology, similar to that caused by *Azospirillum*, was mimicked through the application of Indole-3-acetic acid on roots (Kucey, 1988).

Jain and Patriquin (1984) observed a consistent positive growth responses on wheat seedlings that exhibited root hair deformation or an increase in root mass upon inoculation with different *Azospirillum* spp. These authors later suggested that root hair deformation and root

mass may be used as an indicator for a positive growth response due to *Azospirillum* inoculation. Similarly, Fallik *et al.* (1988) recommended that root surface area as the most reliable criterion in evaluation and measurement of the growth response of *Azospirillum* inoculated maize seedlings.

The observed increases in root fresh weight of inoculated plants could suggest the fact that these plants accumulate more water in their roots than un inoculated controls. And this could be due to a better water extracting ability of the roots as root ramification increases the absorbing surface. *Azospirillum* inoculation has been shown to affect the water status of plants (Okon and Kapulnik, 1986). Sarig *et al.* (1988) reported a 15% significant increase in total soil moisture extraction of inoculated roots of sorghum over un inoculated controls. On the other hand, root dry matter increases of *tef* plants can be associated with better root development with longer and more number of root parts together with a higher accumulation of nutrients (mainly nitrogen) in the root system. A higher root nitrogen accumulation than controls has been obtained for sorghum (Pacovsky *et al.*, 1985), wheat (Jain and Patriquin, 1984) and *Zea mays* (Lin *et al.*, 1983). According to Dart (1986) sorghum plants grown in pots accumulated a substantial (33% of the total) amount of nitrogen in the root medium.

Additional indirect evidences on the effect of inoculation on root development was produced by comparing the root shoot ratios of inoculated and control groups. As shown in table 8 The mean root shoot ratio of inoculated *tef* varieties was higher than the control groups. The percentage increase of root shoot ratio on dry weight bases ranged from 7.6-19.6% over and above that of the control groups. A high root shoot ratio is a clear indication of better root development. The results showed that the associative bacteria accelerated growth of the

underground parts more than those of the upper ground parts of *tef* plants thus caused a decrease in shoot root ratio.

4.3 Effect of inoculation on grain yield of two *tef* varieties

Inoculation of various cereals with *Azospirillum* isolates have been shown to affect the yield and/or different growth parameters (Taylor, 1979; Kapulnik *et al.*, 1981b; Millet and Feldman, 1984; Sarig *et al.*, 1988). Inoculation of *Eragrostis tef* varieties with the homologous (bacteria isolated from the same host as that to be inoculated) (Boddey and Doberiener, 1988) *Azospirillum* isolates significantly increased their grain yield. A higher yield responses were observed for the *tef* variety DZ-01-196 compared to DZ-01-354. Inoculation of the former with either of these bacterial isolates produce a minimum of 10.7% mean grain yield increment over the uninoculated control. The increase in grain yield for the DZ-01-354 variety did not exceed 4.41%.

Similar yield increments of varying degree have been observed for different plants upon inoculation with different *Azospirillum* spp. Mertens and Hess (1984) reported a yield increase ranging from 8 to 32% upon inoculation of a wheat variety with *A. lipoferum* in a consecutive pot experiments. Bahattarai and Hess (1993) observed a grain yield response varying from 8.8 to 25.2% among five different cultivars of wheat when inoculated with a single *A. brasilense* strain 10SW. These authors further investigated the response of two wheat cultivars to inoculation with two different *A. brasilense* strains. The results showed that each wheat cultivar responded differently to different bacterial inoculation. In a similar pot experiment Millet *et al.*(1984) examined the yield responses of twenty wheat lines to

inoculation with a single *Azospirillum* isolate. Out of these lines only two responded with a significant yield increase while others responded with a non-significant or negative increases.

In *Azospirillum*-plant root interaction, the existence of some degree of specificity that could affect the success of inoculation have been discussed (Millet *et al.*, 1984; Doberiener, 1988). While the direction of response appears to be determined largely by the bacterial genome at the strain level, the magnitude of response, on the other hand, is dictated primarily by the host genome at the cultivar level (Millet *et al.*, 1984; Jain *and* Patriquin, 1984). A comparison between the mean yield increases of the two *tef* varieties over their respective controls revealed that the variety DZ-01-196 gave a yield increase of at least two and half times higher than that of DZ-01-354. On the other hand, a higher mean yield was obtained for both varieties upon inoculation with the bacterial isolate A32. However, this difference in yield among different bacterial treatments was not significant for both varieties.

The observed differences in magnitude of response between the two *tef* varieties agreed with the observations of Millet *et al.* (1984) for different wheat lines. And that of Arsac *et al.* (1991) for *Zea mays* hybrids upon inoculation with a single *A. lipoferum* isolate. Moreover, the effect of different bacterial treatments on the yield responses obtained for these *tef* varieties appears to agree with the reports of Bahattarai and Hess (1993) for the two wheat lines. Thus, the observation in this study may suggest a possible existence of specificity in *Azospirillum*-tef association that could affect the magnitude of response.

4.4.Effect of inoculation on total nitrogen and protein content of two *tef* varieties.

Inoculation increased the mean percentage nitrogen and total protein of grains of both varieties (Table 6 and 7). As for the other growth parameters, the *tef* variety DZ-01-196 responded better than DZ-01-354 and hence accumulated more nitrogen in its grains. Accordingly, the total protein content of grains was found higher in *tef* variety DZ-01-196 than DZ-01-354. Since total protein in grains was calculated from the percent grain nitrogen using a multiplying factor of 5.7 (Rao *et al.*, 1977; Alemayehu, 1995), a difference in total grain protein between the two varieties is apparently the direct consequence of the difference in total nitrogen of grains.

A similar difference in total grain nitrogen between inoculated and control groups have been reported for various cereals including wheat, sorghum and panicum (Kapulnik *et al.*, 1981b) in green house and for pearl millet (Wani *et al.*, 1985) in field conditions. Mertens and Hess (1984) reported an increase in total grain nitrogen of *A. lipoferum* inoculated wheat plants ranging from 10.3 to 10.8 percent Bahattarai and Hess (1993) observed an increased grain nitrogen of spring wheat by 10.25 percent upon inoculation with A. *lipoferum*. In the present study, the highest and the lowest percent grain nitrogen and total protein content of grains among inoculated groups were 2.1 and 12.1 (for *tef* variety DZ-01-196) and 2.088 and 11.901 (for *tef* variety DZ-01-354), respectively.

None of the above authors have reported the effect of *Azospirillum* inoculation on total grain protein of the cereals they considered. However, from the grain nitrogen increase it is apparent that the total grain protein should have been also increased On the other hand,

Zambre *et al.* (1984) observed a 5 percent increase in grain protein upon inoculation with *A. brasilense*. In fact, these authors used a conversion factor of 5.83 in calculating the total protein. Contrary to the result in this experiment and that of Zambre *et al.* (1984), Millet and Feldman (1984) observed no apparent effect of inoculation on total grain protein of wheat.

Grain protein content (GPC) can be considered as the amount of protein per seed or unit weight of grain (Anderson, 1977 cited in Alemayehu, 1995). It is markedly influenced by the ability of the plant to absorb adequate nitrogen from the soil, and its translocation from the vegetative parts to the developing grain (Rao *et al.*, 1977). The present result (Table 6 and 7) showed that the two *tef* varieties (control groups) do not differ significantly in the total grain nitrogen. However, inoculation with either of the bacterial isolates produced a marked and significant difference in total grain nitrogen between the two varieties grown on soils of similar fertility. Alemayehu (1995) observed a significant and considerable variation in grain protein content of twelve *tef* genotypes when grown in three different localities. However, he found that the difference in grain protein content due to location to be higher than the difference due to genotype..

The possible reason for the observed difference (Table 6 *and* 7) between inoculated and un inoculated groups can be explained as follows. Inoculated groups of both variety posses a well developed (with respect to weight) and perhaps with much more root hairs than un inoculated groups. These alterations in root morphology possibly caused by the introduced *Azospirilla* (Kapulnik *et al.*, 1985b; Okon and Kapulnik, 1986) subsequently increased the nutrient uptake efficiency of inoculated roots. Hence, with an efficient uptake system inoculated plants were able to absorb a higher amount of nitrogen (Lin *et al.*, 1983; Jain and Patriquin, 1984;

Okon and Kapulnik,1986) and translocate it to the growing seeds resulting in high protein content.

On the other hand, various authors reported a positive correlation between nitrate reductase (the first enzyme in nitrate assimilation) activity (NRA) with grain nitrogen and protein content of grains (Deckard *et al.*, 1973; Eilrich *and* Hageman, 1973; Croy *and* Hageman, 1973; Rao *et al.*, 1977). Recently, Ribaudo *et al.* (1996) found a high NRA both in the root and shoot of *A. lipoferum* inoculated pot grown corn plants This increase in NRA was accompanied by a considerable increase in the weight and volume of roots and dry matter yield of plant tops. Earlier the substrate (ammonia or nitrate) inducibility of nitrate reductase was reported by Legesse and Asfaw 1984 for the *tef* variety DZ-01-354. Later on Amare and Legesse 1984 found the highest NRA for *tef* varieties DZ-01-196 and DZ-01-354 when supplied with 2mM and 8mM KNO_3 , respectively among the four varieties considered Although no investigation was made with respect to NRA of *tef* plants in the present study, the existence of a higher NRA in the two *tef* varieties and its inducibility in combination with the purported increased uptake of nutrients by *Azospirillum* inoculated roots together with a high NRA may have occurred in the inoculated *tef* plants.

As to the differences in percent grain nitrogen and protein content of grains between different bacterial treatments, the two bacterial isolates basically differ in one aspect of nitrogen metabolism as shown Table 2. Isolate A31 was found to be capable of denitrification, but not A32. The dissimilatory conversion of nitrate to nitrite by soil bacteria like *Azospirillum* do not affect the total amount of nitrogen available for plants, the further reduction of nitrite to gaseous nitrogen compounds results in a loss of combined nitrogen in soil (Dart, 1986;

Hartmann *and* Zimmer, 1994). Hence, the comparatively low grain nitrogen and total protein in A31 inoculated plants may have been caused by low soil nitrogen level due to the bacteria's denitrifying effect.

4.5.Effect of inoculation on fresh and dry matter yield of shoots of two *tef* varieties.

As compared to the improvements in other growth parameters observed in this experiment, the total shoot growth response of both *tef* varieties was relatively low. However, a significant ($p<0.05$) increase in fresh weight accumulation due to inoculation was obtained for both varieties, although this increase was small for the *tef* variety DZ-01-354. Moreover, the increase in dry matter yield was also significant ($p<0.05$) for the *tef* variety DZ-01-196 but not for DZ-01-354 variety.

Total shoot weight increments due to inoculation was reported for wheat (Kapulnik *et al.*, 1985a) in hydroponic systems, for wheat, sorghum and panicum (Kapulnik *et al.*, 1981a) in pot cultures. In similar pot experiments a slight (2.5%) dry matter increases have been observed for maize (Albrecht *et al.*, 1981) for digit grass (8.5%) (Schank *et al.*, 1981) and a much higher increase (33.7%) for maize (Lee *et al.*, 1989). Total shoot weight increases have also been reported in field trials for maize (20.1%) and panicum (10.32%) (Kapulnik *et al.*, 1981b). The result of this study further confirmed the growth promoting effect of *Azospirillum* spp. The increase in shoot weight either in dry or fresh weight basis may appear smaller than the above reports. However, such differences in the magnitude of positive responses should be the result of an interactive effect between the plant species, bacterial strain and environmental factors that differ considerably in each experimental condition.

After observing the low yield increases in shoot dry matter and high increases in total shoot nitrogen, Bahattarai and Hess (1993) concluded that the positive effect of inoculation might have occurred by the transfer of atmospheric nitrogen to the plant through bacterial nitrogen fixation. The induction of nitrogenase activity of *Azospirillum* in association with wheat in the in vitro experiments (Hess, 1982) and the significant increase in percentage of nitrogen in grain (Kapulnik *et al.*, 1981a; Millet *and* Feldman, 1984), support the hypothesis that biological nitrogen fixation by *Azospirillum*-root association may contribute to the nitrogen economy of inoculated plants. The result (Table 4 and 5) in this study revealed that the percent increases in dry matter yields was more or less equivalent to the increase in total grain nitrogen. Hence, although the possibility of biological nitrogen fixation can not be ignored, the observations (on root weight increases) seems to favor the role of improved nutrient uptake as a result of efficient root system.

On the other hand, the increase in fresh weight of *tef* varieties (though very small in DZ-01-354) inoculated with either isolate may show the beneficial role of inoculation in improving the water uptake of plants. *Azospirillum* inoculation has been reported to improve the water status of plants (Okon and Kapulnik, 1986). Sarig *et al.* (1988) reported an improvement in the water status of sorghum plants due to inoculation. Such observations could have interesting implications when considering the role of *Azospirillum* inoculation in soils with low rain fall areas or when plants face occasional water stress conditions. Abuhay (1997) reported a decrease in fresh and dry weight of shoots and number of productive tillers by 53.8%, 49.2% and 22.6%, respectively in *tef* plants exposed to water stress. Moreover, the difference in shoot fresh weight of inoculated and un inoculated plants is important if growing

conditions during grain filling are difficult, when stem reserves are translocated to the developing grain (Boyer and Mc pherson, 1975).

Tef has a wide range of utilization in the country. *Tef* straw mixed with clay (mud) can be used in constructing houses. Most importantly *tef* straw is very important as animal feed particularly in the dry season. Much more interesting is that, at Holetta, steers fed on *tef* straw have been found to gain more weight than those fed on wheat straw, oat hay or native hay (Seyfu, 1993). In view of the above-mentioned uses of *tef*, the observed straw yield increases could have an economic significance.

4.6.Effect of inoculation on height and tillering ratio of two *tef* varieties.

A marked and significant ($p<0.05$) difference in height and a higher but non-significant difference in fertile tiller number was observed between inoculated and un inoculated controls (Table 3). This non significant but marked difference in tillering was accompanied by a significant ($p<0.05$) increase in grain yield above un inoculated controls. The bacterial isolate that produced the highest mean increase in grain yield on either variety was the one that induced a higher increase in the number of fertile tillers. These observations seems to agree with the results of Reynders and Vlassak (1982a,b), Millet and Feldman, (1984) and Nuziello *et al.* (1987).

Plant height has been increased significantly ($p<0.05$) upon inoculation of wheat plants with *Azospirillum* spp. in hydroponic systems (Kapulnik *et al.*, 1985a), of wheat, sorghum and panicum (Kapulnik *et al.*, 1981a) and of corn (Lee *et al.*, 1989) undern green house

conditions. Similar increases in height has also been obtained in the field for maize and fox tail millet while sorghum plants remained unaffected by inoculation (Kapulnik *et al.*, 1981b). On the other hand, increased tillering was reported for wheat in hydroponic systems (Kapulnik *et al.*, 1985a), in green house conditions (Millet *et al.*, 1984; Nuziello *et al.*, 1987) and in field trials (Reynders *and* Vlassak, 1982a,b; Zambre *et al.*, 1984).

As shown in Table 3 uninoculated *tef* plants of DZ-01-196 variety were taller than DZ-01-354 counter parts. But this height difference was a bit higher than the range (53-115cm for DZ-01-354 and 50-117cm for DZ-01-196 variety) (Seyfu, 1987 in Seyfu 1993) of height these varieties normally attain. Although inoculation increased the mean height in both varieties, it was the variety DZ-01-196 that has responded better to inoculation. The percentage increase due to inoculation for this variety was almost twice that of DZ-01-354. The possible difference in the degree of compatibility between the bacterial isolates and *tef* variety, as in the other growth parameters, may have caused the difference in the percentage increase. Thus, it can be concluded that a better compatibility between the bacterial isolates and the *tef* variety DZ-01-196 may have promoted the growth of this variety better than DZ-01-354. In this respect a difference in nutrient availability or uptake efficiency, can be explained by the effect of the bacteria on root development or biological nitrogen fixation, and this in turn may explain the cause for the observed difference.

Although the mean fertile tiller number in both inoculated varieties were increased, the percentage increase over un inoculated control was higher for the variety DZ-01-354 than DZ-01-196. In fact, A31 inoculated plants produced slightly higher mean fertile tiller number than un inoculated DZ-01-196 plants. Nonetheless, these increases of mean fertile tiller for

the *tef* variety DZ-01-354 was not accompanied by a comparable grain yield increase. Reynders and Vlassak (1982b) found a negative response in grain yield of pot grown wheat when inoculated with *A. brasilense* spbr14. This decrease in yield was accompanied by a significant increase in tillering and a significant decrease (up to 23%) in root mass. These authors reasoned that the drop in grain yield might have been caused by malnutrition of the excess tillers as a result of reduced nutrient uptake system. However, this was not the case in the present study as the root mass was found to increase significantly ($p<0.05$). Hence, the discrepancy remained obscure.

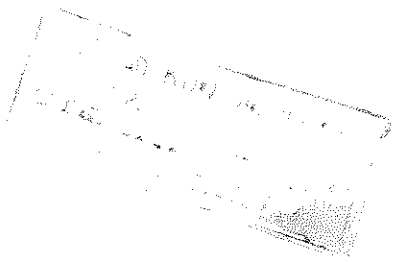
In general, the increase in mean fertile tiller accompanied by a grain yield increase reflected the beneficial effect of *Azospirillum* inoculation in growth promotion or yield improvement. However, the increased height caused by inoculation seems to be a disadvantage for a crop like *tef* because of the susceptibility of taller varieties to lodging (Seyfu, 1993) and a possible loss in yield.

5.CONCLUDING REMARKS

The present study on *tef-Azospirillum* association demonstrated that the two bacterial isolates were capable of improving the growth and different yield components of the two *tef* varieties to varying degrees. The higher yield obtained for inoculated groups, particularly in total grain nitrogen or protein could have a good nutritional implications. In view of the role of *tef* in the diet of the Ethiopian population, the observed small but significant ($p < 0.05$) improvements in dietary quality of *tef* grains will be important. Moreover, the higher yield of *tef* due to inoculation in un fertilized sterile soil would show the potential of these isolates in reducing fertilizer requirements.

The pot experiments in this study revealed that the two *tef* varieties responded differently to inoculation with either of the bacterial isolates. Moreover, it was found that the bacterial isolate A32 performed better than A31 on both varieties while the *tef* variety DZ-01-196 responded better to inoculation. Similar observations on host bacterial interactions has been reported for wheat (Millet *et al.*, 1984; Bahattarai and Hess, 1993) and for maize (Arsac *et al.*, 1991). The existence of a possible similar interaction between *tef* genotypes and the bacterial isolates was suspected that might have governed the degree of positive response to inoculation.

There are numerous reports indicating possible mechanisms by which crop plants may drive benefits from inoculation by associative *Azospirilla* species. (Lin *et al.*, 1983; Boddey and Doberiener, 1988; Zimmer and Bothe, 1988; Bashan and Levanony, 1991). Although the root mass increase in this study showed the role of phytohormones in root development and in effect better nutrient assimilation, the possible role of other mechanisms such as biological



nitrogen fixation can not be ignored. The extent to which each of these various processes contributes to increased yield of inoculated *tef* plants remains to be assessed.

The existence of some degree of specificity in the interaction between *Azospirillum* spp. and different cereals (Boddey *et al.*, 1988) or different cultivars of the same species (Jain *and* Patriquin, 1984; Sukiman and New, 1990) have been discussed. The existence of a similar interaction in the *tef-Azospirillum* association may also necessitate a through study on the interaction of various tef cultivars with *Azospirillum* and other rhizospheric microorganisms found in different agroclimatic regions of the country.

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