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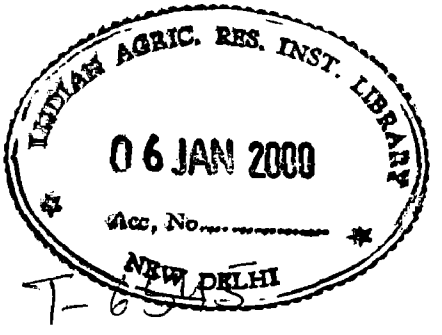

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SCREENING OF N_2-FIXING BACTERIA AND VAM FUNGI TO IMPROVE WHEAT YIELD

A Thesis

By

MAHESHWAR SINGH RATHI

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This is to certify that the thesis entitled "**Screening of N_2-fixing bacteria and VAM fungi to improve wheat yield** " submitted in partial fulfilment of the requirements for the award of the degree of **Master of Science in Microbiology,** to the Faculty of the Post-Graduate School, Indian Agricultural Research Institute, New Delhi, embodies the results of *bona fide* research work carried out by **Shri. Maheshwar Singh Rathi,** under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma. The assistance and help received during the course of investigation has been duly acknowledged by him.

C Singh 18/10/99

Place : New Delhi
Date : 18 October, 1999

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DEDICATED TO

MY BELOVED

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INTRODUCITON

Successful crop production is highly dependent on the availablity of various nutrients. Amongst these, nitrogen is the most needed one, which is limiting in Indian soils. This element can be made available alternatively, through biological N_2 fixation, besides chemically fixed nitrogen. It involves symbiotic and nonsymbiotic bacteria. In later group of bacteria, *Azospirillum,* and *Azotobacter* are the prominent genera.

Azospirillum is the most common diazotroph associated with the roots of variety of grasses, which was first reported by Dobereiner and her associates (1975). Such association was later confirmed with the roots of number of plant species (Lakshmi Kumari *et al.,* 1976; Kavimandan *et al.,* 1978; Singh and Subba Rao, 1979). It has also been established that inoculation of *Azospirillum* is beneficial to crop yield improvement (Singh and Subba Rao, 1979; Subba Rao *et al.,* 1979, 1982, 1983, 1984). However, magnitude of such improvement was noted comparatively less with wheat (Subba Rao *et al.,* 1980).

Azotobacter spp. also form an important group of N_2-fixing bacteria. *Azotobacter* besides their ability to fix molecular nitrogen, produce growth promoting substances like IAA, gibberellin, vitamins and, antifungal metabolities. (Shende *et al.,* 1975; Apte and Shende, 1981). It is also known to enhance seed germination, shoot and root length, N-nutrition and grain yield of various crops. However, response of wheat to inoculation with *Azotobacter* is not consistant under different tropical soil-agro climatic conditions of India (Saxena and Tilak, 1998).

Furthermore, synergistic effect of *Azotobacter* and VAM on Luxor tomatoes (El-Shanshoury *et al.*, 1989), lettuce (Brown and Carr, 1984), tall fescue plant (Ho, 1988) has also been reported. Increased spore number and VAM infection in the presence of *Azotobacter* and *Azospirillum* have also been reported.(Singh, 1992a, 1992b). Such synergistic host response could be attributed to the production of phytohormones or growth regulators by these bacteria, rather than their meagre contribution in N fixation and P availability, which in turn alter rhizosphere microflora of plant.

Beneficial effects of inoculation with vesicular-arbuscular mycorrhizae (VAM) fungi have been shown by several workers, especially in association with other microorganisms (Azcon *et al.*, 1976; Manjunath *et al.*, 1984). Additional improvement in yield of barley, pearl millet due to co-inoculation of *Azospirillum brasilense* and VAM fungi has also been reported (Subba Rao *et al.*, 1985a; 1985b).

In general, VAM fungi improve phosphate nutrition of legume which inturn enhances plant growth and nitrogen fixation. Increased biological nitrogen equivalent to phosphorus application(75-100 Kg ha^{-1}) was noted (Baerea *et al.*, 1987; 1989a; 1989b). Improvement in nodulation, P-uptake and biological nitrogen fixation with soybean (Bethlenfalvay *et al.*, 1990), chickpea (Subba Rao *et al.*, 1979; 1986, Rai *et al.*, 1988;), medicago (Azcon *et al.*, 1990) and pigeonpea (Singh, 1996) were also observed.

Straw yield of wheat in presence of *Azospirillum* and VAM fungi have also been reported (Singh *et al.*, 1990). Increase in grain yield, N and P content of grain and straw of few varieties of wheat were observed with dual inoculation of VAM fungi and *Azospirillum brasilense* (Singh *et al.*, 1990). However, systematic study on dual inoculation of N_2-fixing

bacteria and VAM is lacking. Based on above cited information, it is felt essential to screen various asymbiotic N_2 fixing bacteria (*Azospirillum, Azotobacter*) and VAM fungi to find out the best combination for co-inoculation in order to minimize use of nitrogenous and phosphatic fertilizers. In view of above facts, therefore, investigations have been under-taken with the following objectives :

1. Isolation of nitrogen fixing bacteria from rhizotic zone of wheat (*Triticum aestivum,* L.)

2. To find out most promising N_2-fixing bacteria and VAM fungi and

3. To identify combination of diazotrophs and VAM fungi for maximizing growth and yield of wheat crop.

REVIEW OF LITERATURE

The dependence of high yielding crop varieties on application of higher fertilizer doses to maximise crop yield have imposed, on a global basis, the additional demands for nitrogen over the past few decades. These demands have been met largely by increased production and utilization of fertilizer nitrogen, which is not eco-friendly. The chemically fixed nitrogen is a non renewable resource and largest and costly fossil energy-dependent input in agricultural production, pointing out towards importance of biological nitrogen fixation as an the alternative nitrogen sources, which could atleast partially replace the fertilizer N-input to improve crop production.

In this context, symbiotic and non-symbiotic bacteria are involved in biological N_2- fixation. In later group various species of *Azospirillum, Azotobacter, Klebsiella* and *Derxia,* have been well recognized. However, amongst these *Azospirillum* and *Azotobacter* are the notable N_2-fixing bacteria, posses high potential rate of N_2-fixation and phytohormones production. These have been isolated from the roots of grasses and various crops (Dobereiner and Day, 1976, Lakshmi Kumari *et al.,* 1976, Singh and Subba Rao, 1979). Beneficial effects were noted more when inoculated together, but their effect varied with the host as well as bacteria involved in it.

During recent past, interest of the scientist has also been shifted to apply mycorrhizae, as the fertilizer supplement. The term mycorrhizae was first coined by Frank, (1885) to describe the plant roots and fungal symbiotic association, which means the "fungal root". Lewis (1973) also

clearly classified the mycorrhizae in to four groups *viz.* Sheathing, Vesicular-arbuscular, Orchidaceous and Ericaceous. Out of four groups, vesicular-arbuscular mycorrhizae (VAM) have been considered the most important in agriculture. VAM fungi belongs to the family Endogonaceae (order-Endogonadales) and are obligate symbiont (Lewis, 1973). It can not be cultivated axenically on nutrient media. These are known to mobilize phosphorus, a second important nutrient required by the plant. VAM fungi alter the morphology and biology of rhizotic zone of the plant. However, VAM fungi are not host specific. But their beneficial effects were noted varied between various species.

Inconsistent yield of wheat crop has been reported in past with the bacterial inoculation in general, and with *Azospirillum* in particular. This inconsistency could be due to limited migration of bacteria in soil, adsorption of the bacteria to the soil particles and competitions with native rhizospheric bacteria for nutrients in soil (Bashan, 1986). Therefore, under these circumstances bacterial inoculation potential is expected to be reduced in terms of plant growth and yield.

Improvement in the yield of several crops due to co-inoculation of non-symbiotic bacteria (*Azospirilllum, Azotobacter* etc.) has been well doccumented. Recently, increased grain and straw yield of various genotypes of wheat (*Triticum aestivum* L.) were recorded due to co-inoculation of VAM fungi, *G. fasciculatum* and *A. brasilence*. Based on the above cited facts following aspects have been reviewed in this chapter.

2.1 *Azospirillum*

Dobereiner and Day (1975) first reported the occurrence of *Spirillum lipoferum* associated with the roots of large number of tropical plant

species, having the ability to fix atmospheric nitrogen. The description of the genus *Spirillum* was available in the 17th century. Beijerinck described this new bacterium and named as *Azotobacter spirillum* in 1922. The nitrogen fixing ability of *Spirillum* was reported by Beijerinck (1922) in the enrichment culture of *A. Spirillum.* Becking (1963) isolated a strain of *Vibrio* or *Spirillum* identified to Beijerinck's *Spirillum lipoferum* and established the nitrogen fixing ability of the bacterium by ^{15}N technique. However, the potential agronomic importance of the bacterium was raised when Dobereiner and Day (1976) reported its association with the roots of grasses from various geographical regions.

Krieg (1977) observed that the DNA base composition of eleven *S. lipoferum* isolates was 69-71 moles per cent G + C, a value much higher than the average 38 per cent found in *Spirillum* genus and suggested the generic name *Azospirillum.* Consequently, Tarrand *et al.* (1978) defined the *Azospirillum* genus into two species *viz.*, *A. brasilense* and *A. lipoferum*. In 1983, new isolates corresponding to a third species, *A. amazonense* was discovered (Magalhaes *et al.,* 1983, Folk *et al.*, 1985).

Later on a salt tolerant species *A. halopraeferans,* was isolated from the roots of Kallar grass (Reinhold *et al.,* 1987). The fifth *Azospirillum* species, *A. irakense,* was found in association with rice roots. This is only species that has pectinolytic activities (Khammas and Kaiser, 1991). Recently, a new *Azospirillum sp.* has been isolated from the root, root nodule and stem nodule of *Aeschynomene aspera* and *Naptunia* sp. (Singh, 1991). Nitrogenase activity of this isolate was found similar to the Dobereiner's isolate. *Azospirillum sp.* occur free living in soil or in association with the roots of cereal crops, grasses and tuber plants to perform associative symbiosis (Dobereiner *et al.,* 1976, Lakshmi

Kumari *et al.*, 1976). Cells of this bacterium are plump vibriod or straight rods, 0-9-1.2 μm in width often with pointed ends. Cells stain Gram negative to Gram variable. Intracellular granules of poly-β- hydroxy butyrate (PHB) are present. Cells are motile with a characteristic screw like or vibratory motion in liquid media by means of a single polar flagellum. The colonies of some strains form a light pink or dark pink pigment on potato agar. Pigmentation was shown due to carotenoids. They are nitrogen fixer, exhibiting N_2- dependent growth under microaerophilic conditions.

2.1.1 Nutritional properties

Azospirillum are oxidase positive, grow well on the salts of organic acids such as malate, succinate, lactate and pyruvate (Holt *et al.*, 1994). strains of *A. lipoferum* can utilize a large number of carbohydrates including glucose, mannitol, sorbital etc., which are not used by *A. brasilense,* though some strain grow in glucose (Terrand *et al.*, 1978).

A. brasilence is prototroph whereas *A. lipoferum* require biotin. No amino acid can be used both as sole carbon and nitrogen source. In the presence of malate and under aerobic condition, the nitrogen sources used are ammonia (Okon, *et al.*,1976), nitrate, glutamine and amino acids of the same family (Arg, Pro, Lys, Asp), histidine and purines (Rennie *et al.*, 1981). Dinitrogen can be used only under micro-aerobic conditions (Bulow and Dobereiner, 1975). Denitrification under aerobic conditions was reported (Neyra *et al.*, 1977). It appeared later that all *A. lipoferum* strains were capable of denitrification, whereas *A. brasilense* strain could be divided into two groups, one containing denitrification organisms. The other strains are able to convert NO^-_3 to NO^-_2 but not produce gas from NO^-_2 (Neyra *et al.*, 1977).

2.2 *Azotobacter*

Heterogenicity of soil complex results into its variable nutrient and microbiol straita that can further be revealed in terms of growth and development of plants. A regular consumption of soil nutrient by macro and microorganisms and competition among these organisms weakens the soil complex and stresses on enriching its components by other means. The chemical mode of enriching the soil is performed by fertilizer application but the best substitute is the use of microorganisms as biofertilizers. Use of biofertilizer serves dual purpose as it not only supplements the required nutrients but also enriches the soil with specific beneficial microflora which in turn initiate plant growth and development when applied to seed, seedlings or incorporated into soil.

Beijerinck, was the first to isolate and describe free living nitrogen fixing aerobic bacterium *Azotobacter chroococcum* and *A. agilis* in 1901. The former species was described as a soil inhabitant and the later a water borne one. During subsequent years, several other species have been described from different inhabitates *A. vinelandii* (Lipman, 1903) *A. nigricans* (Krasil'nikov, 1949) and *A. armeniacus* (Thompson and Skerman, 1981).

According to Bergey's Mannual of Systematic Bacteriology, Volume-1 (Tchan, 1984), the family Azotobacteraceae includes various Gram-negative, aerobic and heterotrophic bacteria capable of fixing nitrogen asymbiotically. According to this volume genus *Azotobacter* contains six species, *viz. Azotobacter chroococcum, A. vinelandii, A. beijerinckii, A. nigricans, A. armeniacus* and *A. paspali.*

Page and Prasad (1991) have proposed a new species *A. salinestris*. This Na^+ dependent strain was isolated from the soils in Alberta, Canada. Wang *et al.* (1993) reported the presence of *A. salinestris* from the soils of Australia. *Azotobacter* assumes agricultural significance, however only two through species like *Azotobacter chroococcum* and *A. vinelandii* which had established well in the production of inoculants (biofertilizers) used for inoculation of cereals, vegetables, horticultural, oil seed and plantation crops.

2.2.1 Ecological distribution of *Azotobacter*

Azotobacter can be found in a wide variety of habitats, like soil, leaves, roots, marine and fresh water, at temperature ranging from tropical to temperate, and pH ranging form 3.0- 9.0. Species of *Azotobacter* are specific in their distribution and restricted to environment rich in organic matter (Becking, 1981 and Gordan , 1981).

In both rhizospheric and non-rhizospheric soil *Azotobacter* counts are much lower at pH 5.7 than at pH 6.8 to 7.3 (Sattar and Solaiman, 1988). Sengupta and Sengupta (1991) isolated *Azotobacter* from roots of local grasses and found three distinct *Azotobacter* sp. namely *A. chroococcum, A. vinelandii* and *A. beijerinckii*. Nagraj (1989) reported the occurrence of *Azotobacter* population in the range of 10^5 - 10^6 cells/g in forest soils of Maharashtra irrespective of pH, C/N ratio and soil type. The decidious forest green soil harbours more number of *Azotobacter*. Malik and Bilal (1988) reported *Azotobacter* colonization on the root surface and inside root hair cells of Kallar grass in saline soils.

The bacterium remains viable after prolonged period of dormancy as do the endospore of Gram-positive bacteria (Moreno *et al.*, 1986).

Tippanvar and Reddy (1990) found cyst forming *Azotobacter* sp. in three samples of bovine waste taken from animal and effluent from biogas digester. A transparent, pigment less form of *Azotobacter* which possessed weak N_2-fixing activity and able to grow at high level of fixed-N_2, was isolated from the pouch of ruminants's intestine, ovarian follicles and eggs of hen and silk worm. These pigment less form could be converted to "typical" forms by decreasing level of fixed nitrogen in the medium (Prolanskya and Kortkova, 1993).

2.2.2 Morphology and growth characteristics of *Azotobacter*

The most prominent cell-forms in laboratory cultures are comparatively large (2.3-3.6μ), oval, spherical or rod shaped, frequently in distorted shape, like peanut and spindle. With aging *Azotobacter* cells looses its motility, shortens and becomes almost coccoid in shape. At this stage the cells are surrounded by thick mucoid capsule which is relatively resistant to the phagocytic action of microorganisms (Martin *et al.*, 1965).

Azotobacter chroococcum undergoes cyst formation during adverse growth conditions. Cyst is a living cells with two coats namely exo-cystorium and two layers of exine. The cyst is found to be rich in poly-β-hydroxy butyrate (PHB). With the onset of favourable conditions, the cyst gives rise to vegetative cells. According to Lohnis and Smith (1923) the life cycle of *A. chroococcum* consists of seven vegetative and six reproductive forms, the giant cells being regarded as one of the vegetative forms. Later investigations led to the conclusion that the great variety of forms of *Azotobacter* is largely due to 'Pleomorphism' in this bacterium and partly to the appearance of involution formed.

Strain variation in *A. chroococcum* had been studied in great detail by Zinovyeva (1962) and Jemes (1970). There are more than one brownish black pigments in the strains of *A. choococcum*. Some fraction were water soluble. Sen (1955) observed that the pigment forming strains of *Azotobacter* fixed more nitrogen than the colourless strains. The intensity of colour did not show any correlations with nitrogen fixing capacity of *Azotobacter. Azotobacter* generally fix atmospheric nitrogen at the rate of 10 mg of N_2 per g carbohydrate consumed. It is catalase positive (Holt *et al.*, 1994). A single plant rhizosphere did harbour different strains of *Azotobater* which varied in nitrogen fixation, salt tolerance and antiobiotic resistance. (James and Shende, 1972).

2.3 *Proteus*

According to Bergey's Mannual of Systematic Bacteriology volume - 1 (Tchan, 1984) genus *Proteus* belongs to the family *Enterobacteriaceae* . Cells of this bacterium are straight rods, 0.4-0.8 μm in dia and 1.0-3.00 μm in length, motile by peritrichous flagella and are Gram-negative. Most strains swarms with periodic cycles of migration producing concentric zones, or spread in a uniform film, over moist surface of nutrient media. *Proteus* hydrolize urea and Produce acid from several mono and disaccharides. *Proteus* is pathogenic causing urinary tract infections and are secondary invaders causing septic lesions at other sites of body. It occurs in the intestines of humans and wide variety of animals and also in manure, soil and polluted water. One species has been isolated from gypsy moth larvae.

Genus *Proteus* contains 3 species namely. *P. vulgaris, P. mirabilis and P. myxofaciens. P. myxofaciens,* was excluded form the genus because it was said to be *Erwinia herbicola.*

2.4 *Flavobacterium*

Cells of *Flavobacterium* are rods with parallel sides and rounded ends, typically 0.5μm in dia and 1.0-3.0 μm in length. Intracellular granules of poly-β-hydroxy butyrate (PHB) are absent. It does not glide or spread. *Flavobacterium sp.* is aerobic with strictly respiratory type of metabolsm. Environmental isolates of *Flavobacterium* grow at temperature from 5-30°C but most clinical isolates grow at 37°C. Growth on solid media is typically pigemented (yellow to orange). Colonies are translucent (occasionally opaque), circular 1-2 mm in dia, convex, smooth and shiny with entire edges. *Flavobacterium* is catalase, oxidase and phosphatase postive. It is chemo-organotrophic and widely distributed in soil and water, raw meats, milk and other foods, hospital environment as well as and in human clinical material.

Nitrogenase activity is absent. Cells are Gram-negative to Gram-variable. According to Bergey's Mannual of Systematic Bacteriology volume - 1 (Tchan 1984) *Flavobacterium* genus have seven species *viz.F. aquatile, F. breve, F. bolustinum, F. odoratum, F. meningosepticum, F. multivorum and F. spiritivorum.*

2.5 Vesicular - arbuscular mycorrhizae (VAM)

The term is commonly used to denote symbiotic association between plant root and fungal mycelia. VAM fungi have ancient origin as fungal structure has been recorded in fossil studies dates back to about 300 million years (Butler, 1939).

Vesicular - arbuscular mycorrhizae (VAM) is by for the most wide spread type of mycrorrhizae. It is distributed in tropical, temperate and in arctic regions, hence they are ubiquitous in nature. VAM have unexpected

infecting capacity to wide range of host plant. Distribution has been detected in a low land tropical rain forest in Nigeria (Redhead, 1968), Brazil. Mosse and Hayman (1982) found the VAM root colonization in a deciduous woodlands in England, and reported most of the VAM infection in the fine feeder roots.

The nomenclature was based on the formation of vesicles and arbuscules. VAM fungi penetrate into the cortical of host cells, but the invading mycelium usually lives only a short time intercellularly (Smith, 1980), lysis of intracellular structure (the arbuscules in VAM) then occurs. Host cell survives and can be colonized again by the fungi. Vesicular-arbuscular fungi do not form sheaths around the root, but a network of extra material hyphae usually develops. This grows in to the soil and mycelium can be extend the several centimeters beyond the root surface. The total hypahe length can reach more than 1 meter of hyphae per centimeters of infected root (Smith, 1980, Hayman 1982). VAM are members of the family endogonaceae, which are placed in the genera *Glomus, Sclerocystis, Gigaspora and Aculospora* (Gerdemann and Trappe, 1974), Since they can not be successfully subcultured axenically, they must be considered ecologically obligate symbionts i.e. they do not complete their life cycle unless they can colonize a suitable host plant (Lewis, 1973). The family is considered to be in an order of its own. The endogenales are tentatively placed in the class Zygomycetes in the sub-division of Zygomycotina (Benjamin, 1979).

2.5.1 Process of VAM root colonization

Studies on the development of VAM infection (Powell 1976) showed that neither spore germination nor the initial direction of hyphae growth was influenced by the presence of host root. Hyphae from spore were not

attracted to the root until they approach them closely. There are the four key facts in VAM colonization (1) Spore germination and mycelia development (2) stimulation of germ tubes when they approach the roots closely (3) attachment of the infective hyphae to the root surface and (4) root penetration. Once the infective hyphae arrives at the root an appressorium is usually produced on cortical cells or on root hairs, and hyphae penetration occurs into or between these cells. The infection units - internal mycelium associated with a single entry point grow as the hyphae spread between and through the cells of cortical root layers (Wilson, 1984). When the first successful entry point is established, the root become more prone to further penetration. Shortly after infection, a hyphae growing into single cell may show repeated dichotomic branching, and a tree like structure, the arbuscules, is formed. When the internal infection has been consolidated, the penetration hyphae ramify externally. The external hyphae may grow along the root surface forming more penetration point and also grow through the surrounding soil forming an intensive tri-dimensional network of mycelium (Barea, 1991). Mosse and Hepper (1975) also reported that once primary infection is established, the extensive growth of external mycelium starts and leads to the development of secondary infection. Sanders and Sheikh (1983) suggested that the proportion of root system colonised by VA-fungi depends on (a) the rate of root growth (b) rate of secondary infection development and (c) rate of longitudinal spread of fungi within cortex. Several environmental factors influence these parameters. When the mycorrhizae is well established,the fungi may form vesicles. These are oval to spherical in shaped containing oil droplets that can develop inter or interacellularly. They may have a temporary storage function, after which they remain thin walled or become thick walled as chlamydospore-like structures. Intracellular colonization, as in

the case of arbuscules, have a characteristic feature. The arbuscules are surrounded by the intact host-cell plasmalemma. The cytological changes that occur during arbuscules formation have been well documented by Rhodes and Gerdemann (1980). Therefore arbuscules formation represent large surface of cellular contact between host and fungus. This facilitates the interchanges of metabolites between host and fungi. The function of the arbuscule is the biotropic bidirectional transfer of nutrients. Infact, the arbuscule is considered to be the main site of transfer of mineral nutrients form fungus (Smith and Gianinazzi - Pearson, 1988). The ratio of arbuscules per unit root length of extrametrical hyphal length might be considered for comparison among fungal isolates with regards to functional transport capability. Technological advances that utilize automation need to be applied to this approach (Wright and Millner, 1993).

2.5.2 Mechanism of nutrient translocation by VAM fungi

Often, the beneficial effects of mycorrhizae on plant growth have been related to the increase in the uptake of phosphorus. In addition to phosphorus, VAM fungi also enhance acquisition of relatively immobile micronutrient cations particularly zinc (Grekow and Marschner, 1989) and copper (Pacovsky, 1986).

Various mechanisms have been suggested by Bolan (1991) for the increase in phosphorus uptake by mycorrhizal plants. These include :

1. Physical exploration of soil
2. Faster movement of phosphorus into mycorrhizal hyphae, and
3. Modification of root environment

2.5.3 Role of VA-mycorrhizae in phsophorus nutrition

Approximately 95-99% of soil phosphors occurs in unavailable form to plant roots. Certain sparingly soluble P compounds seemed to be utilized

by VAM as a source of P. This possibility was investigated by experiments in which the labile phosphate pool was labeled with ^{32}P (Powell, 1975; Sanders and Tinker, 1971). It is clear from isotopic studies that VAM hyphae absorb phosphate from the labile pool of P in soil, as do non-mycorrhizal roots. It is known that mycorrhizal plant can respond to the addition of sparingly soluble P like rock phosphate. However, this is not necessarily a P solubilization effect. There is a controversy among the various workers whether the VAM fungi help in both solubilization and mobilization of nutrient form which are otherwise unavailable to the plants (Azcon *et al.*, 1976). But, it is a general agreement that VAM fungi help in mobilizing P and other less mobile nutrient rather than solubilization. Vesicular-arbuscular mycorrhizae (VAM) enhance P uptake in two different ways. One mode of fungal action is merely physical and is based on the increased number of sites for absorption achieved by external mycelium. The hyphae growing through soil pore space are able to affect phosphate absorption beyond the depletion zone upto 8 cm from the roots (Rhodes and Gerdemann, 1975). Thus, mycorrhizal roots explore a much greater volume of soil to take up phosphate. VAM hyphae come in a closer contact with the P-containing particles than roots to take advantage of the phosphate ions that are slowly and naturally dissociated by physio-chemical or bio-chemical mechanisms (Barea, 1991). The main site of phosphate transfer to the host, which occurs by an active mechanism across the membrane of both partners, seems to be the arbuscules (Cox, *et al.* , 1980). It is now accepted that the break down of the arbuscules can account for only 1% of the P inflow to the host cells. Phosphate released by other structure such as hyphae or vesicles might also be involved, but the extensive increase of contact surface area makes the arbuscules more probable sites for nutrient transfer between mycorrhizal symbionts.

There are some indications that the VAM fungi are able to take P from soil solution with low phosphate concentration, where simple roots have difficulties. In other words VAM fungi seems to induce a lower threshold concentration for effective P uptake (Bolan *et al.,* 1983). VAM hyphae have advantageous geometry and better distribution than roots to acquire P form transitory, localised and diluted source of the nutrient (Jakobsen *et al.,* 1992). Studies conducted by Clapperton and Reid (1991) concluded that interaction between the inoculum density of VAM fungi and soil nutrient availability effects the plant growth, in *Phleum pratense* and *Agropyron trachycaulum.*

2.5.4 Nitrogen and other nutrients

Mycorrhizal plants derive significantly more ^{15}N form various N sources. In ^{15}N applied mycorrhizal plant significant and positive correlation with per cent mycorrhizal fungal colonization, number of hyphal crossing throughout the mesh into area of ^{15}N placement, total length of hyphae per gram of soil and the hyphal diameter in the soil were noted. The mean flux of N through the hyphae of *G. mossae* was 7.342×10^{8} mol N cm^{2} S^{-1} for the inorganic -N treatment over an 88 days period (Ames *et al.,* 1983).

Further, seed inoculation with *Rhizobium* and soil inoculation with *Glomus fasciculatum* increased nodulation, nitrogen and phosphorus concentration in plants and yield of chick pea (*Cicer arietinum*) at 50 kg P_2O_5 ha^{-1} application. Inoculation with *Rhizobium* or *G. fasciculatum* separately or in combination significantly increased the N_2 fixed in grain and straw than uninoculated control as determined by ^{15}N atom per cent excess of plants grown on soils amended with ^{15}N-labelled ammonium sulphate. The increase was more pronounced when phosphorus was applied

at 50 kg P_2O_5 ha^{-1} (Subba Roa *et al.*, 1986). Laboratory and green-house experiments using ^{15}N isotope-dilution technique have revealed that inoculation of VAM fungi improved nitrogen fixation with soybean, (Ganry *et al.*, 1985) *Hedysarum coronarium* (Barea *et al.*, 1987) and pigeonpea (Singh 1990).

Mycorrhizal roots could absorb potassium, sulphate, zinc and strontium 90 (Powell, 1975) and copper (Pacovsky, 1986) at faster rates than non mycorrhizal plants. Coper and zinc could markedly improve the myorrhizal formation. Phytohormone level also had been reported to induce changes in the VA-mycorrhizal colonisation (Allen *et al.*, 1982) since these hormones also being synthesised by VAM mycorrhizae (Barea and Azcon-Aguilar 1982).

2.5.5 Influence of VAM fungi on plant growth

Vesicular-abuscular mycorrhizal symbiosis is often associated with improved plant growth. Interest on VAM fungi has reached a peak in recent years due to its benefical effect on plant growth mainly by enhancing accumulaiton of plant nutrient such as phosphorus, zinc, copper etc. through greater soil exploration by mycorrhizal hyphae (Barea, 1991; Allen , 1992; Tarafar and Marschner, 1994; Joner and Jakobsen, 1995). Field studies have demostrated an increase in crop yield due to inoculation with VAM fungi (McGonigle 1988). However, response to VAM fungi may vary depending upon crop species (Habte and Manjunath, 1991) and soil phosphorus levels (Bethlenfalvay, 1982). It has been shown that the external hyphae of VAM fungi could deliver upto 80% of the plant phosphorus to the host plant over a distance of more than 10 cm from the root surface (Li *et al.*, 1991). Jakobsen (1983) reported increased in dry matter production, phosphorus, zinc, copper and bromium uptake by barley inoculated with

Glomus caledonium. Raju *et al.* (1990) showed that sorghum plant inoculated with VAM recorded higher amount of P, K, Mg, Mn, S, Ca, Fe, Cu and Zn than non-mycorrhizal plants.

Bagyaraj and Manjunath (1980) observed that growth response to inoculation with efficient VA-mycorrhizal fungi in natural soil can be expected even in the presence of indigenous endophytes. Singh and Singh (1986) found that lentil grown on P deficient soil responded to *Glomus fasciculatum* inoculation upto 80 mg kg^{-1} of soil application. Bell *et al.* (1989) obtained response to mycorrhizal inoculation in terms of dry matter without threshold to increasing P rate upto 120 kg P ha^{-1} in sterilised soil. Rao *et al.* (1990) noticed positive relationship between VAM inoculation and yield component in groundnut. Devi and Sitaramaiah (1991) found that blackgram grown in field soil inoculated with VAM showed increased mycorrhizal root, greater root volume and greater dry weight of shoot and roots than non inoculated plants. VAM also has a significant effect on greengram (Saxena *et al.*, 1997; Santhi and Kothandaraman, 1995; Rao and Rao, 1996) and cowpea (Meenakumari and Nair, 1992).

2.5.6 Effect of VAM on wheat (*Triticum aestivum*, L.)

Hetricka *et al.* (1996) reported that mycorrhizal responsiveness decreased with P fertilization for cultivars that were dependent on the symbiosis but it was uneffected by P fertilization in cultivars that were negatively impacted by the mycorrhizae. The relastionship between biomass production and percentage root colonization was positive for cultivars which responded favourably to the symbiosis. P transport effectiveness of mycorrhizal fungus is meaningful only in the context of its associated host plant species (Ravnskov and Jakobsen, 1995).

The VAM inoculation (*Glomus mosseae*) was closely related with increased plant height, total dry weight of shoot and root, number of grains per ear and density of mycorrhizal colonization in roots (Omar, 1998; Chhabra and Jalali, 1997). Tu-Shittua *et al.* (1997) reported that both P addition and VAM infection increased the grain yield of wheat. VAM infection had both positive and negative effect on P uptake depending on the growth stage of wheat and translocation of nutrients. The role of VAM in enhancing the translocation of P from root and straw to grain was beneficial towards seed sets (Goh *et al.,* 1997).

Inoculation with VAM fungi (*Glomus sp.*) increased shoot dry weight, N and P uptake of wheat by 82.8%, 98.3% and 154.5% respectively over the control (Mikhael *et al.,* 1997). Spike weight, grain weight per spike, root length, root and shoot dry matter were higher with VAM inoculation than PSB inoculation (Zaghloul *et al.,* 1996). Inoculation with *Glomus mosseae* increased the dry matter production of the wheat cultivars more than inoculation with *Glomus fasciculatum.* (Virhileg and Ocampo, 1991; Tarafdar and Marschner, 1995). However, Singh *et al.* (1990) reported that inoculation with *Glomus fasciculatum* resulted increased grain and straw yield of wheat. Similar result was obtained by Yocom and Boosalis (1991) under field condition in western USA. Panwar (1991) also has found that plants inoculated with *Glomus fasciculatum* showed increase in chlorophyll content and photosynthetic rate of wheat.

Tarafdar (1995) reported that inoculation with VAM significantly increased shoot and root dry weight and total root length, although shoot : root ratio was uneffected.

Pradhan and Mohan (1996) in a pot experiment studied the effect of seed inoculation with *Glomus fasciculatum, Glomus epigeanum* and

Glomus mosseae on wheat. Inoculation with VAM significantly increased plant height, total dry matter production and uptake of N, P and K compared with control. Majjigudda and Sreenivasa (1996) observed that inoculation with *Glomus fasciculatum* plus 75% of recommended phosphorus improved the grain yield of wheat. The total and available N, P and K were more with VAM inoculation than with PSB inoculation (Zaghloul *et al.*, 1996).

Javid-Iqbal *et al.* (1997) studied the seasonal fluctuation in VAM fungi association on wheat. The predominant VAM species were *Glomus mosseae* and *Glomus fasciculatum.* The fungal colonization was high from February to May and from the end of July to September. Fares (1997) reported that the native endophyte (VAM) competed more efficiently in colonizing the roots and were able to produce high yields in soil containing low available P levels after 90 days of cultivation.

2.6 Host -VAM-water stress relationship

Water is an inevitable prerequisite for the proper growth of any plant. VA-mycorrhizae fungi play key role to ellivate water stress tolerance of plant (Johnson and Hummel,1985). Under water stress the external hyphae of mycobiont greatly enlarge the potential surface area of water and nutrient scavenging system of plant and water absorption capacity of root is enhanced by mycorrhizae (Nye and Tinker, 1977). However, Singh *et al.* (1991) also made comparative study of several VA-mycorrhizae, on the host plant-water relationship and noted the difference in the effectiveness to ellivate water stress tolerance of *Cajanus cajan.* Among the eight (*G. etunicatum, Glomus californea, G. aggorcarpum, G. macrocarpum, G. mosseae, Giga spora margarita, G. intraradices, G. deserticola*), *Gigaspora margarita* was considered most efficient VAM to bring the

increase on plant growth and to lower the drought severity under soil drought stress.

2.7 Interactive effect of asymbiotic N_2-fixer and VA mycorrhizae

Interactive effect of VAM-*Rhizobium*-legume symbiosis has been reported by several worker (Abbott, 1977, Bagyaraj *et al.*, 1979; Subba Rao, *et al.*, 1985 and Singh, 1990). However, there is lack of information on VAM with asymbiotic nitrogen-fixing bacteria.

Since mycorrhizal association are known to improve the growth and yield of crops in nutrient deficient condition, they also play important role for improvement of crop yield. Barea *et al.*(1980) reported better survival of *Azotobacter paspali* in the rhizosphere of *Paspalum nototum* in the presence of mycorrhizae. Improvement in onion due to symbiotic effect of *Beijerinckia mobilis, Aspergillus niger* and *Glomus fasciculatum*, was also noted (manjunath *et al.*, 1981). Increased in grain yield and P content in barley and pearl millet due to inoculation of VAM fungi (*Glomus fasciculatum, G. mosseae, Acaulospora* sp., *Gigaspora margarita* and *G. callospora*) in presence of *Azospirillum brasilense* inoculation was recorded (Subba Rao, 1985a, 1985b).

Singh and Subba Rao (1987) observed that eight genotypes of wheat (*Triticum aestivum,* L.) differed with regard to colonization of root by VA-mycorrhizal fungi. However, inoculation of *G. fasciculatum* enhanced the yield and grain content of wheat. This was further increased in the presence of *A. brasilense*. Nitrogen accumulation of plant was also increased with dual inoculation. Such effect was further confirmed (Singh *et al.*, 1990).

Bagyaraj and Menge (1978) studied the interaction between *Azotobacter chroococcum* and *Glomus fasciculatum* in tomato and found a synergistic effect on plant growth. Mycorrhizal colonization and spore number was also enhanced by VA-mycorrhizae. This effect was attributed to hormone production as well as N_2-fixation. Interaction studies with bacteria have indicated the longer survival of the bacterium in the rhizosphere of mycorrhizal root (Linderman, 1978). The phosphate solubilizing bacteria rendered more P soluble, while mycorrhizae enhanced P uptake. Due to combined inoculation of phosphate solubilizing bacteria and mycorrhizae there was a synergistic effect of P supply and dry matter production (Gaur and Rana, 1990). This bacterium also produce hormones and vitamins.

Krishna *et al.* (1982) studied the inoculation effect of *G. fasciculatum* and *Streptomyces cinnamomonicus* with finger millet. Simultaneously inoculation with both the organisms has an antagonistic effect on each other. Each one has suppressed the growth and multiplication of other in the rhizosphere. In sorghum and pearl millet co-inoculation of *A. lipoferum* and *Gigaspora callospora* significantly increased the growth, chlorophyll content and mycorrhizal infection of the roots (Rao, *et al.* 1989). Increased growth and nutrition of barley plant was observed when co-inoculated with *A. brasilense* and *Glomus versiforme* (Negi *et al.*, 1990). Dual inoculation with various VAM fungi (*Aculospora, Gig margarita* and *G. fasciculatum*) and *A. brasilense* in the prsence of SSP and rock phosphate resulted in higher dry matter production and root colonization of fungi in pearl millet (Tilak and Singh, 1988). Dual inocualtion of *A. chroococcum* and *G. fasciculatum* enhanced root infection of VAM fungi, stimulated the plant growth and increased shoot N, Ca, Mg and K in luxar tomato (El-shanshoury *et al.*, 1989).

A new species of nitrogen fixing acetic acid bacterium *Acetobacter diazotrophicus* have been isolated from aerial plant parts of sugar cane and sweet potatoes (Gillis *et al.*, 1989). Inoculation of sterile plants is only successful when the diazotroph is inoculated together with VAM fungi. Recently it has been found that spore germination and fungal hyphal growth was increased in the presence of N_2-fixing bacteria (*Azotobacter, Azospirillum, Rhizobium spp.*) and phosphate solubilizing bacteria (*B. polymyxa, P. striata*) (Singh and Tamilvendan, 1999). In their study such increase was noted with *A. brasilense* strain, *Azo. lac.lac.* as compared to other nitrogen fixing bacteria. *Flavobacterium* was also found enhanced spore germination and fungal hyphal growth. Such effect were correlated with the production of growth promoting substances (Tamilvendan, 1999).

2.8 VAM-bacteria-host relationship

The growth of the host of mycorrhizal plant provides space and substrate for the fungus. This effects the further growth of plant, resulting the incrase in uptake of nutrient which increas the rate of growth of the host in consequence. The following event and process are involved in mycorrhizal host symbosis : 1. Germinationof propagules and subsequent hyphal growth in soil. 2. Primary infection process. 3. Spread of the fungus to other parts of the root system (secondary infection). 4. Transfer of host assimilate of fungus. 5. Growth of the fungal biomass on the root cortex and surrounding soil. 6. Uptake of nutrients (particularly phosphorus) by the mycelium from the soil and their transfer to the host. 7. Improvement in the nutrition of the host which influences its growth rate, partitioning to assimilate, and leads to increase the root growth. 8. Increase in root growth influence the uptake and substrate availability to the fungus. Different VA-mycorrhizal fungi seem to differ in their effects on the growth of the

host plant (Sanders, 1986; Singh *et al.*, 1991). Some of these differences may arise from different rates of spread of mycorrhizal infection within the root system and/or differences in the abilities of the fungus to take up and translocate phosphorus from the soil to host.

The soil-borne or inoculated propagules germinate after the onset of favourable conditions of moisture and temperature (Schenck *et al.* 1975), but probably is not influenced by the presence of host root (Daniels and Trappe, 1980). However, spore germination and further infection is greatly influenced by bacteria associated with the spores or/and present in the soil. The influence of such microorganisms of VA spore germination was first pointed out by Moose (1959) and Mejstrik (1965), and there after Daniel and Trappe (1980) noted that the loss of microorganisms inhibited spore germination. Barea and Azcon-Aguilar (1982) indicated that the presence of some microorganisms was able to stimulate the non-symbiotic development of *Glomus mosseae* (Gerdemann and Trappe, 1974).

2.9 Plant root exudates and their impact on microorganisms

The rhizosphere effect, as it is called, the influence of plant root exudates on soil microorganims, has been well recognized. Several studies have been conducted to determine the influence of plant root exudates on vesicular-arbuscular mycorrhizal (VAM) colonization (Johnson *et al.*, 1982; Ratnayake *et al.*, 1978). In addition, studies have specifically looked at the effect of plant age (Johson *et al.*, 1982), plant species (Schwab *et al.*, 1984) and light (Johnson *et al.*, 1982; Rovira, 1959) on root exudation and in turn on VAM formation.

Scientist had a belive that P-deficiency increases root exudation and that it is leaked into the rhizoshphere and increase VAM infection.

However, Schwab *et. al.*, (1983) did not find quantitative differences on the exudates from P-deficient plants and those of non-Pdeficient plants. Plant height of VAM and/or PSB inoculated tree seedling was superior in unsterilised soil over sterilised soil. This indicate significance of natural soil over sterilised soil system.

2.10 Bacteria and growth promoting substances

Plant growth regulators, auxin and gibberellins are produced on culture supernatants of *Azotobacter choococcum, Azotobacter vinelandii* and *Azotobacter paspali.* Ctyokinins are produced by *Azotobacter chroococcum* (Barea *et al.*, 1974) and *Azotobacter paspali* (Barea and Brown, 1974). The effect of culture supernatants of *Azotobacter vinellandii* and *Azotobacter beijerinckii,* certain auxins, gibberellin and cytokinin like substances accelerates plant growth and increased trait. The effect of culture was noted at par wtih a plant hormones (Azcon and Barea, 1975). The production of plant growth regulators by *Azotobater vinelandii* under natural condition was also shown subsequently (Gonzalez-Lopez *et al.*, 19886).

Report also suggest that plant responses to inoculation with nitrogen fixing bacteria due to host plant-bacteria strain interaction. The stimulation of N_2-fixing *Bacillus* sp. on the rhizosphere of certain wheat lines, and the difference in the establishment of *Azospirillum* strain in wheat roots (Baldani *et al.*, 1983) and in the *rhizosphere* of *Zea mays* (O'Hara *et al.*, 1981). *Azospirillum* as compared to *Azotobacter , Klebsiella, Rhizobium, Pseudomonas* and *Escherichia,* is preferentially observed to pearl millet and guania pig grass root (Umali - Carcia *et al.*, 1980). Substances in the root exudates bind *Azospirillum* and promote its absroption. The variation in exudation was noted within different genotype

of sorghum (*Sorghum bicolor*), which has effected the growth and nitrogenase activity of *Azospirillum lipoferum* (Kipe-Nolt, 1985).

Introduction of plant growth substances produced by *Azospirillum brasilence* and their effect on the growth of pearl millet (*Pennisetum americanum*) was also detected by Tein *et. al.*(1979). Tryptophan was converted into indole acetic acid and indole lactic acid ; the production of organic acid was related to the production of tryptophan in the medium. Biologically active compounds such as gibberellin and three cytokinins production were detected in the culture medium. The solution containing this compound produced by *Azospirillum*, when inoculated, resulted in change of morphology or roots of pearl millet. More root hair formation, elongation of roots, reduction in lateral root formation was noticed due to incorporation solution. Such effect was equivalent to the effect of cytokinin and gibberellin.

Dewan and Subba Rao (1979), were first to observe the increase in root length and biomass of rice (*Oryza sativa*) due to inoculation of *Azospirillumn brasilense* and *Azotobacter chroococcum*. Subsequently, Singh and Subba Rao (1979), noticed more nodule number and root biomass of soybean (*G. max*) with inoculation of *Rhizobium japonicum*.

Stimulation of VAM root colonization of various legumes (*G. max, C. cajan, P. mungo, P. aureus, V. uniguiculata*) was also observed due to inoculation of yeast (*Saccharomyces cerevisiae*). However, such effect was more pronounced with the plant inoculated with *Rhizobium*. Increase in the number of vesicles and arbuscules was noticed due to inoculation of commercial, Baker's yeast (*Saccharomyces cerevisiae*). Subsequently, increase in spore number and VAM root colonization were also observed

MATERIALS AND METHODS

3.1 ISOLATION OF N_2-FIXING BACTERIA:

3.1.1 *Azospirillum*

Various isolates were made from the root pieces of wheat (*Triticum aestivum,* L.) grown at IARI farm following standard procedure, as described by Bulow and Dobereiner (1975). Isolation was done from three month old plants of various wheat varieties *viz.*, HW-2004, HD-2669, DL-784-3 (Vaishali), DL- 153-2 (Kundan), DL - 803-3 (Kanchan), HD-2710 and HDR-77.

The root pieces (1.0 cm) were first surface sterilized with mercuric chloride (0.01%) followed by several washings with the sterilized water to remove traces of mercuric chloride. After sterilization three root pieces from each variety were incorporated in screw caped tubes (15 x 120 mm) containing five ml of sterilized Dobereiner's nitrogen free semi-solid medium . There after these tubes were incubated for three days at 35 ± 2°C to allow the growth of *Azospirillum*. The positive tubes showing the pellicle formation were reserved for further purification by transferring a loopful of pellicle in to the fresh Dobereiner's nitrogen free semi-solid medium. This process was repeated five to seven times, till the characteristic pellicle formation was observed below 2-3 mm from the top surface of the medium.

3.1.2 Purification of *Azospirillum*

These isolates of *Azospirillum* were further purified by streaking over solid surface of Dobereiner's nitrogen free medium in sterilized petridishes

(Bulow and Dobereiner 1975). Streaked plates were incubated at 35 ± 2°C for three days. Single colony types were picked up from the plates and transferred to the slant containing Okon's medium (Okon *et al.*, 1977).

Media composition

1. **Composition of Dobereiner N-free medium** (Dobereiner and Day, 1976)

Malic acid	5.0g
KOH	4.0g
KH_2PO_4	0.5g
$FeSO_4.7H_2O$	0.05g
$MnSO_4.7H_2O$	0.01g
$MgSO_4.7H_2O$	0.10g
NaCl	0.20g
$CaCl_2$	0.01g
Na_2MoO_4	0.002g
Bromothymol blue (BTB) (5% alcoholic solution)	2.0 ml
Distilled wateer	1000 ml
pH	6.8-7.0

Agar

For semi-solid medium	1.75g
For solid medium	15.0g

2. **Composition of Okon's medium** (Okon *et al.*, 1977)

Part A

$K_2H\ PO_4$	6.0g
$KH_2\ PO_4$	4.0g
Distilled water	500 ml

Part B

$MgSO_4$	0.02g
NaCl	0.10g
$CaCl_2$	0.02g
NH_4Cl	1.00g
Malic acid	5.00g
NaOH	3.00g
Na_2MoO_4	0.005g
$MnSO_4$	0.001g
H_3BO_3	0.0014g
$Cu\ (NO_3)_2$	0.004g
$ZnSO_4$	0.0021g
$FeCl_3$	0.002g
Distilled water	500 ml
Bromothymol blue (BTB) (5% alcoholic solution)	2.0 ml
pH	6.8 - 7.0
Agar	2%

Part A and B were sterilized separately and mixed while hot and poured in to plates and allowed to settle.

Plate -1

Colonies of *P. vulgaris* on Nutrient Agar medium

Plate-2

Colonies of *A. chroococcum* strain, W-5 on Jensen's N-free medium.

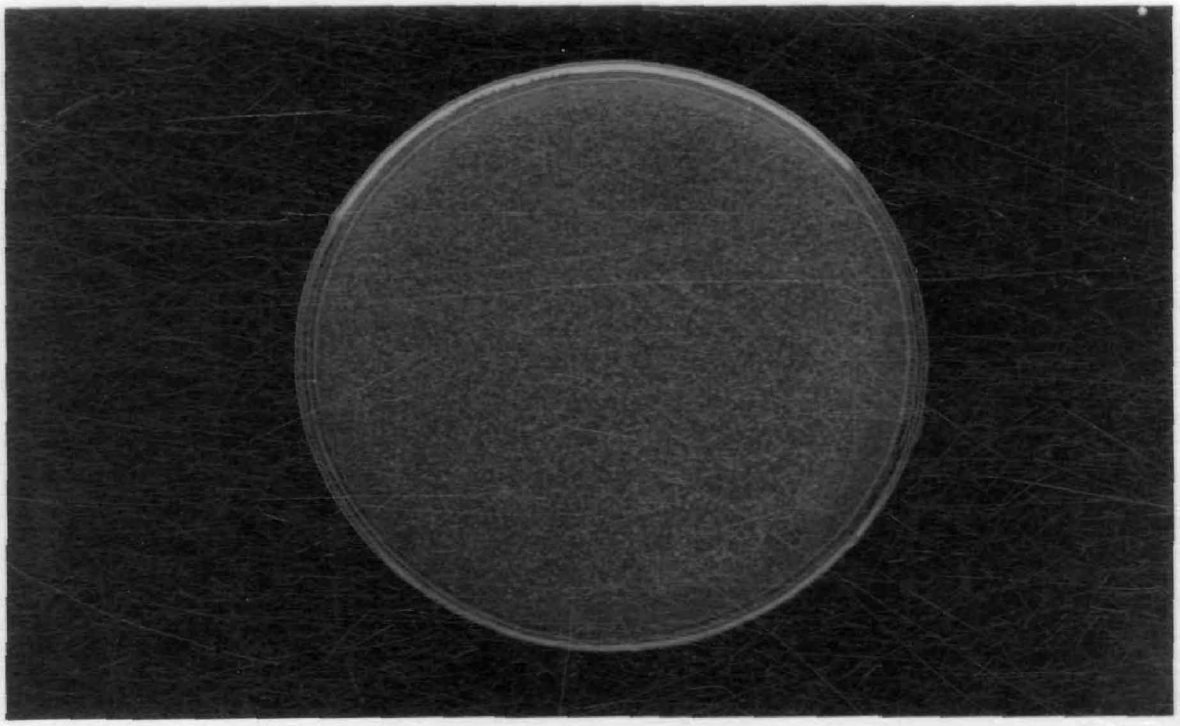

Plate -1

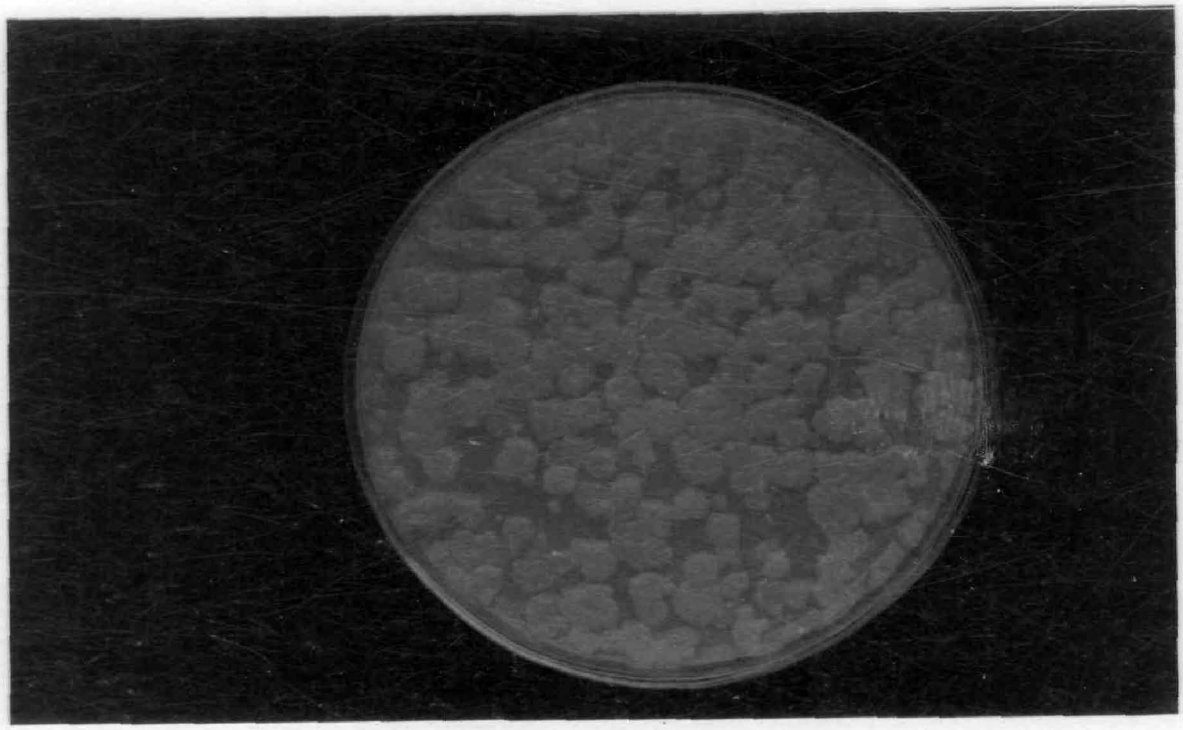

Plate -2

3.2 Isolation of *Azotobacter*

Rhizospheric soil samples of various varieties of wheat (*Triticum aestivum.* L.) *viz.*, HD - 2669, DL-784-3 (Vaishali), , HD-2687,HD-2643, HD - 2680, HD- 2710, HD - 2690 and HDR - 77 grown at IARI farm, were collected and serial dilution were made under aseptic condition. One ml aliquot from the serially diluted (10^{-2}, 10^{-3}, 10^{-4}) samples were transferred to the clean and sterilized petridishes (100 x 17 mm) and was mixed with 15 ml of melted and cooled (45°C) Jensen's N-free medium under aseptic condition. Two such plates were prepared from each dilution (10^{-2}, 10^{-3}, 10^{-4}) and incubated at 30 ± 2°C for 72 hours.

3.2.1 Purification of *Azotobacter*

Single isolated colonies which appeared on Jensen's N-free medium were picked up and streaked over the respective medium to obtain more isolated colonies. Well isolated, pigment forming colony (brown to black) were transferred to the slants containing Jensen's N-free solid medium and incubated (30± 2°C) for 72 hours to allow the growth of the bacteria.

Composition of Jensen's medium (Jensen's 1942)

Sucrose	20.00 g
K_2HPO_4	1.00 g
$MgSO_4.7H_2O$	0.50 g
NaCl	0.50 g
$FeSO_4.7H_2O$	0.10g
Na_2MoO_4	0.005g
$CaCO_3$	2.00 g
Agar	15-18 g
Distilled water	1000 ml
pH	7.0

Plate-3

Colonies of *Azotobacter* isolate, Azoto. 2687-1 on Jensen's N-free medium

Plate-4

Colonies of *Azospirillum* strain, *Azo. lac.lac.* on Dobereiner's N-free medium

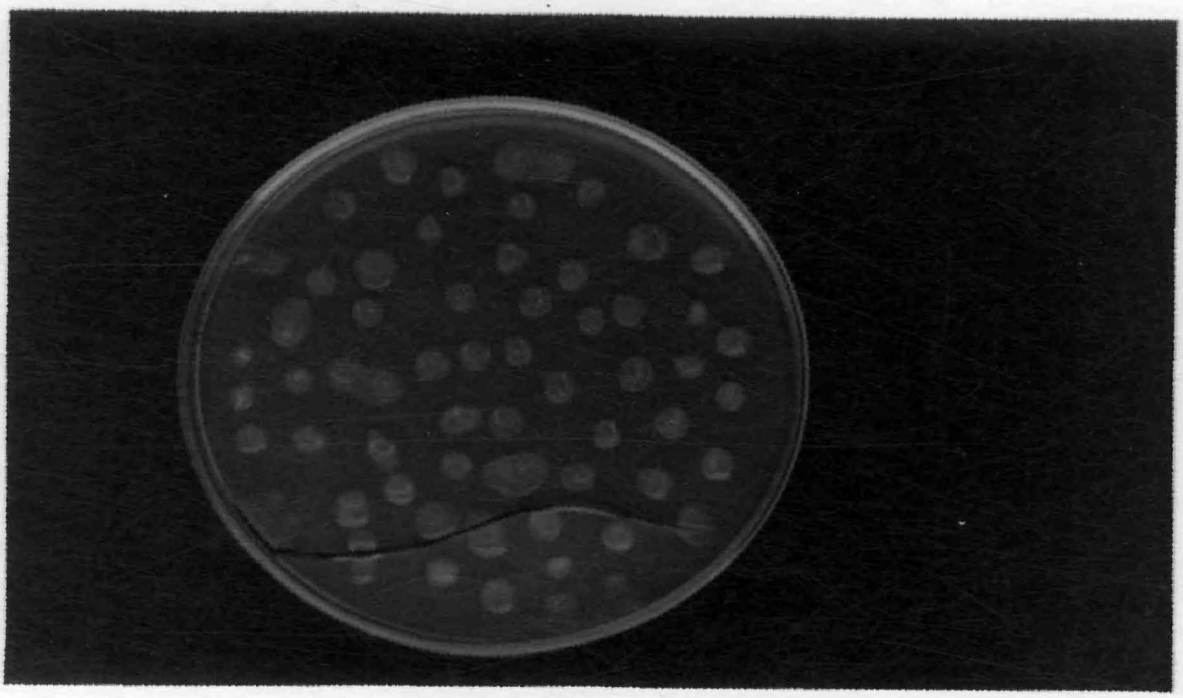

Plate -3

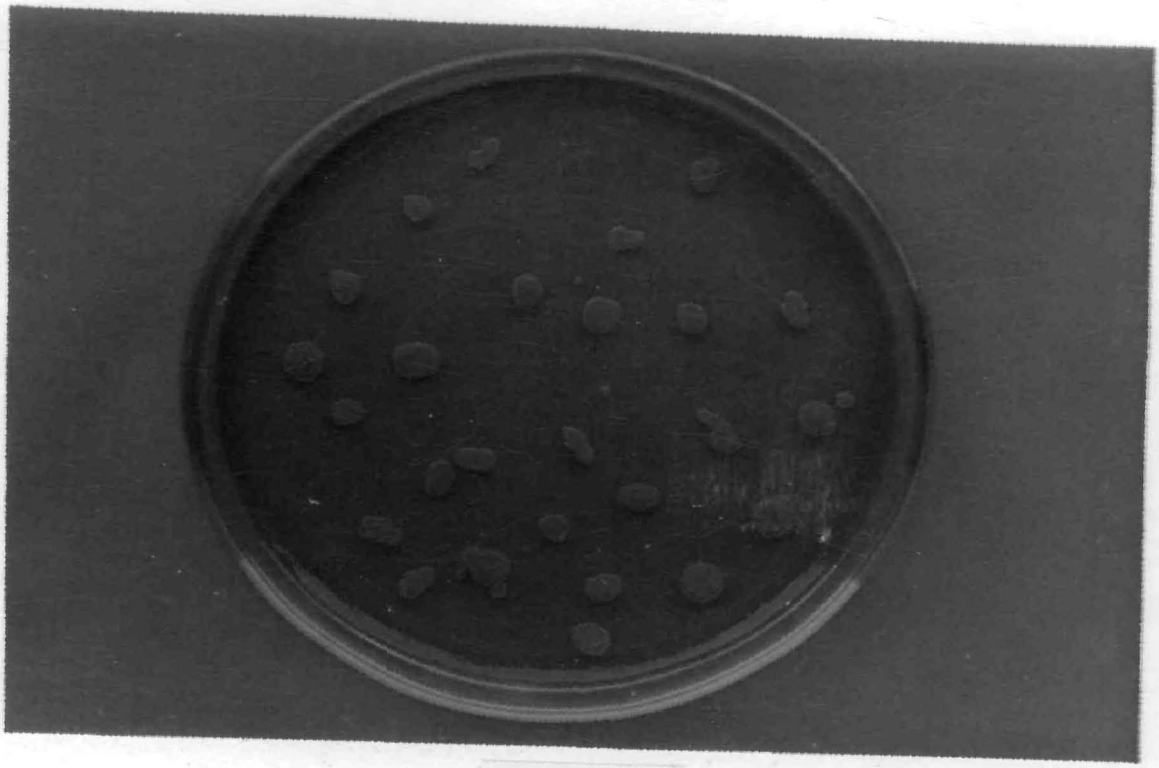

Plate -4

3.2.2 Maintenance of N_2-fixing bacteria

Azospirillum and *Azotobacter* were maintained on Okon's medium and Jensen's N-free solid medium respectively for further use.

3.3 Collection of cultures

Nitrogen fixing bacterial cultures were obtained from various sources (Table 1). In the present study standard cultures of *Azospirillum* and *Azotobactor* were also collected and used. *Proteus vulgaris* spp. and *Flavobacterium* sp . (P-25) were also included in the study. In the present study standard cultures and newly isolated N_2-fixing bacteria were included to compare the overall effect on the growth of wheat . Vesicular-arbuscular mycorrhizae (VAM) fungi now renamed as arbuscular mycorrhizae (Walker , 1995) were also obtained and used to find out the best performing VAM with wheat (*Triticum aestivum,* L.) *var* HD-2329. Details of the standard cultures used in the present study are presented in the following (Table1).

Table 1 Details of the standard bacterial cultures used in the study

Cultures	*Characters*	*Sources*
A. Nitrogen fixer		
1. ***Azospirillum***		
A. brasilense Sp-7	isolated from grasses	Dr.J.Dobereiner, Embrapa Agrobiology Riode Janeiro, Brasil.
A. lac. lac	isolated from sprocarp of *Laccaria laccata*	Dr. Cy. Li. USDA Pacific Northwest Research station
A. Rv. Zae	isolated from sporocarp of *Rhizopogon vinicolor*	Forestry science laboratory, corvallis orgoen, USA

Plate-5

Colonies of *A. lipoferum* strain, Azo.242 on Dobereiner's N-free medium

Plate-6

Colonies of *Azospirillum* isolate, Azo.2669 on Dobereiner's N-free medium

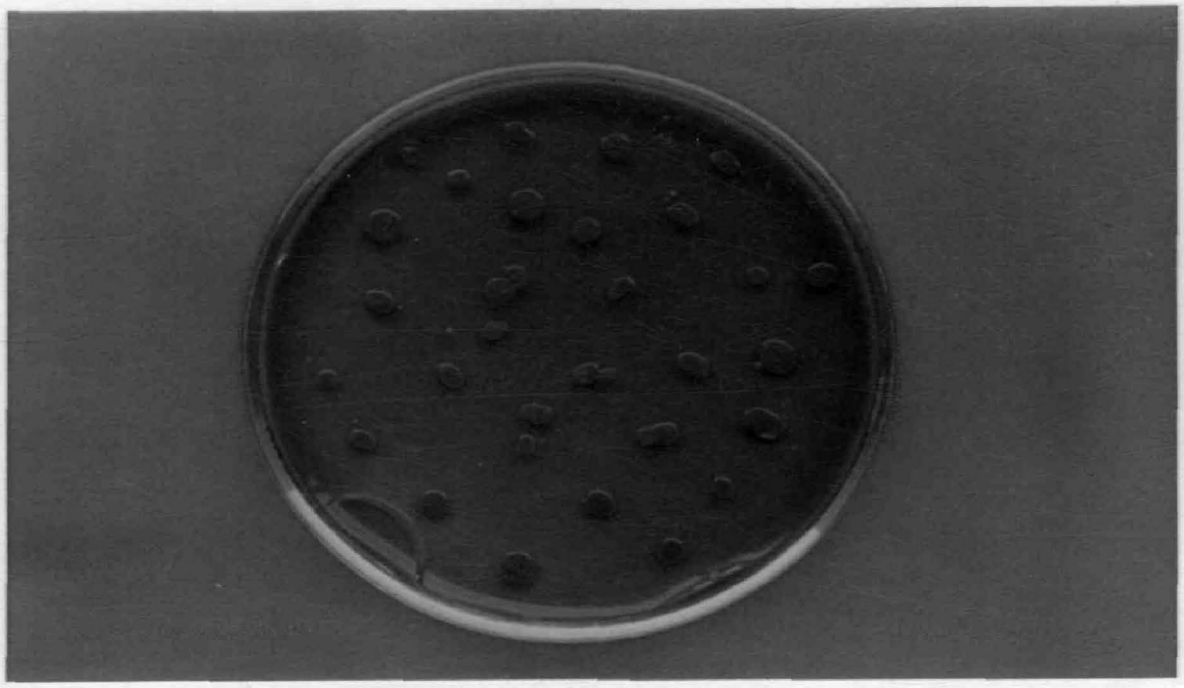

Plate -5

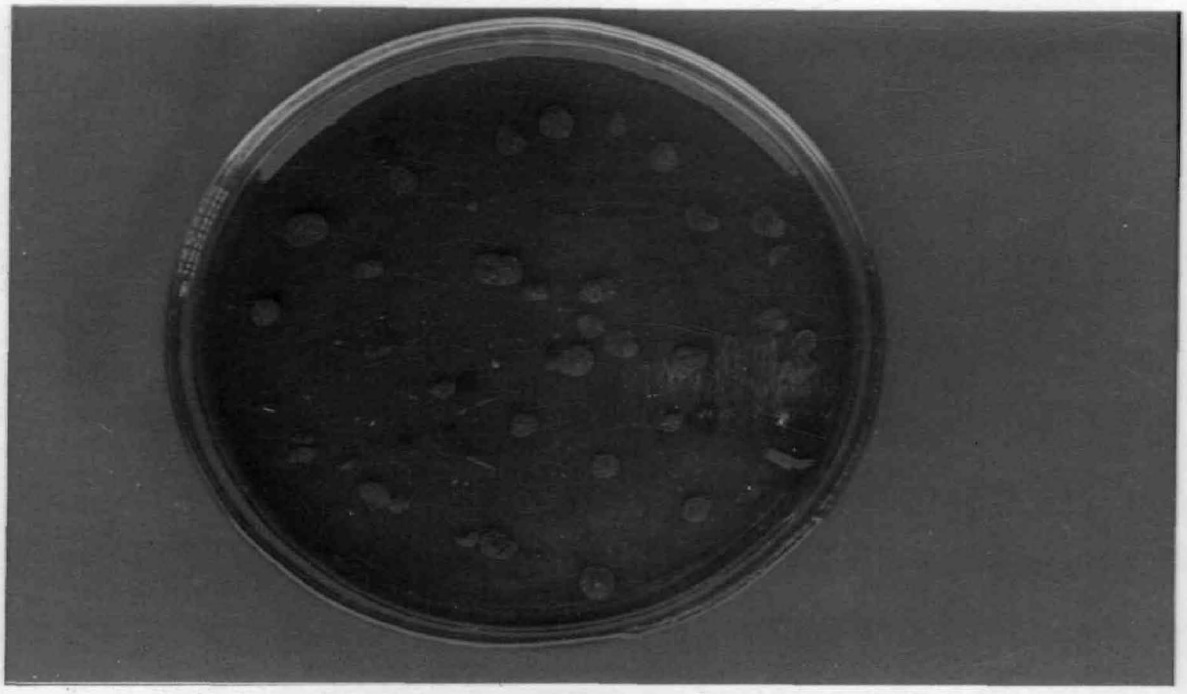

Plate -6

additional observation on spore count in the soil and per cent VAM root infection were made. Details of the experiment are as follows.

3.4.1 Screening of nitrogen-fixing bacteria *Azospirillum* and *Azotobacter*- under *in vitro* condition.

The enzyme nitrogenase, in addition to reducing N_2 to NH_3, can reduce acetylene to ethylene. The gaseous products can be separated and identified by passing the mixture through a porapak column of Gas Chromatograph using flame ionisation detector (Hardy *et al.*, 1968).

Nitrogenase activity as aceylene reduction activity (ARA) was estimated using a *Shimadzu* gas chromatograph model GC - 14A, having a porapak N column. Separation conditions used were : Injector temperature-110°C, detector (FID) temperature-110°C, column temperature 80°C and flow rate of carrier gas N_2-30ml min^{-1}. Standard ethylene (105 vpm) was obtained from E.D.T. Research London, U.K.

Azospirillum isolates, obtained from the roots of wheat (*Triticum aestivum*, L.) and other collected strains of *Azospirillum*, were grown on Okons's medium. The cultures were incubated in serum stoppered tubes (25 x 150 mm) containing 10 ml of above medium and incubated for three days at 35±2°C. Five ml of C_2H_2 (10% v/v) was injected after removal of same amount of air from the vials and incubated for 24 hours at prescribed temperature. Acetylene reduction activity (nitrogenase activity) was measured by injecting 1 ml of gas from the tubes to the preconditioned gas chromatograph and peak height of ethylene (C_2H_4) was recorded .

Similarly nitrogenase activity of *Azotobacter*, *Proteus sp.* and *Flavobacterium* sp. (p-25) were also determined by following standard procedure (Hardy *et al.*, 1968) using their respective medium.

Above mentioned bacteria were grown for three days on decline solid surface (slant) of Jensen's Nitrogen free medium. Nitrogenase activity were determined by the same procedure as described in this chapter.

3.5 Screening of efficient N_2-fixing bacteria (*Azospirillum, Azotobactor) and other bacteria (Proteus, Flavobacterium)* under *in vivo* condition

3.5.1 Soil

Farm soil of Indian Agriculture Research Institute (IARI) New Delhi having following characteristics *viz.*, organic C % - 0.11, available P Kg ha^{-1} - 9.0, available K Kg ha^{-1} - 67, $CaCO_3$ - high, E.C. - 0.22, texture - sandy, pH - 8.7, were used for pot culture experiments. The soil was first seived through 30 mesh size and sterilized for four hours at 15 lb pressure $inch^{-2}$ in an autoclave. It was amended with nitrogen (20 Kg N ha^{-1}) and phosphorus (30 Kg P_2O_5 ha^{-1}) in the form of urea and single super phosphate respectively as a basal dose. Another dose of nitrogen (20 kg N ha^{-1}) was also applied at the 40 days of the plant growth. Such amended soil (2.0 Kg) pot^{-1} were distributed in plastic pots (15×15 cms) to carry out the experiments.

3.5.2 Method of inoculation of N_2-fixing bacteria

Three ml of active culture of *Azotobacter* (two days), *Azospirillum* (three days) and *Flavobacterium* (two days) were used to inoculate surface sterilized healthy seeds (3.0g) of wheat (*Triticum aestivum,* L.) *var* H.D. 2329. Carrier based culture of *Proteus vulgaris* was also obtained and used in the study. One g of the culture was used to inoculate 3.0g of wheat seed. The inoculated seeds were incubated at room temperature for 2 hours. Six holes up to 5 cm depth were made in each pot and two seeds were sown in each hole. Care was also taken to maintain normal

soil moisture at the time of sowing. The experiment was conducted following randomized block design (RBD) and each treatment was replicated six times. Out of six replications, three replications were terminated at 45 days of plant growth and initial observations were taken.

3.5.3 Observations

Observations on shoot height, number of tillers, fresh and dry weight of shoot and root and root volume, were noted. *Azospirillum* and *Azotobacter* populations in soil were also determined.

3.6 Screening of various VAM fungi

Various VAM fungi such as *Glomus mosseae, G. macrocarpum, Gigaspora margarita, G. fasciculatum, Sclerocystis, Acaulospora, Gigaspora callospora, G. aggrocarpum, G. etunicatum, G. deserticola* and *Glomus interaradices,* were obtained from IARI and used for screening of potent VAM fungi.

3.6.1 Method of inoculation of VAM fungi

IARI farm soil as mentioned earlier, was used for screening of potent VAM fungi. Ten VAM spores of various species were first transferred in the holes (six holes pot^{-1})made in the pots containing sterilized soil (as mentioned above) and in these holes two seeds of wheat (*Triticum aestivum*, L.) *var* HD-2329 were sown. In this experiment also seeds surface sterilized with mercuric chloride (0.01% solution) were used. The experiment was conducted following the randomized block design (R.B.D.). Six replications were kept for each treatment and three were terminated at 45 days of plant growth and observation on various plant parameters *viz,*. plant height , Number of tillers, fresh and dry weight of shoot and root and root volume were taken. Per cent VAM infection (Phillips and Hayman, 1970)

and spore count (Gerdemann and Nicolson, 1963) in the rhizospheric soil were also taken to screen out the best performining VAM fungi to be used for further investigation.

3.6.2 Per cent VAM root colonization

The per cent mycorrhizal root colonization of the wheat by VAM fungi was determined following the standard method of Phillips and Hayman (1970). The roots were washed gently in tap water. The washed roots were cut in to 1 cm size and then immersed in 10 per cent KOH solution for clearing the host cytoplasm, nuclei and also for stain penetration. Roots were boiled for 10-20 minutes. After digestion, KOH was removed and the root bits were washed for 3-5 times and immersed in hydrogen peroxide (H_2O_2) solution for 30 minutes. Root bits were taken out and washed with tap water for about 3 times or until no brown colour appeared in the rinsed water. The roots were acidified with 2 per cent HCl for proper staining. The acid was poured out. Without rinsing with water, root bits were stained with 0.05 per cent trypan blue solution in lactophenol (Lactic acid: Glycerol : Water 1:1:1, v/v/v) for 30 minutes and boiled for 10 minutes. After decanting the dye, these roots were destained with lacto glycerol. The root bits were mounted on glass slides and observed under stereoscopic zoom microscope. Fifty root segments in each replication were examined to determine VAM fungal colonization percentage.

$$\text{Per cent VAM mycorrhizal colonization} = \frac{\text{No. of root bits with infection}}{\text{Total no. of root bits examined}} \times 100$$

3.6.3 Collection of VAM spores

Sand and soil based inoculum was collected from mother cultures and mixed thoroughly. The spores were collected by following the standard procedure "wet sieving and decanting" (Gerdemann and Nicolson, 1963).

One 100 gram of rhizospheric soil sample was taken and mixed thoroughly in one litre of tap water and heavier particles in the suspension were allowed to settle down . The suspension was decanted through a coarse sieve 500-800 μm size to remove large pieces of organic matter. The liquid which passed through the sieve was collected separately and stirred well to resuspend all particle. The suspension was decanted through a sieve fine enough to retain desired spores (50-350 mesh size). The material retained on the sieve was washed with a stream of water to ensure that all colloidal material were passed through the sieve.

3.6.4 Counting of VAM spores

The small amount of remaining debris were transferred to a shallow layer of water in a petridish and examined under a stereoscopic zoom microscope. The spore number was counted and expressed per 100 g of soil.

3.7 Study on interaction of potent N_2-fixing bacteria and *Glomus macrocarpum*

Among the seventeen isolates of *Azospirillum,* (Table 3) four strains / isolates *viz.*, Azo. lac. lac, Azo-242, Azo. HW. 2004-2 and Azo. 2669 were found more efficient *Asospirillum.* Out of twenty *Azotobacter* strains/isolates (Table 5) three isolates, (*Azoto.* 2687-1, Azoto. 2687-2 and W-5) were selected for further study as potent *Azotobacter.* Accordingly four *Azospirillum* strains, three *Azotobacter* strains, one *Proteus vulgaris* and one *Flavobacterium sp* . (p-25) were used in this experiment in the presence of *Glomus macrocarpum,* which was found most potent among various species of VAM tested on wheat (*Triticum aestivum,* L.) *var* H D -2329.

To carry out this experiment IARI farm soil, having the following characteristics *viz.*, organic C% - 0.04, available P Kg ha^{-1}- 29.9, available K kg ha^{-1} - 140, $CaCo_3$ - high, E.C. - 0.15, texture-clay loam, pH-8.5 were first sieved and distributed in 14" diameter pots @ 12 Kg soil pot^{-1}. The objective of this experiment was to screen out most potent N_2-fixing bacteria in the presence of most potent VAM fungi, with out amendment of nitrogen and phosphorus. Detail of the experiment is being out lined as below.

Table 2 Details of the experiment

S.No.	Treatment
1.	Control
2.	*Glomus macrocarpum*
3.	Azo. lac. lac
4.	Azo.242
5.	Azo. HW. 2004-2
6.	Azo. 2669
7.	Azoto.2687-1
8.	Azoto.2687-2
9.	Azotobacter W-5
10.	*Proteus vulgaris*
11.	*Flavobacterium sp* . (p-25)
12.	*G. Macrocarpum* + Azo. lac. lac
13.	*G. Macrocarpum* + Azo. 242
14.	*G. Macrocarpum* + Azo.HW. 2004-2
15.	*G. Macrocarpum* + Azo.2669
16.	*G. Macrocarpum* + Azoto.2687-1
17.	*G. Macrocarpum* + Azoto.2687-2
18.	*G. Macrocarpum* + Azotobacter W-5
19.	*G. Macrocarpum* + *Proteus vulgaris*
20.	*G. Macrocarpum* + *Flavobacterum sp* . (P-25)
21.	1/3 N of recommended dose (40 Kg Nha^{-1})

22. 1/2 N of recommended dose (60 Kg Nha^{-1})
23. Full N of recommended dose (120 Kg Nha^{-1})

No. of treatments	23
No. of replications	5
No. of plants/pot	5
Wheat variety	HD -2329
Date of sowing	16.12.98

The method of inoculation of bacteria and VAM fungi remains the same as described in this chapter. However, in this experiment the plants were thined to five to take observation at harvest (grain and straw yield of wheat and number of spikes). Grain and straw were ground to the level of powder, passed through 60 mesh size sieve to obtain homogenous fine powder. Nitrogen and phosphorus contents in these powdered samples were determined by following standard procedure (Jackson, 1967).

3.8 Plant samples analysis

3.8.1 Determination of total phosphorus

The total phosphorus in the plant samles (grain and straw) was estimated by the method of Jackson (1967).

Reagents

Barton's reagent

i) 25g of ammonium molybdate was dissolved in 400 ml of warm distilled water.

ii) 1.25g ammonium metavandate was dissolved in 300 ml of warm distilled water. The solution was cooled and 250 ml of conc. nitric acid was added to it. The solution was allowed to cool. Solution (i) and (ii) were mixed and the volume was made to one litre.

Tri acid mixture

Concentrated nitric acid, sulphuric acid and perchloric acid was mixed in the ratio of 10:2:3 and the mixture was cooled before use.

Procedure

12 ml of tri acid mixture was added to one g of finely ground samples in the digestion tubes (75 ml capicity) and digestion was carried out on Tecator Digester 12,1009 at 300-350°C till the solution become clear. The digestion tubes were cooled and the volume was made up to 25 ml with distilled water. 4 ml of diquot was taken in a 50 ml volumetric flask and to this a little amount of distilled water was added followed by the addition of 2.5 ml of Barton's reagent. The flask was shaken to mix the contents thoroughly. The volume was made 50ml with distilled water. The O.D. of the coloured solution was read at 440 nm in a Sprectophotometer after setting zero with a reagent blank. Simultaneously reading for standard P solution was also recorded at various concentration. The phosphorus content in plant samples was calculated from the standard curve.

3.8.2 Estimation of total nitrogen

Total nitrogen in the dried plant samples was estimated using an N-autoanalyser following the procedure out lined in Technicon Manograph 1, 1971. The method consisted of two main steps,

(i) acid digestion of plant material

(ii) measurnment of N as amonia in the digested plant samples after converting it into coloured indophenol dye.

Digestion

The digestion was carried out following a modified version of the Kjeldahl procedure. The sample size used for analysis varied according to the expected N content. Finaly ground and weighed samples were transferred to the digestion tubes of 75 ml capicity, to each digestion tubes 4 ml of acid mixture and a digestion tablet was added. The acid mixture consisted of concentrated sulphuric acid and phosphoric acid in the ration of 20:1. Each digestion tablet contained 1.5g potassium sulphate and 0.0075g of selenium. Forty samples were digested at a time on a Tecator Digestion unit at a temperature 300-350°C. Towards the end of digestion 1.0 or 1.5 ml of 30 per cent v/v hydrogenperoxide (H_2O_2) was added to each tube. Subsequent to digestion (approximately 3-5 min), the tubes were cooled and the volume was made up to 75 ml with distilled water.

Dtermination of N content

The N content was estimated as ammonium ion content in the digest in a Technicon N-autoanalyser. The method is based on the reaction of ammonium ions in the digested samples with alkaline hypochlorite to form Chlormine and then with phenol to produce a blue indophenol dye.

The absorbance of the coloured compound was automatically read at 660nm in a Colorimeter and recorded as a peak on a chart. The N concentration was then estimated using a calibration curve obtained with ammonium chloride treated similarly. Nitrogen concentration was expressed as N per cent in plant sample.

3.9 Statistical analysis of data

The results (data) obtained during the present investigation were statistically analysed using the "analysis of variance" method as described for Completely Randomized Design (C.R.D.) by Fisher (1970), Gomez and Gomez (1984).

RESULTS

1. Isolation of N_2-fixing bacteria

1.2 *Azospirillum*

Eleven isolates of *Azospirillum viz.* Azo. HW.2004-1, Azo. HW. 2004-2, Azo. HW. 2669, Azo. Vaishali-1, Azo. Vaishali-2, Azo. Kundan, Azo. Kanchan, Azo. 2710-1, Azo. 2710-2, Azo. HDR.77-1 and Azo.HDR.77-2 were obtained from roots of seven different varieties of wheat (*Triticum aestivum,* L.) *viz.* HW-2004, HD-2669, DL- 784-3 (Vaishali), DL - 153-2 (Kundan), DL-803-3 (Kanchan), HD-2710 and HDR-77, grown at IARI farm (Table 3).

When the root pieces of these varieties, after surface sterilization, incorporated in to Dobereiner's N-free medium, formation of white pellicle was observed on the top of the surface of the medium after three days of incubation. A loopful of pellicle was transferred further to the fresh Dobereiner's N-free medium. Upon incubation white pellicle formation was again observed in the tubes, below 2-3 mm from the top surface. Such tubes were considered positive for the presence of *Azospirillum.* From such tubes, a loopful semisolid medium (pellicle especially) containing bacterium was taken out and streaked over solid surface of N-free medium, in the petri dishes (4"size), as described earlier. After incubation at 35±2°C for three days, round (1-2 mm in dia), convex, whitish, transparent and gum producing colonies were appeared on such pertidishes (Table 4).

Under the microscope, the most characterstic feature of the organism was its spiral movement. The cell showed clock-wise half turn or full spiral

Table 3 : Details of *Azospirillum* isolates obtained from various wheat varieties (*Triticum aestivum, L.*) and standard strains of *Azospirillium*

S. No.	Name of *Azospirillum* isolates	Source/name of wheat variety	Nitrogenase activity
A. *Azospirillium* isolates, isolated from wheat			
1	Azo. HW. 2004-1	HW-2004	+
2	Azo. HW. 2004-2	HW-2004	+
3	Azo. 2669	H.D - 2669	+
4	Azo. Vaishali-1	DL-784-3 (Vaishali)	+
5	Azo.Vaishali-2	DL-784-3 (Vaishali)	+
6	Azo. Kundan	DL-153-2 (Kundan)	+
7	Azo. Kanchan	DL- 803-3 (Kanchan)	+
8	Azo. 2710-1	HD - 2710	+
9	Azo. 2710-2	HD - 2710	+
10	Azo. HDR. 77-1	HDR - 77	+
11	Azo. HDR. 77 -2	HDR - 77	+
B. Standard *Azospirillum* strains			
1	*A.brasilense* Sp-7	Isolated from grass	+
2	*A. lac. lac*	Isolated from sporocarp of *Laccaria laccata*	+
3	*A. Rv.Zae*	Isolated from sporocarp of *Rhizopogon vinicolor*	+
4	*A. amazonense*	Isolated from grass	+
5	*A. lipoferun*-242	Isolated from grass	+
6	*A. brasilense*-Sp cd	Isolated from grass	+

Note : + = Positive

Table 4 Morphological and cultural characterstics of *Azospirillum* isolates on N- free semi-solid malate medium

S.No.	Strains	Microaerophilic growth (pellicle formation)	Shape	Size	Motility	Gram strain reaction	Colony on N-free agar medium	Nitrogenase activity
1	Azo. HW. 2004-1	Below 2 mm from agar surface	Spirally curved rods, coccoid cell	2-6×0.5-1 µm	Rapid, back and forward	Negative	Moist, mildly raised, slimy 0.5-1 mm dia.	Positive
2	Azo. HW. 2004-2	"	"	"	"	"	"	"
3	Azo. 2669	"	"	"	"	"	"	"
4	Azo. Vaishali-1	"	"	"	"	"	"	"
5	Azo.Vaishali-2	"	"	"	"	"	"	"
6	Azo. Kundan	"	"	"	"	"	"	"
7	Azo. Kanchan	"	"	"	"	"	"	"
8	Azo. 2710-1	"	"	"	"	"	"	"
9	Azo. 2710-2	"	"	"	"	"	"	"
10	Azo. HDR. 77-1	"	"	"	"	"	"	"
11	Azo. HDR. 77 -2	"	"	"	"	"	"	"
		"	"	"	"	"	"	"
		"	"	"	"	"	"	"

movement. When attached to one point the cells seems to rotate on their own axis, but when moving from one point to another, typical spirillar movement became clear.

Small and big size cells were often present. Conspicuous poly -β-hydroxy butyrate (PHB) granules which often distorted the shape of the cell was observed. All the isolates retained the colour of the counter stain-saffranine, indicating the Gram negative reaction. These cells were spirally curved rods or coccoid (Table 4).

The cultures of *Azospirillum* in Dobereiner's N-free medium reduced acetylene to ethylene. Nitrogenase positive *Azospirillum* were only included for further investigation.

1.3 *Azotobacter*

Sixteen isolates of *Azotobacter viz.* Azoto.2669, Azoto.Vaishali, Azoto. 2687-1, Azoto.2687-2, Azoto.2643-1, Azoto.2643-2, Azoto.2680-1, Azoto.2680-2, Azoto.2680-3, Azoto.2710-1, Azoto.2710-2, Azoto.2690-1, Azoto.2690-2, Azoto.HDR.77-1, Azoto. HDR-77-2 and Azoto.HDR. 77-2 were isolated from the rhizospheric soil samples of eight different varieties of wheat *viz.* HD-2669, DL-784-3 (Vaishali), HD-2687, HD-2643, HD-2680, HD-2710, HD-2690 and HDR-77, which were grown at IARI farm (Table 5). The single isolated, pigment forming (brown to black) colonies from Jensen's N-free medium were first picked up and streaked over the same medium in the petridishes. These petridishes were incubated at 30±2°C for 72 hours. Upon incubation round (2-3 mm in dia), convex gum producing pigment forming colonies were appeared on such petridishes (Table 6).

Such bacterial colonies were considered as *Azotobacter* and maintained on slants of Jensen's N-free medium. Under the microscope, cells of these

Table 5 : Details of *Azotobacter* isolates, obtained from various wheat varieties (*Triticum aestivum*, L.) and standard strains of *Azotobacter*

S.No.	Name of *Azotobacter* isolates	Source/name of wheat variety	Nitorgenase activity
A. *Azotobacter* isolates, isolated from wheat			
1	Azoto. 2669	HD - 2669	+
2	Azoto. Vaishali	DL-784-3 (Vaishali)	+
3	Azoto. 2687-1	HD - 2687	+
4	Azoto. 2687-2	"	+
5	Azoto. 2643-1	HD - 2643	+
6	Azoto. 2643-2	"	+
7	Azoto. 2680-1	HD - 2680	+
8	Azoto. 2680-2	"	+
9	Azoto. 2680-3	"	+
10	Azoto. 2710-1	HD - 2710	+
11	Azoto. 2710-2	"	+
12	Azoto. 2690-1	HD - 2690	+
13	Azoto. 2690-2	"	+
14	Azoto. HDR. 77-1	HDR - 77	+
15	Azoto. HDR. 77-2	"	+
16	Azoto. HDR. 77-3	"	+
B. Standard *Azotobactor* strains			
1	M-4	isolated from maize (*Zea mays*) rhizosphere	+
2	CBD-15	isolated from cotton (*Gossypium barbadense*) rhizosphere	+
3	W-5	isolated from wheat (*Triticum aestivum*, L.) rhizosphere	+
4	A-41	isolated from Deharadun forest soil	+

Note : + = Positive

Table 6 Morphological and cultural characterstics of *Azotobacter* isolates on Jensen's N-free medium

S.No.	Strains	Aerobic growth	Shape	Size	Motility	Gram reaction	Colony on N-free agar medium	Nitrogenase activity
1	Azoto. 2669	on the agar surface	oval, spherical or rod shaped	2.0-3.0 × 3.0-6.0µm	Motility present in young culture, lost due to aging	Negative	Mildly raised colony due to productionof extra cellular polysaccharides, 2-3 mm in dia	Positive
2	Azoto. Vaishali	"	"	"	"	"	"	"
3	Azoto.2687-1	"	"	"	"	"	"	"
4	Azoto.2687-2	"	"	"	"	"	"	"
5	Azoto.2643-1	"	"	"	"	"	"	"
6	Azoto.2643-2	"	"	"	"	"	"	"
7	Azoto.2680-1	"	"	"	"	"	"	"
8	Azoto.2680-2	"	"	"	"	"	"	"
9	Azoto.2680-3	"	"	"	"	"	"	"
10	Azoto.2710-1	"	"	"	"	"	"	"
11	Azoto.2710-2	"	"	"	"	"	"	"
12	Azoto.2690-1	"	"	"	"	"	"	"
13	Azoto.2690-2	"	"	"	"	"	"	"
14	Azoto.HDR. 77-1	"	"	"	"	"	"	"
15	Azoto.HDR. 77-2	"	"	"	"	"	"	"
16	Azoto.HDR. 77-3	"	"	"	"	"	"	"

cultures were seen as a rods (2.0-3.0×3.0-6.0μm), pleomorphic and motile. Gram reaction of these bacteria was found to be negative as observed with *Azospirillum*. Accumulation of poly -β- hydroxy butyrate (PHB) was also observed (Table 6).

2.0 Screening of N_2-fixing bacteria and vesicular-arbuscular mycorrhizae (VAM) fungi

Experiments were carried out to screen out more efficient N_2-fixing bacteria and vesicular-arbuscular mycorhizae (VAM) fungi. To achieve the above target two separate experiments were carried out simultaneously in potted soil condition. Out come of these experiments are being summerized as under :

2.1 Acetylene reduction assay of N_2-fixing bacteria (*Azospirillum, Azotobacter*) and *Proteus* and *Flavobacterium*)

Azospirillum spp. (Sp-7, Azo.lac.lac, Rv.zae, Azo. *amazonense*, Azo.242 and Sp cd) obtained from various sources were found to be positive to nitrogenase activity (Table 3). Similarly other isolates of *Azospirillum* (Azo. HW. 2004-1, Azo. HW. 2004-2, Azo.2669, Azo.Vaishali-1,Azo.Vaishali-2, Azo. Kundan, Azo. Kanchan, Azo.2710-1, Azo.2710-2, Azo. HDR.77-1 and Azo. HDR.77-2) isolated from various varieties of wheat (*Triticum aestivum,* L.) *viz.,* HW- 2004, HD-2669, DL-784-3 (Vaishali), DL-153-2 (Kundan), DL-803-3 (Kanchan), HD-2710, HD- 2690 and HDR-77) were also found to be nitrogenase positive Table (3).

All the standard culture of *Azotobacter* (M-4, CBD-15, A-41 and W-5) obtianed from various sources were nitrogenase positive. All the other isolates of *Azotobacter* (Azoto.2669, Azoto. Vaishali, Azoto. 2687-1, Azoto. 2687-2, Azoto 2643-1, Azoto 2643-2, Azoto.2680-1, Azoto.2680-2, Azoto.2680-3, Azoto.2710-1, Azoto.2710-2, Azoto.2690-1, Azoto.2690-2, Azoto. HDR.

77-1, Azoto.HDR. 77-2 and Azoto. HDR.77-3), isolated from rhizospheric soil of different varieties of wheat have also shown nitrogenase activity (Table 5).

Other bacteria such as *Proteus vulgaris* and *Flavobacterium sp.* (p-25) were also included in the study and were found to be nitrogenase positive. At this stage it remains contraversial and further confirmation is required. Only nitrogenase positive, bacteria were included for pot culture study alongwith standard N_2-fixing bacterial cultures, as mentioned earlier.

2.2 Screening of N_2-fixing bacteria under potted soil condition

Different isolates of *Azospirillum viz.* Azo. HW. 2004-1, Azo. HW. 2004-2, Azo. 2669, Azo. Vaishali-1, Azo. Vaishali-2, Azo. Kundan, Azo. Kanchan, Azo. 2710-1, Azo. 2710-2, Azo. HDR.77-1 and Azo. HDR. 77-2 (Table 7A) and *Azotobacter* isolates *viz.*, Azoto. 2669, Azoto.Vaishali, Azoto. 2687-1, Azoto. 2687-2, Azoto. 2643-1, Azoto.2643-2, Azoto.2680-1, Azoto. 2680-2, Azoto. 2680-3, Azoto.2710-1, Azoto.2710-2, Azoto.2690-1, Azoto.2690-2, Azoto. HDR. 77-1, Azoto. HDR.77-2 and Azoto. HDR. 77-3 were included to screen efficient N_2-fixing bacteria (Table 7 B). Besides these standard cultures of *Azospirillum spp.* (Sp-7, Spcd, Azo. lac. lac, Rv.Zae, Azo. *Amazonense*, Azo. 242) (Table 7 A) and *Azotobacter spp.* (W-5, CBD-15, M-4, A-41) were also used in the present study (Table 7 B). *Flavobacterium sp* . (P-25) and *Proteus vulgaris* were also included to compare their efficiency with above mentioned N_2-fixing bacteria, **for the first time with wheat** (Table 7C).

2.2.1 Inoculation effect of N_2-fixing bacteria (*Azospirillum, Azotobacter) Proteus and Flavobacterium* on growth and other plant parameters (Table 7A, 7B, 7C, 8A, 8B, 8C)

Various isolates of *Azospirillum* and *Azotobacter* were taken to find out their inoculation effect on growth performance of wheat (*Triticum aestivum,* L.) *var.* HD-2329, under potted soil condition. *Flavobacterium sp.* (P-25) and *Proteus vulgaris* were also included in the study alongwith above mentioned bacteria.

In general, growth parameters such as plant height, fresh and dry weight of shoot and root, number of tillers and root volume were increased due to seed inoculation of these bacteria. Difference in increase between the bacteria were also observed. It is worth to note that above parameters were noted maximum with *Flavobacterium sp.* (P-25), followed by increase obtained due to inoculation of *Azotobacter sp.* (W-5), *Azospirillum sp.* (Azo. lac.lac), *Proteus vulgaris, Azotobacter sp.*, (Azoto 2687-2), *Azospirillum sp.* (Azo.242) , *Azospirillum sp.* (Azo. 2669), *Azospirillum sp.* (Azo. 2004-2) and *Azotobacter sp.* (Azoto. 2687-1). Details of the experimental results are being summerized as follows :

2.2.2 Inoculation effect of *Azospirillum* on various plant growth parameters of wheat (7A, 8A)

Various strains of *Azospirillum spp.* (Sp-7, Azo. lac.lac, Rvzae, Azo. *amozonense*, Azo. 242 and Sp cd) obtained from various sources and newly isolates of *Azospirillum,* isolated from different wheat varieties *viz.* HW-2004, HD-2669, DL-784-3 (Vaishali), DL-153-2 (Kundan), DL- 803-3 (Kanchan), HD-2710 and HDR-77 (Table 7A), were used in the experiment to find out efficient *Azospirillum* strain on the basis of various plant

Plate-7

Effect of *Azospirillum spp.* on growth of wheat (from left to right) - control, *Azo. lac. lac.* Azo.242, Azo. HW.2004-2 and Azo. 2669

Plate-8

Effect of *Azotobacters spp.* on growth of wheat (from left to right) - control, Azoto.2687-1, Azoto.2687-2, W-5 and CBD-15

Plate-9

Effect of VA-mycorrhizae on growth of wheat (from left to right) - control, *G. mosseae, G. macrocarpum, G. fasciculatum* and *G. aggrocarpum*

Plate -7

Plate -8

Plate -9

parameters (as described earlier). Increase in plant height, fresh and dry weight of shoot and root, number of tillers and root volume were noted due to inoculation of above mentioned strains of *Azospirillum spp.* However, it was noted maximum due to inoculation of *A. lipoferum* strain, Azo. 242 (isolate of ectomycorrhizae) followed by increase noted with other *Azospirillum spp.* Azo. lac. lac (isolated from ectomycorrhizae), Azo. 2004-2 (wheat isolate) and Azo. 2669 (wheat isolate). Increase due to these four *Azospirillum* strains/isolate were significant over control. Increase in above plant parameters due to inoculation of other *Azospirillum* strians/isolates were at par. Inoculation effect of various strains/isolates of *Azospirillum* on different plant growth parameters are being summarized as follows :

2.2.2.1 Plant height

Increase in plant height was recorded due to inoculation of various strains/isolates of *Azospirillum* (Table 7A). Differences in increase was observed between various *spp.* of *Azospirillum* used in the study. Plant height was noted maximum with *A. lipoferum* strain, Azo. 242 (isolate of grass) and lowest with *Azospirillum* isolate, Azo. HW. 2004-1. The order of increase of plant height due to inoculation of various strains/isolates of *Azospirillum* is being listed as under :

Azo.242>Azo. lac. lac>Azo.HW. 2004-2>Azo.2669>Azo.Vaishali-1>Azo.*amazonense*>Azo.2710-2>Azo.HDR.77-2>Rvzae>Sp-7>Azo.HDR-77-1>Azo.Kanchan>Azo.2710-1>Spcd>Azo.Kundan>Azo.Vaishali-2>Azo.HW.2004-1.

2.2.2.2. Number of tillers

Number of tillers of wheat were also increased due to inoculation of almost all the strains/isolates of *Azospirillum* tested. (Table 7A). Increase in number of tillers was noted maximum with *A. lipoferum* strain, Azo.242 (isolate of *grass*) and lowest with Sp cd and Azo. Kundan. Baring few exceptions, differenes in increase within various species of *Azospirillum* were also noted. The order of increase is being listed as under:

Azo.242>Azo.lac.lac>Azo.*amazonense*>Azo.HW.2004-2>Rvzae,Azo.2669, Azo. Vaishali-1, Azo.2710-1, Azo. 2710-2, Azo. HDR.77-1>Azo. Kanchan>Sp-7, Azo. HW. 2004-1, Azo. Vaishali-2, Azo. HDR.77-2> Sp cd, Azo. Kundan.

2.2.2.3 Shoot fresh weight

Fresh shoot weight of the plant was also found to be increased due to inoculation of various species of *Azospirillum* (Table 7A). Differences in increase of shoot fresh weight was also recorded between the various species of *Azospirillum*. However, it was maximum with *A. lipoferum* strain, Azo 242 and minimum with *Azospirillum* isolates Azo. kundan. The order of increase in the fresh weight of shoot is being listed as under :

Azo.242>Azo.lac.lac.>Azo.HW.2004-2 > Azo.*amonense* > Azo.2669 > Rv.zae>Azo.Vaishali-1>Sp cd>Azo. HDR 77-2>Sp-7>Azo.2710-1>Azo.Vaishali-2>Azo.2710-2>Azo.HDR.77-1>Azo. Kanchan>Azo. HW 2004-1>Azo.Kundan.

2.2.2.4 Shoot dry weight

Increased dry weight of the shoot was noted due to inoculation of various strains/isolates of *Azospirillum* (Table 7A). Increased differences in shoot dry weight was also noted between the various strain/isolate of *Azospirillum*.

A. lipoferum strain, Azo. 242 was found most effecient among all the *Azospirillum* isolate tested. *Azospirillum* isolate, Azo. 242 Kundan was noted for bring minimum increase in shoot dry weight. The order of increase in shoot dry weight is being listed as under :

Azo.242>Azo.lac.lac>Azo.HW. 2004-2>Azo.2669 > Azo. Vaishali-1>Azo.Vaishali-2>Azo.2710-1>Spcd>Sp-7>Azo.HDR.77-2> Rv.zae> Azo.*amazonense*>Azo.HW 2004-1>Azo.2710-2>Azo.HDR.77-1>Azo. Kundan.

2.2.2.5 Root fresh weight

Fresh weight of root was found to be increased due to inoculation of various strain/isolate of *Azospirillum* (Table 7A). It was noted maximum with *A. lipoferum* strain, Azo.242 and lowest with *Azospirillum* isolates Azo. Kundan. However, differences in increase brought due to various strain/ isolate of *Azospirillum* was also observed in this experiment. The order of increase in the parameter is being listed as under :

Azo.242>Azo.lac.lac>Azo.HW.2004-2>Sp cd>Sp-7>Azo.2669> Azo.2710-1>Azo.*amazonense*>Azo.HDR77-2>Azo. Kanchan>Azo.HW 2004-1>Azo.Vaishali-1>Rv.zae>Azo.Vaishali-2>Azo.HDR.77-1>Azo.2710-2>Azo.Kundan.

2.2.2.6 Root dry weight

Increase in dry weight of root was observed due to inoculation of various species of *Azospirillum* (Table 7A). Differences in increase of above parameter was also varied due to inoculation of various strain/isolates of *A. lipoferum*. However, the effect on dry weight of root was noted maximum with *Azospirillum* isolates, Azo. 242 and lowest with *Azospirillum* strain,

Plate-10

Effect of *G. macrocarpum* and *Azospirillum* on growth of wheat (from left to right) - control, *G. macrocarpum, Azo. lac.lac.*, Azo.242 and Azo.2669

Plate-11

Effect of *Azotobacter, P. vulgaris* and *Flavobacterium sp.* (P-25) on growth of wheat (from left to right) - control, Azo.2687-1, W-5, *P. vulgaris* and *Flavobacterium sp.* (P-25)

Plate -10

Plate -11

Azo. Kundan. The order of increase in root dry weight is being listed as under :

Azo.242>Azo.lac.lac>Azo.HW. 2004-2>Azo.2669>Azo.2710-1>Azo.Kanchan>Sp cd>Azo.Vaishali-2>Azo.HW.2004-1>Azo.HDR.77-2>Rv.zae>Azo.Vaishali-1Azo.HDR.77-1>SP-7>Azo. *amazonense*>Azo.2710-2>Azo.Kundan.

2.2.2.7 Root volumes

Increased root volume of wheat plant was also found due to inoculation of various strain/isolates of *Azospirillum* (Table 7A). Increase in root volume was noted maximum with the *A. lipoferum* strain , Azo.242 and lowest with *Azospirillum* isolate, Azo. Vaishali-1. Differences in the increase due to inoculation of various *Azospirillum* strains/isolates were also noted. The order of increase in root volume is being listed as under :

Azo.242 >Azo.lac.lac > Azo. HW.2004-2, Azo. 2669, Azo.*amazonense*> Azo. vaishali-2> Azo. 2710-2> Azo. 2710-1 Sp-7>Azo. Kanchan> Azo. HDR.77-2> Azo.HW.2004-1> Azo.Kundan>Rv.zae>Azo.HDR. 77-1> Sp cd> Azo.vaishali-1

Note : Only four strains/isolates of *Azospirillum* (Azo. lac.lac, Azo. 242, Azo. HW. 2004-2 and Azo. 2669) were selected on the basis of the above mentioned parameters, amongest seventeen strains/isolates of *Azospirillum* tested for screening. These four strains/isolates were used for further screening to find out most efficient N_2-fixing bacteria in the presence of VAM fungi.

2.2.2.8 Number of spikes

Number of spikes of wheat was increased due to inoculation of various strains/isolates of *Azospirillum* (Table 8a). However, it was noted maximum with *A. lipoferum* strain, Azo.242 and minimum with Sp-7 and Azo. HW. 2004-1. Differences in increase in number of spikes between the various strains/isolates of *Azospirillum* was also noted. The order of increase in the number of spikes is being listed as under :

Azo.242>Azo.lac. lac>Azo.*amazonense*,Azo.HW.2004-2>Azo.2710-2, Azo. 2669, Azo. HDR. 77-2>Rv.zae, Sp cd, Azo. Vaishali-1, Azo.HDR. 77-1>Azo.2710-1>Azo.Vaishali-2,Azo. Kanchan>Azo. Kundan>Sp-7>Azo. HW.2004-1.

2.2.2.9 Grain yield

Grain yield of wheat was increased due to inoculation of various *Azospirillum* species (Table 8A). It was noted maximum with *A. lipoferum* strain, Azo.242 and lowest with *Azospirillum* isolate, Azo. Kundan. Differences in increase in the grain yield was also noted within the various *Azospirillum* species. The order of increase in grain yield is being listed as under :

Azo.242>Azo.lac.lac>Azo.HW. 2004-2>Azo.2669>Azo.HDR.77-2 >Azo.2710-2> Azo.*amazonense*>Sp cd> Azo.Vaishali-2>Sp-7>Azo.Vaishali-1>Azo.HDR.77-1>Azo.HW.2004-1>Azo.Kanchan>Azo.2710-1>Rv.zae>Azo. Kundan.

Table 7A **Inoculation effect of *Azospirillum* on various plant growth parameters of wheat (*Triticum aestivum*, L.) *var.* HD 2329 at 45 days of plant growth (Average of three replications)**

SNo.	Treatments	Plant height (cms)	No. of tillers	Shoot fresh wt. (g/pot)	Shoot dry wt. (g/pot)	Root fresh wt. (g/pot)	Root dry wt. (g/pot)	Root volume (cc)
1	Control	38.10	1.50	8.45	1.32	1.85	0.44	0.90
2	Sp-7	40.00	1.75	**11.51**	1.71	**2.67**	0.60	**1.87**
3	*Azo. lac. lac*	**48.90**	**3.00**	**18.89**	**2.83**	**4.48**	**1.25**	**3.00**
4	Rv.Zae	40.80	**2.25**	**14.24**	1.68	1.91	**0.77**	**1.48**
5	*Azo. amazonense*	**42.50**	**2.75**	**17.86**	1.60	**2.34**	0.58	**2.10**
6	Azo. 242	**51.40**	**3.25**	**20.96**	**3.14**	**4.68**	**1.46**	**3.30**
7	Sp.cd	39.10	1.50	**12.43**	1.77	**3.00**	**0.92**	1.10
8	Azo. HW. 2004-1	33.30	1.75	9.50	1.55	2.06	**0.88**	**1.70**
9	Azo. HW. 2004-2	**47.10**	**2.50**	**18.19**	**2.61**	**3.27**	**1.22**	**2.90**
10	Azo. 2669	**45.30**	**2.25**	**16.08**	**2.03**	**2.61**	**1.19**	**2.45**
11	Azo. vaishali-1	**42.90**	**2.25**	**12.78**	**1.98**	1.98	**0.65**	1.07
12	Azo. vaishali-2	34.23	1.75	**10.79**	**1.85**	1.79	**0.89**	**2.05**
13	Azo. Kundan	37.05	1.50	7.23	1.41	1.45	0.36	**1.67**
14	Azo. Kanchan	39.40	2.00	9.72	1.52	2.22	**0.96**	**1.83**
15	Azo. 2710-1	39.30	**2.25**	**11.18**	**1.84**	**2.42**	**0.98**	**1.87**
16	Azo. 2710-2	**42.10**	**2.25**	**10.57**	1.54	1.56	0.45	**1.93**
17	Azo. HDR. 77-1	39.50	**2.25**	**10.40**	1.52	1.75	**0.65**	**1.33**
18	Azo. HDR. 77-2	**41.60**	1.75	**11.26**	1.69	2.26	**0.81**	**1.73**
	SEm±	1.107	0.262	0.559	0.160	0.148	0.058	0.110
	CD at 5%	3.115	0.738	1.574	0.451	0.417	0.162	0.311

Table 8A Grain and straw yield and N and P content of wheat (*Triticum aestivum*, L.) *var.* HD-2329 as influenced due to inoculation of *Azospirillum species* (Average of three replicaitons)

S. No.	Treatments	No. of spikes	Grain yield (g/pot)	Straw yield (g/pot)	Grain		Straw	
					% N	% P	% N	% P
1	Control	8.00	6.91	12.46	2.45	0.14	0.85	0.08
2	Sp-7	7.67	**8.14**	12.56	**2.87**	0.21	0.60	0.05
3	*Azo lac. lac*	**12.00**	**11.50**	**16.20**	2.60	0.16	0.70	0.09
4	Rv. Zae	**10.33**	6.58	14.28	**2.77**	0.18	0.79	0.08
5	*Azo. amazonense*	**11.00**	**9.92**	12.61	2.43	0.24	0.70	0.05
6	Azo. 242	**13.00**	**12.27**	**17.10**	**2.83**	0.23	0.70	0.06
7	Sp cd	**10.00**	**9.61**	12.90	**2.90**	0.17	0.55	0.04
8	Azo. HW. 2004-1	7.00	7.43	10.05	2.25	0.12	**0.95**	0.07
9	Azo. HW. 2004-2	**11.00**	**11.46**	**16.10**	2.23	0.15	0.50	0.07
10	Azo. 2669	**10.33**	**11.18**	**15.96**	2.10	0.12	**0.95**	0.06
11	Azo. vaishali-1	**10.00**	7.88	10.56	2.23	0.13	0.65	0.04
12	Azo. vaishali-2	**9.33**	**8.15**	14.22	2.23	0.18	0.70	0.07
13	Azo. Kundan	9.00	5.90	**14.93**	**2.87**	0.10	**0.95**	0.05
14	Azo. Kanchan	**9.33**	7.16	13.71	2.53	0.16	0.70	**0.10**
15	Azo. 2710-1	**9.67**	6.18	11.66	2.53	0.14	**0.95**	**0.10**
16	Azo. 2710-2	**10.67**	**10.27**	**15.96**	2.60	0.19	0.80	0.08
17	Azo.HDR. 77-1	**10.00**	7.57	**14.26**	**3.03**	0.11	**0.95**	**0.15**
18	Azo. HDR. 77-2	**10.33**	**10.35**	**14.70**	**3.13**	0.14	0.85	0.07
	SEm±	0.391	0.427	0.743	0.079	0.038	0.026	0.003
	CD at 5%	1.100	1.202	2.090	0.221	0.107	0.072	0.007

2.2.2.10 Straw yield

Straw yield of wheat was also increased due to inoculation of various strains/isolates of *Azospirillum* (Table 8A). It was noted maximum with *A. lipoferum* strain, Azo.242 and lowest with *Azospirillum* isolate, Azo. HW. 2004-1. Differences in increase in straw yield between the various strains/ isolates of *Azospirillum* was also noted. The order of increase in straw yield is being listed as under :

Azo. 242>Azo.lac. lac>Azo. HW.2004-2> Azo. 2669, Azo. 2710-2>Azo. Kundan>Azo.HDR.77-2>Rv.zae>Azo.HDR.77-1>Azo. Vaishali-2>Azo. Kanchan>Sp cd. Azo.*amazonense*>Sp-7>Azo. 2710-1>Azo. Vaishali-1>Azo.HW. 2004-1.

Note : Three parameters *viz* number of spikes, yield (grain and straw) were not used for screening of efficient strains/isolates of *Azospirillum* due to lack of time. The observations at harvest stage were taken later to verify the results of earlier experiment on screening (taken at 45 days of plant growth) and the decision taken for the selection of efficient *Azospirillum* species (Azo. lac.lac, Azo.242, Azo. HW. 2004-2 and Azo. 2669). It was observed that *Azospirillum* strains which were found efficient in increasing plant parameters (taken at 45 days of plant growth) were also found to bring increase the grain yield etc. in a similar manner.

2.2.3 Inoculation effect of *Azotobacter* on various plant growth parameters of wheat (Table 7B, 8B)

Sixteen isolates of *Azotobacter spp.* (Azoto. 2669, Azoto.Vaishali, Azoto. 2687-1, Azoto.2687-2, Azoto.2643-1, Azoto. 2643-2, Azoto. 2680-1, Azoto. 2680-2, Azoto.2680-3, Azoto.2710-1, Azoto. 2710-2, Azoto. 2690-1, Azoto. 2690-2, Azoto. HDR. 77-1, Azoto. HDR. 77-2 and Azoto. HDR.

77-3) isolated from rhizospheric soil samples of various varieties of wheat (*Triticum aestivum,* L.), and four standard strains of *Azotobecter spp.* (M-4, CBD-15, A-41 and W-5) were used in this study to screen out efficient *Azotobacter*. In this experiment increase in plant height, number of tiller, fresh and dry weight of shoot and root volume was observed due to inoculation of various *Azotobacter* species as mentioned above.Out of twenty *Azotobacter* species, only three *Azotobacter spp.,* Azoto. 2687-1, Azoto. 2687-2 and W-5, were found to bring significant increase in almost all the plant growth parameters of wheat (Table 7B). Differences in increase due to inoculation of various *Azotobacter spp.*, Azoto. 2687-1, Azoto. 2687-2, W-5 and others were also observed. Increase in above parameters by other *Azotobacter* strains was found significant, when the comprison was made with control. Based on the increased plant growth only, three species of *Azotobacter* (Azoto. 2687-1, Azoto.2687-2 and W-5) were selected for further study. Details of the experimental results are given as under :

2.2.3. 1 Plant height

Increase in plant height was noted due to inoculation of various species of *Azotobacter* (Table 7 B). Differences in increase was also noted between various species of *Azotobacter,* used in the study. The plant height was noted maximum with *Azotobacter* species Azoto. 2643-1 and lowest with Azoto. HDR.77-1. The order of increase in plant height due to various *Azotobacter* species is being listed as under :

Azoto. 2643-1>Azoto.HDR. 77-2>Azoto. 2690-1>Azoto. 2687-2>Azoto. 2643-2>Azoto. 2687-1>Azoto. 2710-2>CBD-15>Azoto. 2669>Azoto.2710-1, A-41, W-5> Azoto.2690-2>Azoto.2680-2, M-4> Azoto.Vaishali>Azoto.2680-1>Azoto.2680-3>Azoto. HDR. 77-1.

2.2.3.2 Number of tillers

Number of tillers was also increased due to inoculation of almost all the species of *Azotobacter* tested (Table 7B). Number of tillers was noted maximum with *A. chroococcum* strain, W-5 and lowest with *Azotobacter* species, Azoto. 2690-1.Baring few exceptions, differences in increase within various species of *Azotobacter* were also observed. The order of increase in number of tillers is being listed as under :

W-5>Azoto.2687-2>Azoto.2669, Azoto.2687-1, Azoto.2680-1, Azoto.2710-2, CBD-15, A-41>Azoto.Vaishali, Azoto.2680-2, Azoto.2710-1, Azoto.2690-2,Azoto.HDR. 77-2, M-4> Azoto.HDR.77-3> Azoto. 2643-1, Azoto.2680-3, Azoto.HDR.77-1>Azoto.2643-2, Azoto. 2690-1.

2.2.3.3 Shoot fresh weight

Shoot fresh weight the of plant was also found to be increased due to inoculation of various species of *Azotobacter* (Table 7 B). Differences in increase of shoot fresh weight were also recorded between the various species of *Azotobacter*. However, it was noted maximum with *A. chroococcum* strain W-5 and lowest with *Azotobacter* species-Azoto. HDR. 77-1. The order of increase in shoot fresh weight is being listed as under :

W-5>Azoto.2687-2>M-4>Azoto.2710-1>Azoto.2687-1>Azoto.Vaishali>Azoto.2690-1>Azoto.2680-2>A-41>CBD-15>Azoto.2669>Azoto.2643-1>Azoto.2680-3> Azoto.2680-1>Azoto. 2710-2 > Azoto.HDR.77-2>Azoto.2643-2>Azoto.2690-2>Azoto.HDR.77-3>Azoto. HDR.77-1.

2.2.3.4 Shoot dry weight

Dry weight of the shoot was also increased due to inoculation of various species of *Azotobacter* (Table 7 B). Increased differences in shoot dry weight

was also noted between the various species of *Azotobacter*. *A. chroococcum*, strain W-5 was found to be the most efficient among all *Azotobacter* species tested. *Azotobacter sp* . Azoto. HDR.77-1, resulted in minimum increase in shoot dry weight. The order of increase in shoot dry weight is being listed as under :

W-5>Azoto.2687-2>Azoto.2687-1>Azoto.2680-2> Azoto. HDR. 77-2>A-41>Azoto.2710-2>Azoto.2643-1>Azoto.2643-2>M-4>Azoto.Vaishali>Azoto.2710-1>Azoto.2669>Azoto.2690-2>Azoto. 2690-1>CBD-15>Azoto.2680-3>Azoto.2680-1>Azoto.HDR. 77-3>Azoto. HDR. 77-1.

2.2.3.5 Root fresh weight

Increased in fresh weight of root due to inoculation of various species of *Azotobacter* was noted (Table 7B). It was noted maximum with *A. chroococcum* strain, W-5 and lowest with *Azotobacter* species, Azoto. HDR. 77-2. However, differences in increase brought due to inoculation of various species, of *Azotobacter* was also noted in this experiment. The order of increase in root fresh weight is being listed as under :

W-5>Azoto.2687-2>Azoto.2687-1>Azoto.2669>M-4>Azoto.2690-2>Azoto.2680-2>Azoto.2710-1>CBD-15>Azoto.2680-1, Azoto. 2710-2>Azoto. 2643-1, Azoto. HDR. 77-3>Azoto. Vaishali> Azoto. 2690-1>A-41>Azoto.2643-2>Azoto.HDR. 77-1>Azoto. 2680-3>Azoto. HDR. 77-2.

2.2.3.6 Root dry weight

Root dry weight was also increased due to inoculation of various species of *Azotobacter* (Table 7B). Increase in above parameter was also varied due to inoculation of various species of *Azotobacter*. However, the effect on dry root weight was noted maximum with *A. chroococcum* strain, W-5 and

lowest with *Azotobacter* species, Azoto. 2680-3. The order of increase in root dry weight is being listed as under :

W-5>Azoto.2687-1>Azoto. Vaishali>M-4>Azoto.HDR. 77-3>Azoto.2690-1>Azoto.2710-1,CBD-15>Azoto.2687-2>Azoto.2710-2>Azoto.2669>Azoto.2690-2, Azoto. HDR. 77-2>Azoto. 2643-1, Azoto. 2680-2>Azoto. 2643-2> Azoto. 2680-1>Azoto. HDR. 77-1, A-41>Azoto.2680-3.

2.2.3.7 Root volume

Root volume of wheat plant was also increased due to inoculation of various species of *Azotobacter* (Table 7 B). Increase in root volume was maximum with *A. chroococcum* strain, W-5 and lowest with *Azotobacter* species, Azoto. 2680-3 and Azoto. HDR. 77-1. Differences in increase in root volume due to inoculation of various *Azotobacter* species was also noted. The order of increase in root volume is being listed as under :

W-5>Azoto.2687-2>Azoto.2687-1>Azoto.Vaishali, Azoto.2643-1, Azoto. 2710-2> Azoto. 2680-1> A-41> Azoto. 2680-2> Azoto. 2710-1, Azoto. HDR. 77-2> Azoto. 2690-1> Azoto. 2643-2> Azoto. 2669, Azoto. HDR. 77-3> Azoto.2690-2> M-4>CBD-15>Azoto.2680-3, Azoto. HDR. 77-1.

Note : Based on plant height, number of tillers, fresh and dry weight of shoot and root and root volume, three species of *Azotobacter* (Azoto. 2687-1, Azoto. 2687-2 and W-5) were selected out of twenty species of *Azotobacter*. These three species were used for further screening to find out the most efficient N_2-fixing bacteria in the presence of VAM fungi.

2.2.3.8 Number of spikes

Number of spikes of wheat was increased due to inoculation of various species of *Azotobacter* (Table 8 B). However, it was noted maximum with

A. chroococcum strain, W-5 and lowest with *Azotobacter* species . Azoto. HDR. 77-1 and Azoto. HDR. 77-2. Differences in increase in the number of spikes was also noted within various *Azotobacter* species . The order of increase in number of spikes is being listed as under :

W-5>Azoto.2687-2>Azoto.2690-1,CBD-15>A-41>Azoto.2687-1, Azoto. 2643-2, Azoto.2680-1,Azoto. 2710-1, Azoto. 2690-2, M-4>Azoto. Vaishali, Azoto.2710-2>Azoto.2680-2, Azoto. HDR.-77-3> Azoto. 2669, Azoto. 2680-3>Azoto. 2643-1>Azoto. HDR. 77-1, Azoto. HDR. 77-2.

2.2.3.9 Grain yield

Grain yield was found to be increased due to inoculation of various *Azotobacter* species (Table 8B). It was noted maximum with *A. chroococcum* strain, W-5 and lowest with *Azotobacter* species, Azoto. HDR. 77-1. Differences in increase in the grain yield was also noted within various species of *Azotobacter* . The order of increase in grain yield is being listed as under:

W-5>Azoto.2687-2>Azoto.2687-1>Azoto.2690-1>Azoto.2680-2>Azoto.2710-2>M-4>Azoto.2669>Azoto.2690-2>A-41>Azoto.HDR. 77-2>Azoto. Vaishali>Azoto. 2643-2>Azoto.2643-1>CBD-15>Azoto.2710-1>Azoto.2680-3>Azoto.2680-1>Azoto.HDR. 77-3>Azoto.HDR. 77-1.

2.2.3. 10 Straw yield

Straw yield of wheat was also increased due to inoculation of various species of *Azotobacter* (Table 8B). It was noted maximum with *A. chroococcum* strain, W-5 and lowest with *Azotobacter* species, HDR. 77-1. Differences in increase in straw yield between the various species of *Azotobacter* was also noted. The order of increase in straw yield is being listed as under :

Table 7B Inoculation effect of *Azotobacter* on various plant growth parameters of wheat (*Triticum aestivum*, L.) *var.* HD 2329 at 45 days of plant growth (Average of three replications)

S. No.	Treatments	Plant height (cms)	No. of tillers	Shoot fresh wt. (g/pot)	Shoot dry wt. (g/pot)	Root fresh wt. (g/pot)	Root dry wt. (g/pot)	Root volume (cc)
1	Control	38.10	1.50	8.45	1.32	1.85	0.44	0.90
2	Azoto. 2669	40.80	**2.50**	**12.12**	1.67	2.23	0.55	**1.43**
3	Azoto. vaishali	39.30	**2.25**	**13.61**	1.75	1.93	**0.65**	**1.93**
4	Azoto. 2687-1	**45.63**	**2.50**	**14.75**	**2.30**	**2.45**	**0.66**	**2.05**
5	Azoto. 2687-2	**47.50**	**2.75**	**15.37**	**2.61**	**2.65**	0.58	**2.30**
6	Azoto. 2643-1	**50.80**	1.75	**10.85**	**1.81**	1.96	0.50	**1.93**
7	Azoto. 2643-2	**46.30**	1.50	**10.15**	**1.79**	1.87	0.49	**1.47**
8	Azoto.2680-1	39.00	**2.50**	**10.31**	1.45	1.97	0.48	**1.90**
9	Azoto. 2680-2	39.25	**2.25**	**12.78**	**2.00**	2.02	0.50	**1.77**
10	Azoto. 2680-3	38.50	1.75	**10.59**	1.48	1.77	0.40	1.00
11	Azoto. 2710-1	40.30	**2.25**	**15.15**	1.70	1.99	0.59	**1.70**
12	Azoto. 2710-2	**43.75**	**2.50**	**10.32**	**1.84**	1.97	0.57	**1.93**
13	Azoto. 2690-1	**48.30**	1.50	**13.06**	1.58	1.91	0.60	**1.67**
14	Azoto. 2690-2	39.30	**2.25**	**10.12**	1.64	2.05	0.91	**1.40**
15	Azoto. HDR.77-1	25.00	1.75	2.18	0.38	1.80	0.47	1.00
16	Azoto. HDR.77-2	**49.80**	**2.25**	**10.22**	**1.97**	1.73	0.51	**1.70**
17	Azoto. .HDR.77-3	38.75	2.00	6.40	1.09	1.96	**0.62**	**1.43**
18	M-4	39.25	**2.25**	**15.20**	1.77	2.06	**0.64**	**1.35**
19	CBD-15	**42.30**	**2.50**	**12.24**	1.57	1.98	0.59	**1.20**
20	A-41	40.30	**2.50**	**12.58**	**1.94**	1.88	0.47	**1.80**
21	W-5	40.30	**3.25**	**15.50**	**2.91**	**2.78**	**0.74**	**2.40**
	SEm±	1.107	0.262	0.559	0.160	0.148	0.058	0.110
	CD at 5%	3.115	0.738	1.574	0.451	0.417	0.162	0.311

Table 8B Grain and straw yield and N and P content of wheat (*Triticum asetivum*, L.) *Var.* HD -2329 as influenced due to inoculation of *Azotobacter species* (Average of three replicaitons)

S. No.	Treatments	No. of spikes	Grain yield (g/pot)	Straw yield (g/pot)	Grain		Straw	
					% N	% P	% N	% P
1	Control	8.00	6.91	12.46	2.45	0.14	0.85	0.08
2	Azoto. 2669	**10.00**	**8.58**	**17.31**	2.50	0.10	0.55	**0.10**
3	Azoto. Vaishali	**11.33**	**8.20**	**16.90**	2.23	0.10	0.85	**0.10**
4	Azoto. 2687-1	**12.33**	**10.31**	**15.40**	**3.43**	0.23	**1.00**	**0.13**
5	Azoto. 2687-2	**13.33**	**10.50**	**14.85**	**2.80**	0.07	0.70	0.04
6	Azoto. 2643-1	**9.33**	7.83	11.98	**3.05**	0.10	**1.00**	0.06
7	Azoto. 2643-2	**12.33**	7.92	12.12	2.55	0.06	**1.30**	0.07
8	Azoto. 2680-1	**12.00**	7.47	13.34	2.60	0.06	0.75	0.05
9	Azoto. 2680-2	**11.00**	**10.03**	**17.32**	**3.70**	0.21	**1.30**	0.09
10	Azoto. 2680-3	**10.00**	7.68	**17.30**	**3.20**	0.19	**1.10**	0.04
11	Azoto .2710-1	**12.00**	7.73	**18.27**	**3.50**	0.15	0.85	0.05
12	Azoto .2710-2	**11.33**	**9.19**	**17.63**	**3.63**	0.21	1.00	0.05
13	Azoto .2690-1	**13.00**	**10.07**	**17.83**	**2.90**	0.09	0.80	0.03
14	Azoto .2690-2	**12.00**	**8.45**	**14.88**	**2.87**	0.11	0.70	0.03
15	Azoto. HDR.77-1	9.00	4.99	8.65	**4.03**	0.30	0.75	0.05
16	Azoto. HDR.77-2	9.00	**8.24**	14.46	**3.33**	0.11	0.55	0.07
17	Azoto. HDR.77-3	**11.00**	7.27	12.47	**3.00**	0.06	0.85	0.03
18	M-4	**12.00**	**8.71**	14.32	**3.15**	0.16	0.80	0.02
19	CBD-15	**13.00**	7.81	**14.74**	**3.23**	0.19	**1.00**	0.08
20	A-41	**12.67**	**8.33**	14.20	**2.93**	0.10	0.80	**0.10**
21	W-5	**13.67**	**11.96**	**19.61**	2.58	0.10	1.00	0.06
	SEm±	0.391	0.427	0.743	0.079	0.038	0.026	0.003
	CD at 5%	1.100	1.202	2.090	0.221	0.107	0.072	0.007

W-5>Azoto.2710-1>Azoto.2690-1>Azoto.2710-2>Azoto.2680-2>Azoto.2669>Azoto.2680-3>Azoto. Vaishali>Azoto.2687-1>Azoto.2690-2>Azoto.2687-2>CBD-15>Azoto.HDR.77-2>M-4>A-41>Azoto.2680-1>Azoto.HDR.77-3>Azoto.2643-2>Azoto.2643-2>Azoto.2643-1>Azoto.HDR.77-1.

Note : Three plant parameters *viz.* number of spikes, grain and straw yield were not used for screening the efficient species *Azotobacter* due to lack of time. These observations were taken later to verify the result of the previous experiment on screening (taken at 45 days of plant growth) and the decision was taken for the selection of efficient *Azotobacter* strains. It was observed that *Azotobacter* species which were found efficient in increasing plant parameters (taken at 45 days of plant growth), were also found to increase the grain and straw yield in a similar manner.

2.2.4 Inoculation effects of *Flavobacterium* and *Proteus sp.* on various plant growth parameters of wheat (Table 7C, 8C)

Plant parameters *viz.*, Plant height, number of tillers, fresh and dry weight, of shoot and root and root volume were found to be increased due to seed inoculation with *Flavobacterium* sp. (P-25) and *Proteus vulgaris*, as compared to control (Table 7C). Details of the experimental results is being listed as under :

2.2.4.1. Plant height

Increase in plant height was noted due to inoculation of *Flavobacterium sp.* (P-25) and *Proteus* vulgaris (Table 7C). The plant height was higher with *Flavobacterium sp.* (P-25).

2.2.4.2 Number of tillers

Number of tillers was increased due to inoculation of *Flavobacterium sp.* (P-25) and *Proteus vulgaris* (Table 7C). Number of tillers was maximum with *Flavobacterium sp.* (P-25).

2.2.4.3. Shoot fresh weight

Shoot fresh weight was increased due to inoculation of *Flavobacterium sp.* (P-25) and *Proteus vulgaris* (Table 7C). Shoot fresh weight was increased maximum with *Flavobacterium sp.* (P-25).

2.2.4.4. Shoot dry weight

Dry weight of shoot was increased due to seed inoculation of *Flavobacterium sp.* (P-25) and *Proteus vulgaris* (Table 7C). Shoot dry weight was noted maximum with *Flavobacterium sp.* (P-25).

2.2.4.5 Root fresh weight

Fresh weight of root was increased due to inoculation of *Flavobacterium sp.* (P-25) and *Proteus vulgaris* (Table 7C). It was noted maximum with *Flavobacterium sp.* (P-25).

2.2.4.6 Root dry weight

Root dry weight was increased due to inoculation of *Flavobacterium sp.* (P-25) and *Proteus vulgaris* (Table 7C). Dry weight of root was noted maximum with *Flavobacterium sp.* (P-25).

2.2.4. 7 Root volume

Root volume was increased due to inoculation of *Flavobacterium sp.* (P-25) and *Proteus vulgaris* (Table 7C). It was noted maximum in the presence of *Flavobacterium sp.* (P-25).

Table 7C **Inoculation effect of *Flavobacterium sp.* (P-25) and *proteus valgaris* on various plant growth parameters of wheat (*Triticum aestivum*, L.) *var.* HD 2329 at 45 days of plant growth (Average of three replications)**

S No.	Treatments	Plant height (cms)	No. of tillers	Shoot fresh wt. (g/pot)	Shoot dry wt. (g/pot)	Root fresh wt. (g/pot)	Root dry wt (g/pot)	Root volume (cc)
1	Control	38.80	1.50	8.45	1.32	1.85	0.44	0.90
2	*Flavobacterium sp.* (P-25)	**42.50**	**3.50**	**18.89**	**3.26**	**4.83**	**1.25**	**3.40**
3	*Proteus vulgaris*	**41.90**	**2.75**	**15.18**	**2.35**	**3.00**	**0.98**	**2.20**
	SEm±	1.107	0.262	0.559	0.160	0.148	0.058	0.110
	CD at 5%	3.115	0.738	1.574	0.451	0.417	0.162	0.311

Table 8C **Grain and straw yield and N and P content of wheat (*Triticum asetivum*, L.) *Var.* HD -2329 as influenced due to inoculation of *Flavobacterium sp.* (P-25) and *Proteus vulgaris* (Average of three replicaitons)**

S. No.	Treatments	No. of spikes	Grain yield (g/pot)	Straw yield (g/pot)	Grain		Straw	
					% N	% P	% N	% P
1	Control	8.00	6.91	12.46	2.45	0.14	0.85	0.08
2	*Flavobacterium sp.* (P-25)	**14.67**	**13.33**	**22.22**	2.43	0.08	0.77	0.03
3	*Proteus vulgaris*	**12.33**	**9.03**	**14.31**	**2.80**	0.15	**0.95**	**0.11**
	SEm±	0.391	0.427	0.743	0.079	0.038	0.026	0.003
	CD at 5%	1.100	1.202	2.090	0.221	0.107	0.072	0.007

2.2.4.8 Number of spikes

Number of spikes of wheat was increased due to inoculation of *Flavobacterium sp.* (P-25) and *Proteus vulgaris* (Table 8C). It was noted maximum with *Flavobacterium sp.* (P-25).

2.2.4.9 Grain yield

Grain yield of wheat was increased due to inoculation of *Flavobacterium sp.* (P-25) and *Proteus vulgaris* (Table 8C). It was noted maximum with *Flavobacterium sp.* (P-25).

2.2.4.10 Straw yield

Straw yield of wheat was increased due to inoculation of *Flavobacterium sp.* (P-25) and *Proteus vulgaris* (Table 8C). Straw yield was noted maximum in presence of *Flavobacterium sp.* (P-25).

2.3 Screening of various vesicular-arbuscular mycorrhizae (VAM) fungi

Different types of VAM (*Glomus macrocarpum, G. mosseae, Gigaspora margarita, G. fasciculatum, Sclerocystis, Aculospora, Gig. callospora, G. aggrocarpum, G. etunicatum, G. deserticola* and *G. intraradices*) were included in the study to find out their effect on various growth parameters of wheat (*Triticum aestivum,* L.) *var.* HD-2329. Inoculation effect of various VAM fungi on different plant parameters (individually) are being given as under (Table 9A, 9B):

2.3.1 Plant height

Increase in plant height was noted due to inoculation of various VAM fungi (Table 9A). However, it was noted maximum with *G. macrocarpum* and minimum with *Gig. callospora.* Increased differences in plant height

beteween various species of VAM fungi was also noted. The order of increase in plant height is being listed as under:

G.macrocarpum > G.mosseae> G.intraradices > G.aggrocarpum > G.etunicatum > Gig.margarita> sclerocystis >Aculospora > G.deserticola > G.fasciculatum > Gig. callospora.

2.3.2 Number of tillers

Number of tillers was increased due to inoculation of different VAM fungi (Table 9A). However, it was noted maximum with *G. macrocarpum* and lowest with *Gig. margarita* and *G. aggrocarpum* as compared to control. Differences in increase in number of tillers between the various VAM fungi was also observed. The order of increase in number of tillers is being listed as under:

G.macrocarpum>G.intraradices>G. etunicatum > Gig. callospora>G. deserticola>G. fasciculatum>G.mosseae>Sclerocystis>Aculospora>Gig. margarita, G. aggrocarpum.

2.3.3 Shoot fresh weight

Shoot fresh weight was increased due to inoculation of various VAM fungi (Table 9A). However, it was noted maximum with *G. macrocarpum* and lowest with *Gig. margarita.* Differences in increase in shoot fresh weight between various VAM fungi was also recorded. The order of increase in shoot fresh weight is given as under :

G.macrocarpum > G.intraradices > G. deserticola> Gig.callospora > G.etunicatum > Sclerocystis > G.fasciculatum > Aculospora > G.mosseae > G.aggrocarpum >Gig. margarita.

2.3.4 Shoot dry weight

Increase in shoot dry weight was noted due to inoculation of various VAM fungi (Table 9A). However, it was noted maximum with *G. macrocarpum* and lowest with *Gig. margarita.* Differences shoot dry weight between different species of VAM fungi was also noted. The order of increase in shoot dry weight is given as follows:

G.macrocarpum>G.intraradices>G.deserticola>G.etunicatum>G.fasciculatum >Gig.callospora>Sclerocystis>G.aggrocarpum>Aculospora>G. mosseae >Gig. margarita.

2.3.5 Root fresh weight

Increase in fresh weight of root was also observed due to inoculation of various VAM fungi (Table 9A). However, it was noted maximum with *G. macrocarpum* and lowest with *Gig. margarita.* Differences in increase in root fresh weight between different VAM fungi was also noted. The order of increase in root fresh weight is given as under :

G.macrocarpum>G.aggrocarpum>Sclerocystis>G.intraradices> G.fasciculatum>G.mosseae >G.deserticola>Aculospora> Gig.callospora >G.etunicatum>Gig.margarita.

2.3.6 Root dry weight

Increase in root dry weight was noted due to inoculation of various VAM fungi (Table 9A). However, it was noted maximum with *G. macrocarpum* and lowest with *Gig. margarita.* Differences in increase in root dry weight between various VAM fungi was also noted. The order of increase in root dry weight is given as under:

G. macrocarpum > *G. intraradices* > *G. aggrocarpum* > *G. mosseae* > *G.fasciculatum* > *Aculospora* > *Gig. callospora* > *G. etunicatum* > *G. deserticola* > *Sclerocystis* > *Gig. margarita.*

2.3.7 Root volume

Root volume was increased due to inoculation of various VAM fungi (Table 9A). However, it was noted maximum with *G. macrocarpum* and lowest with *Gig. margarita* . Differences in increase in root volume between the various VAM fungi was also noted. The order of increase in root volume is given as follows:

G. macrocarpum > *G. aggrocarpum* > *Sclerocystis* > *G. interaradices* > *G. fasciculatum* > *Gig. callospora* > *Aculospora* > *G. etunicatum, G. mosseae* > *G. deserticola* > *Gig. margarita.*

2.3.8 Number of spikes

Number of spikes of wheat was increased due to inoculation of various VAM fungi (Table 9B). However, it was noted maximum with *G. macrocarpum* and minimum with *Aculospora* and *G. deserticola.* Differences increased in number of spikes between the various VAM fungi was also noted. The order of increase in number of spikes is being given as under:

G. macrocarpum > *G. fasciculatum* > *Gig. margarita* > *Sclerocystis* > *G. aggrocarpum* > *G. mosseae, Gig. callospora, G. etunicatum, G. intraradices* > *Aculospora, G. deserticola.*

2.3.9 Grain yield

Grain yield was increased due to inoculation of various VAM fungi (Table 9B). However, it was noted maximum with *G. macrocarpum* and

Table 9A :Inoculation effect of vesicular - arbuscular mycorrhizae (VAM) fungi on various plant growth parameters of wheat (*Triticum avestivum,* L.) *var.* HD-2329 at 45 days of plant growth (average of three replications)

S. No.	Treatments	Plant height (cms)	No. of tillers	Shoot fresh wt. (g/pot)	Shoot dry wt. (g/pot)	Root fresh wt. (g/pot)	Root dry wt. (g/pot)	Root volume (cc)
1	Control	38.1	1.50	8.45	1.32	1.85	0.44	0.90
2	*G. Mosseae*	**48.8**	1.75	9.80	1.57	2.28	**1.14**	**2.00**
3	*G. macrocarpum*	**50.0**	**3.25**	**25.68**	**3.40**	**4.45**	**1.60**	**3.20**
4	*Gig. margarita*	**45.8**	1.50	8.21	1.02	0.97	0.50	1.20
5	*G. fasciculatum*	**44.3**	2.0	**11.52**	**2.02**	2.43	**0.94**	**2.50**
6	*Sclerocystis*	**45.3**	**1.75**	**11.94**	**1.91**	**2.85**	**0.96**	**2.60**
7	*Aculospora*	**45.0**	**1.75**	**11.11**	**1.85**	2.25	**0.93**	**2.10**
8	*Gig. Callospora*	**43.6**	**2.25**	**14.75**	**1.96**	2.25	**0.83**	**2.30**
9	*G. aggrocarpum*	**46.8**	**1.50**	9.25	1.44	**3.35**	**1.16**	**3.20**
10	*G. etunicatum*	**46.5**	**2.75**	**14.03**	**2.06**	1.90	**0.81**	**2.00**
11	*G. deserticola*	**44.5**	**2.25**	**18.97**	**2.69**	2.20	**0.77**	**1.90**
12	*G. intraradices*	**48.3**	**3.00**	**20.74**	**2.92**	**2.84**	**1.18**	**2.25**
	SEm±	1.424	0.267	0.716	0.115	0.188	0.060	0.127
	CD at 5%	4.155	0.789	2.091	0.336	0.547	0.175	0.370

Table 9B Grain and straw yield and N and P content of wheat (*Triticum aestivum*, L.) *var.* HD-2329 as influenced due to inoculation of vesicular-arbuscular mycorrhizae (VAM) fungi. (average of three replications)

S. No.	Treatments	No. of spikes	Grain yield (g/pot)	Straw yield (g/pot)	Grain		Straw	
					% N	% P	% N	% P
1	Control	8.00	6.89	11.04	2.50	0.10	0.85	0.06
2	*G. Mosseae*	**12.00**	6.49	10.12	2.43	0.10	0.87	0.02
3	*G. macrocarpum*	**13.33**	**10.29**	**17.37**	**2.70**	**0.15**	**1.25**	0.07
4	*Gig. margarita*	**12.33**	7.18	**14.96**	**3.23**	**0.17**	**1.10**	**0.09**
5	*G. fasciculatum*	**13.00**	**8.11**	12.76	**2.60**	**0.15**	**0.96**	0.04
6	*Sclerocystis*	**12.33**	**9.89**	**14.21**	**3.27**	**0.13**	**0.98**	0.04
7	*Aculospora*	**11.33**	5.88	**14.76**	**3.10**	0.11	**1.15**	0.05
8	*Gig. Callospora*	**12.00**	**8.62**	**15.45**	2.90	0.08	**0.95**	0.04
9	*G. aggrocarpum*	**12.33**	0.08	12.30	**3.50**	**0.24**	**1.15**	**0.08**
10	*G. etunicatum*	**12.00**	7.69	**15.63**	**3.47**	**0.18**	**1.10**	0.04
11	*G. deserticola*	**11.30**	**9.01**	12.47	**3.25**	**0.16**	**1.00**	0.05
12	*G. intraradices*	**12.00**	**9.06**	**17.30**	**3.47**	**0.31**	**1.24**	0.06
	SEm±	0.527	0.370	0.986	0.033	0.003	0.018	0.002
	CD at 5%	1.538	1.081	2.877	0.093	0.009	0.049	0.007

lowest with *G. Aculospora*. Differences in increase of grain yield between the various VAM fungi was also noted. The order of increase in grain yield is given as follows :

G. macrocarpum > *Sclerocystis* > *G. interaradices* > *G. deserticala* > *Gig. callospora* > *G. fasciculatum* > *G. aggrocarpum* > *G. etunicatum* > *Gig. margarita* > *G. mosseae* > *Aculospora.*

2.3.10 Straw yield

Straw yield was increased due to inoculation of various VAM fungi (Table 9B). However, it was noted maximum with *G. macrocarpum* and lowest with *G. aggrocarpum*. The order of increase in straw yield is being given as under:

G. macrocarpum > *G. intraradices* > *G. etunicatum* > *Gig. callospora* > *Gig. margarita* > *Aculospora* > *Sclerocystis* > *G. fasciculatum* > *G. deserticola* > *G. aggrocarpum* > *G. mosseae.*

Note : Only one VAM fungi *G. macrocarpum* was selected (on the basis of above mentioned parameters) amongst eleven VAM fungi tested for screening. This VAM fungus was used for co-inoculation study to find out best combination with various N_2-fixing bacteria.

Three parameters *viz.* number of spikes and grain and straw yield were not used for screening of efficient VAM fungi. Above mentioned observations was taken later with the aim to verify the results of earlier experiment on screening (taken at 45days of plant growth) and the decision was taken for the selection of efficient VAM fungi- *G. macrocarpum*. In general, it was

Plate -12

Co-inoculation effect of *G. macrocarpum* and *Azospirillum* isolates on growth of wheat (from left to right)-control, *G. macrocarpum* +*Azo. lac. lac.*, *G. macrocarpum* + Azo.2669, 60Kg Nha^{-1} and 120 Kg N ha^{-1}

Plate-13

Co-inoculation effect of *G. macrocarpum, Azotobacter, P. vulgaris* and *Flavobacterium sp.* (P-25) on growth of wheat (from left to right) - control, *G. macrocarpum*+W-5, *G. macrocarpum* +*P. vulgaris, G. macrocarpum*+*Flavobacterium sp.* (P-25), 60 Kg N ha^{-1} and 120 Kg N ha^{-1}

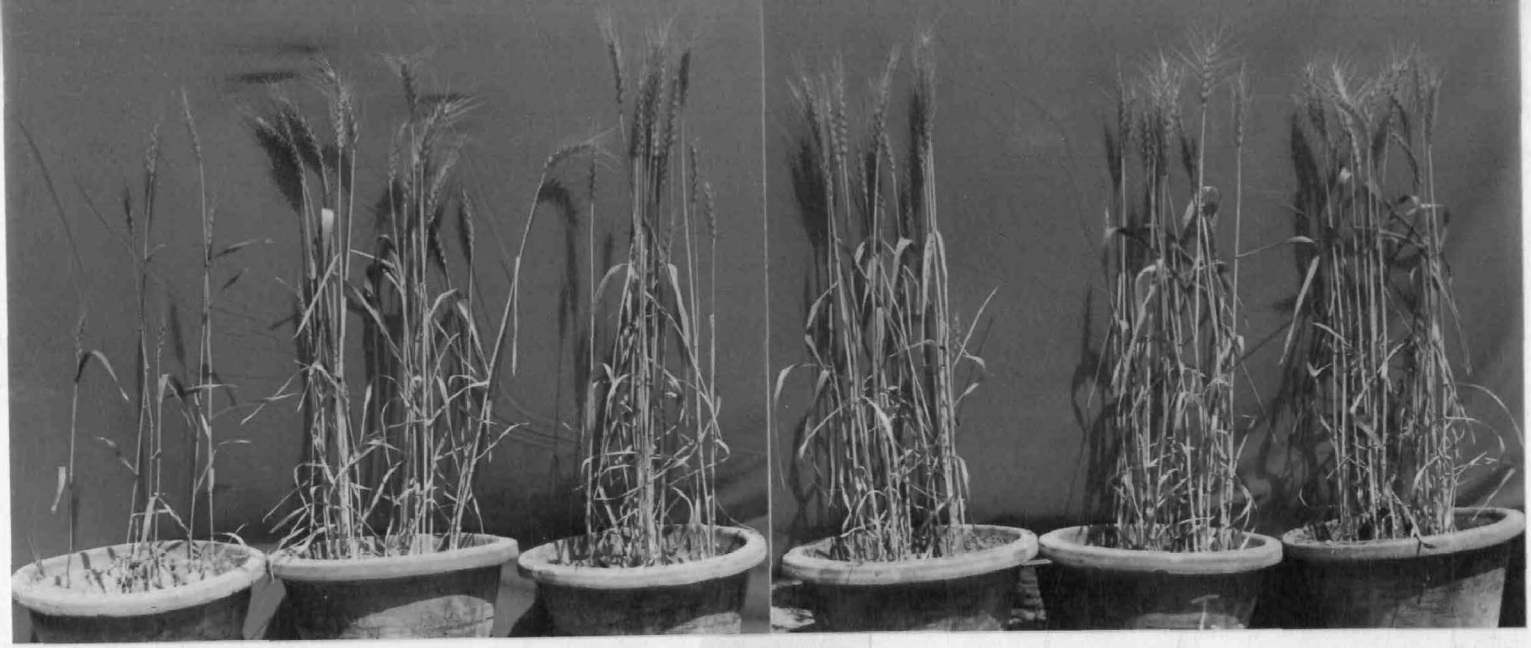

Plate -12

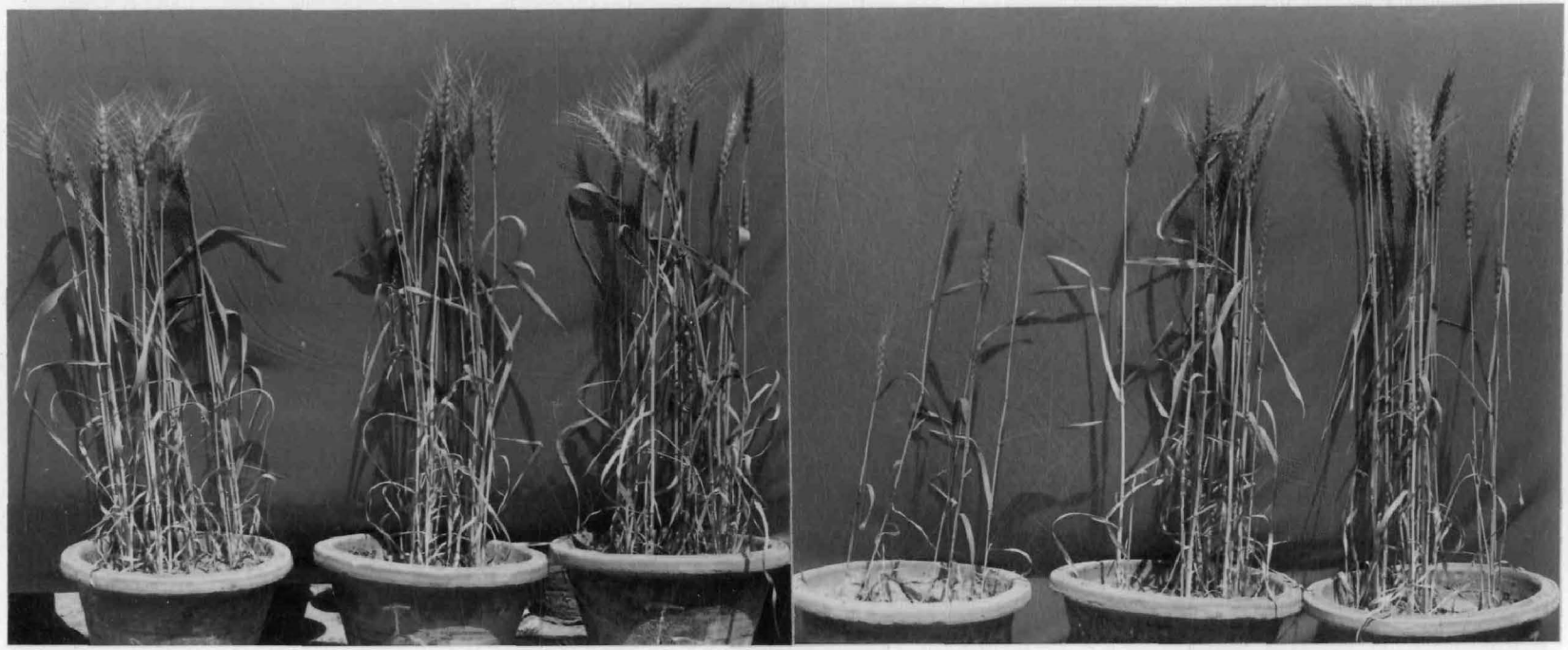

Plate -13

observed that VAM fungi which was found efficient in increasing various plant parameters (at 45 days of plant growth), was also brought increase in grain and straw yield in a similar manner.

3.0 Co-inoculation effect of N_2 fixing bacteria (*Azospirillum, Azotobacter), Flavobacterium, proteus* and *Glomus macrocarpum* on yield and nutrient content (N and P) of wheat (*Triticum aestivum,* L.) *var.* HD-2329 (Table 10)

This experiment was carried out to find out the best combination of N_2-fixing bacteria, and *G. macrocarpum. Flavobacterium sp.* (P-25) and *proteus vulgaris* were also included for co-inoculation study, because informations on inoculation effect of these two bacteria is lacking. In general grain and straw yield of wheat *(Triticum aestivum,* L.) *var.*-2329 was increased due to inoculation of various N_2-fixing bacteria- (*Azospirillum, Azotobacter*), *Flavobacterium sp.* (P-25) and *Proteus vulgaris* as compared to uninoculated control (Table 10).

However, increase in these parameters were noted more with seed inoculation with *Flavobacterium sp.* (P-25) as compared to N_2-fixing bacteria, tested. The effect of individual bacterium on above parameters were more pronounced in presence of soil inoculation of *Glomus macrocarpum*. The above parameters were also increased due to soil inoculation with *Glomus macrocarpum* alone as compared to uninoculated control, which was less than the increased noted with N_2-fixing bacteria (*Azospirillum, Azotobacter*), *Flavobacterium* and *Proteus*.

Nitrogen (N) and phosphorus (P) content in grain and straw were also increased due to inoculation of *Glomus macrocarpum* and all other individual bacteria. In this experiment N and P contents (grain and straw) were also increased further due to co-inoculation with *Glomus macrocarpum*

Plate-14

Co-inoculation effect of *G. macrocarpum* and *P. vulgaris* on growth of wheat (from left to right) - control, *P. vulgaris, G. macrocarpum*+ *P. vulgaris,* 60 Kg N ha^{-1} and 120 Kg N ha^{-1}

Plate-15

Effect of N_2-fixing bacteria (*Azospirillum, Azotobacter*), *G. macrocarpum, Flavobacterium* and *P. vulgaris* on growth of wheat (from left to right) - control, *G. macrocarpum,* Azo. 2669, Azoto.2687-1, *P. vulgaris* and *Flavobacterium sp.* (P-25).

Plate -14

Plate -15

and few bacteria as compared to control. Increase in grain and straw yield due to individual bacteria and co-inoculation (bacteria+*G. macrocarpum*) was equivalent to the application of nitrogen @ 60 Kg N ha^{-1}.Number of spikes was also increased due to inoculation of *G. macrocarpum* as compared to control. Details of the experimental results are being cited as under :

3.1 Number of spikes

Number of spikes was found to be increased due to inoculation of almost all the bacteria and *G. macrocarpum* tested, individually (Table 10). Differences in increase in number of spikes was also noted between different types of N_2- fixing and other bacteria. Effect of individual bacterium on number of spikes was more pronounced in the presence of *G. macrocarpum*. However, increase in number of spikes was noted maximum with *Flavobacterium sp.* (P-25) *and lowest with Azospirillum sp.* Azo.2669. It is also evident from result of the experiment that effect of *Flavobacterium sp.* (P-25) on the number of spikes was found quite similar to the effect noted with *A. chrocoocum* strain, W-5 and *A. Lipoferum* strain, Azo.242. Increase in number of spikes due to individual bacteria alone and with co-inoculation (bacteria+*Glomus macrocarpum*) was noted more than the increase obtained due to N application @ 60 kg Nha^{-1}.

3.2 Grain yield

In general grain yield was increased due to inoculation of different types of bacteria under study. Increase in grain yield was recorded maximum with *Flavobacterium sp.* (P-25) and lowest with *Azospirillum sp.* Azo.2669 (Table 10). Differences in increase between the different type of bacteria was also noted. Grain yield was also increased in presence of *Glomus macrocarpum* as compared to uninoculated control, which was more than

Table 10: Co-inoculation effect of N_2- fixing bacteria and *Glomus macrocarpum* on grain and straw yield and N and P content of wheat (*Triticum aestivum*, L.) *var.* HD-2329 (Average of five replications)

S. No.	Treatments	No. of spikes	Grain yield (g/pot)	Straw yield (g/pot)	Grain		Straw	
					% N	% P	% N	% P
1	Control	10.80	5.28	8.55	2.75	0.17	0.95	0.05
2	*G. macrocarpum*	**13.20**	**7.14**	**12.35**	**3.17**	0.2i	**1.05**	**0.07**
3	*Azo. lac. lac*	**15.60**	**7.65**	**12.40**	**3.33**	**0.23**	1.00	0.05
4	Azo. 242	**17.60**	**8.92**	**14.30**	**3.53**	**0.23**	1.00	0.05
5	Azo. HW. 2004-2	**16.40**	**7.40**	**11.42**	2.77	**0.31**	**1.10**	0.05
6	Azo. 2669	**14.80**	**6.45**	**10.92**	2.67	**0.30**	0.80	0.04
7	Azoto. 2687-1	**15.00**	**6.80**	**11.04**	2.47	**0.26**	0.97	0.05
8	Azoto . 2687-2	**16.40**	**7.40**	**10.85**	**3.13**	**0.26**	0.97	**0.07**
9	W-5	**18.00**	**9.02**	**15.00**	**3.07**	**0.27**	1.00	**0.07**
10	*Protues vulgaris*	**16.60**	**7.90**	**12.30**	**3.22**	**0.25**	1.00	0.06
11	*Falvobacterium sp.*	**18.40**	**9.10**	**14.52**	**3.30**	0.18	0.95	0.05
12	Gm + *Azo. lac. lac*	**16.60**	**8.02**	**13.24**	2.55	**0.32**	**1.05**	0.02
13	Gm+Azo.242	**17.60**	**8.96**	**14.60**	**3.07**	**0.24**	**1.10**	0.02
14	Gm+ Azo. HW. 2004-2	**15.40**	7.75	**12.70**	**3.03**	0.18	0.75	0.04
15	Gm+Azo. 2669	**15.20**	**7.02**	**11.33**	**3.07**	**0.23**	0.95	0.02
16	Gm +Azoto. 2687-1	**14.80**	**7.20**	**12.42**	**3.37**	**0.22**	**1.22**	0.03
17	Gm+Azoto. 2687-2	**15.80**	**7.80**	**12.04**	**2.92**	0.17	0.95	0.04
18	Gm+W-5	**18.00**	**9.30**	**14.86**	**3.07**	0.13	0.95	0.04
19	Gm+ *Proteus vulgaris*	**17.20**	**8.25**	**15.00**	**2.95**	**0.21**	0.75	0.05
20	Gm+*Falvobacterium sp.*	**18.60**	**9.45**	**16.76**	2.77	**0.20**	0.75	0.03
21	40 kg N ha^{-1}	**15.80**	**6.33**	**10.44**	2.47	**0.21**	0.96	0.02
22	60 kg N ha^{-1}	**17.20**	**8.34**	**14.16**	2.77	**0.22**	1.00	0.03
23	120 kg N ha^{-1}	**19.00**	**9.77**	**18.25**	**3.30**	**0.27**	**1.10**	0.05
	SEm±	0.531	0.058	0.096	0.024	0.003	0.022	0.009
	CD at 5%	1.494	0.163	0.272	0.069	0.010	0.062	0.008

* = Average of five replications

the yield obtained due to nitrogen application (40 Kg N ha^{-1}). Increase in grain yield was increased further due to co-inoculation of N_2 -fixing bacteria and *Glomus macrocarpum*. Increase in grain yield brought due to *Flavobacterium sp.* (P-25) alone or in combination with *G.macrocarpum* was found more than the increase recorded due to application of 60 Kg N/ha^{-1}.

3.3 Straw yield

In general straw yield was also found to be increased due to inoculation of various types of nitrogen fixing bacteria (*Azospirillum, Azotobacter*) and other bacteria *Flavobacterium sp.* (P-25) and *Proteus vulgaris* (Table 10.) Differences in increase in straw yield was also noticed between the different types of bacteria, tested. Straw yield was increased further due to co-inoculation of individual bacterium in presence of *G.macrocarpum*. *A. chrococcum* strain, W-5, was noted to bring maximum increase in straw yield, which was similar to the effect obtained with *Flavobacterium sp.* (P-25) and *A. lipoferum* strain, Azo.242. *Azotobacter sp.*, Azoto. 2687-2 was recorded for the minimum increase in the straw yield. Increased obtained with *A. chroococcum* strain W-5, *Flavobacterium sp.* (P-25) and *A. lipoferum* strain, Azo.242 was noted more than the increase obtained due to application of 60 Kg N ha^{-1} .

DISCUSSION

In general, most of the microorganisms are ubiquitous, means they are present in all the environments (soil, water and air), although various factors decides their magnification in terms of population. However, beneficial microorganisms such as nitrogen-fixing bacteria (symbiotic and non symbiotic), phosphate solubilizing bacteria are being a great concern of agricultural microbiologist to lower the cost of cultivation of the crops, without altering the environment.

Azospirillum as associative symbiont and nitrogen fixer, with roots of large number of grasses, was first reported by Dobereiner and Day (1975). Occurrence of *Azospirillum* with the roots of various crops (Bulow and Dobereiner, 1975, Dobereiner *et al.,* 1976, Lakshmi Kumari *et al.,* 1976, Tilak *et al.*, 1987, Singh and Subba Rao, 1980), root and nodule of Aeschynomene species (Singh, 1992), within tissue of sporocarps of actomycorrhizae (Lee and Hung, 1987) and fungal sporocarps (Pal and Singh, 1993) have been reported. This bacterium is Gram-negative, microacrophilic and posses the ability to reduce actylene to ethylene. It is spiral shaped bacteria and shows clock wise half turn or full spiral movement, when seen under microscope. Cell size varies from (2.0-6.0×0.5-1.0μm.

In the present study several isolates of *Azospirillum* (Azo. HW. 2004-1, Azo.HW.2004-2, Azo.2669,Azo. Vaishali-1, Azo.Vaishali-2,Azo.Kundan, Azo.Kanchan, Azo.2710-1, Azo.2710-2,Azo. HDR. 77-1 and Azo.HDR.77-2) were obtained from roots of different varieties of wheat (*Triticum aestivum,* L.)

viz. HW-2004, HD-2669, DL-784-3 (Vaishali), DL-153-2 (Kundan), DL-803-3 (Kanchan), HD-2710 and HDR-77.

These isolates formed pellicle below 2-3 mm from the top surface in the Dobereiner's nitrogen free semi-solid medium. Under the microscope, these were motile (rapid back and forward motion), spirally curved rods/ coccoid cells. Gram reaction was found to be negative. Colonies on N-free agar medium were moist, mildely raised, slimly and 0.5-1.0 mm in dia. All the *Azospirillum* isolates reduced acetylene to ethylene. Based on these characteristic features as described by several workers (Doberener *et al,* 1976,Lakshmi Kumari *et al.*, 1976, Tilak and Subba Rao, 1987, Holt *et al.*, 1994), these isolates were tentatively identified as *Azospirillum* species. The naming of *Azospirillum* were done according to their source of isolation e.g., *Azospirillum* isolates Azo. Kundan was named after the wheat variety DL-153-2 (Kundan).

Amongst the non-symbiotic nitrogen fixing bacteria, *Azotobacter* was first isolated a century back by Beijerinck in the year 1901. Later on it was isolated from the rhizospheric soil of various crops (Lipman, 1903 Krasil' nikov, 1949, Sattar and Solaiman, 1988, Nagraj, 1989, Tippanavar *et al.*, 1990, Page *et al* ., 1991, Sengupta *et al.*, 1991, Wang *et al.*, 1993). It is a pigment forming (brown to black), Gram-negative, pleomorphic and motile bacterium. Cell size of *Azotobacter* varies from 2.0-3.0×1.2-2.5µm. It has ability to reduce acetylene to ethylene (Schroeder, 1987).

In the present study various isolates of *Azotobacter* (Azoto. 2669, Azoto. Vaishali, Azoto.2687-1, Azoto.2687-2, Azoto.2643-1, Azoto.2643-2, Azoto.2680-1, Azoto.2680-2, Azoto.2680-3, Azoto.2710-1, Azoto.2710-2, Azoto.2690-1, Azoto.2690-2, Azoto.HDR.77-1 and Azoto. HDR. 77-2)

were isolated from the rhizospheric soil of different varieties of wheat (*Triticum aestivum,* L.*) viz.,* HD-2669, DL-784-3 (Vaishali), HD-2687, HD-2643, HD-2680, HD-2710, HD-2690 and HDR-77.

All these isolates, isolated from the wheat rhizospheric soil, were nitrogenase positive. Their cell size also varies from 2.0-3.0×3.0-6.0 µm. Cells of these isolates were also found Gram-negative, pleomorphic and pigment forming (brown to black). Therefore, based on the above characteristics, these isolates were tentatively identified as *Azotobacter* species. The naming of *Azotobacter* isolates were also done according to their source of isolation e.g., *Azotobacter* isolates Azoto. 2669 was named after the wheat variety HD-2669.

It is well established fact that *Azospirillum* species (*A. brasilense and A. lipoferum*) increase the yield of various crops, such as sorghum, pearlmillet etc. (Tilak *et al.,* 1982, Suba Rao *et al.,* 1982, 1983 Tilak and Singh, 1988, Rai *et al* ., 1988, Tilak, 1995, Saxena and Tilak , 1998). Their effect have been noted to be varied with crops grown under different agro-climatic conditions (Subba Rao *et al.,* 1979). The increase due to inoculation of *Azospirillum* species varied from 10-30%, which has been attributed to the nitrogen fixation and growth promoting substances produced by this bacterium (Tien *et al.,* 1979, Kipe Nolt *et al.*, 1985). Increase in root biomass has also been noted (Singh and Subba Rao, 1979, Dewan and Subba Rao,1979).

Similarly in the present investigation various plant growth parameters such as plant height, number of tillers, fresh and dry weight of shoot and root, root volume, number of spikes and grain and straw yield of wheat were found to be increased due to seed inoculation of various *Azospirillum*

isolates, isolated from, the roots of different varieties of wheat *viz.* HW-2004, HD-2669, DL-784-3 (Vaishali), DL-153-2 (Kundan), DL-803-3 (Kanchan), HD-2710 and HDR-77, sporocarps of ectyomycorrhizae (*Azo. lac. lac. Rv. Zae*) and standared cultures of *A. brasilense* (Sp-7, Sp cd) and *A. lipoferum* strain, Azo. 242 (Courtesy, Dr J. Dobereiner).

When these isolates of *Azospirillum* were used to inoculate seed of wheat (*Triticum aestivum* L.) *var.* HD-2329, various plant growth parameters and grain and straw yield were increased, which is quite obivious because similar results were also reported by other workers also. In present investigation plant growth and yield parameters of wheat were increased due to inoculation of *Azospirillum* isolates, isolated from wheat (homologus strain) and sporocarp of ectomycorrhizae (heterologus strain). Increase in plant growth parameters and yield of wheat by homologus strains of *Azospirillum* is very scanty.

Beneficial effect of *A. brasilense* and *A. lipoferum* in increasing crop yield has been well doccumented. However, information in this regard with *A. amazonense* is not available till date. In the present investigation increase in plant growth parameters as well as grain and straw yield of wheat have been recorded for the **first time**, but it is difficult to equate the effect of *A. amazonense* with other *Azospirillum* strains.

Beneficial effect of *Azotobacter* species on various crops has already been worked out by several workers (Azcon *et al.*, 1975; Shende, *et al.*, 1977; Rai, *et al.*, 1988). *Azotobacter* species to some extent are host specific (Apte and Shende, 1981, Raj Kumar *et al.*, 1990). This bacterim show multiple action on plant (Shende *et al.*, 1975).

In present study several *Azotobacter* isolates were obtained from rhizospheric soil samples of different varieties of wheat *viz.*, HD-2669, DL-784 -3 (Vaishali), HD-2687, HD-2643, HD-2680, HD-2710,HD-2690 and HDR-77, and tested for their compatibility to increase growth and yield of wheat (*Triticum aestivum,* L.) *var*-HD23229. Seed inoculation of wheat with these *Azotobacter* isolates, separately, above plant growth parameters and grain and straw yield were also increased. Differences in increase in these parameters between various strains/isolates of *Azotobacter* was also noted. However, among various *Azotobacter* isolates, (Azoto.2669, Azoto. Vaishali, Azoto.2687-1, Azoto.2687-2, Azoto.2643-1, Azoto.2643-2, Azoto.2680-1,Azoto.2680-2, Azoto.2680-3, Azoto.2710-12, Azoto.2710-2, Azoto.2690-1,Azoto.2690-2, Azoto. HDR.77-1 and Azoto. HDR 77-2), obtained from rhizospheric soil of wheat, *Azotobacter* isolate, Azoto. 2687-2 was found more effective in bringing maximum increase in yield. It is also evident that increased plant growth parameters and grain yield was noted maximum due to inoculation of standard *A. chroococcum* strain, W-5 (isolate of wheat rhizosphere), when compared to A-41 and CBD-15, (isolate of wheat and cotton rhizosphere, respectively). Therefore, this piece of work has indicated that *Azotobacter* strains obtained from wheat rhizosphere can perform better with the homologous host which requires more confirmation, although to some extent this finding has been supported by other workers also (Apte and Shende, 1981, Raj Kumar *et al.*, 1990).

Further more increase in grain yield due to seed inoculation of *Azotobacter spp.* could be attributed to the production of growth promoting substances (Brown *et al.*, 1968, Azcon *et al.*, 1975, Apte and Shende, 1981, Glick, 1995), shoot and root length, seed germination (Shende *et al.*, 1977), N-nutritian (Kavianandan *et al.*, 1978, Rai *et al.*,1988) and increase in root biomass (Dewan and Subba Rao, 1979).

Flavobacterium sp do not have nitrogenase enzyme, but posses phosphatase enzyme and is known to solubilize phosphorus. Besides this rapid root colonization of wheat by the *Flavobacterium* species have been reported (Jane *et al.*, 1994). In the present investigation, inoculation of *Flavobacterium sp* . (P-25) has brought in maximum increase in plant growth parameters and grain yield of wheat as compared to nitrogen fixing bacteria (*Azotobacter, Azospirillum*), *and Proteus*. At present, explanation on beneficial effect of this bacterium is not available. However, this bacterium is known to alter the enzymatic activities of endorhizospheric and rhizospheric population (Jane, *et al.*, 1994).

Beneficial effect of *Proteus valgaris* as a nitrogen fixer have also came into picture recently, increase in grain yield of sunflower was observed due to seed inoculation with *P. vulgaris* (Malik, *et al.*, 1995, 1996). In the present investigation, plant growth parameters and grain yield of wheat *(Triticum aestivum,* L.) *var.* HD-2329 were increased in the presence of *P. vulgaris*. However, exaplation on the beneficial effect of this bacterium is also not available till date, and its N_2-fixation ability is also doubted presently.

. VAM fungi are known to enhance plant growth and yield of various crops. (Abbot *et al.*, 1977, Ames *et al.*, 1983, Singh *et al.*, 1987, Barea *et al.*, 1980, 1987, 1989a and 1989b, Suba Rao *et al.*, 1990, Negi *et al.*, 1990, Singh, 1996). Beneficial effects of VAM have been attributed to increase in phosphorus content and other elements in the plant tissue (Powell, 1975, Bell *et al* ., 1987, Raju *et al.*, 1990, Singh, 1990, Berea, 1991, Joner *et al.*, 1995, Tu-Shittua *et al.*, 1997). However, VAM is not specific like *Rhizobium* and *Azotobacter,* although differences in the effectiveness of various VAM have also been observed by several workers (Virhcileg *et al.*, 1991, Singh

1992a). Increase in root biomass and number of rhizobacteria has also been reported (Azcon *et al.*, 1987, 1990).

In the present study inoculation of VA-mycorrhizae were found to increase, growth and yield parameters of plant *viz.*, plant height, number of tillers, fresh and dry weight of shoot and root, root volume, number of spikes and grain and straw yield of wheat crop. Differences in their effectiveness for the enhancement of various plant growth and yield parameters were also observed. These finding can be explained on the basis of observations obtained by several workers (Raju *et al.*, 1990, Singh 1990, Suba Rao *et al.*, 1990, Tu-Shittau *et al.*, 1997). Amongst eleven VAM screened in the present investigation, *G. macrocarpum* resulted in maximum increase in all the plant growth and yield parameters of wheat, and was selected for further study.

It has also been reported that seed inoculation with N_2- fixing bacteria, (*Azospirillum and Azotobacter*) is beneficial for improvement of crop yield (Tilak *et al.*, 1982, 1988, Rai *et al.*, 1988, Saxena and Tilak, 1998). However, their effect were further enhanced in the presence of VA-mycorrhizae. Few reports have suggested that nitrogen fixing bacteria enhances VAM infection and multiplication and vice-versa (Singh *et al.*, 1991a, Singh, 1992 and Singh, 1995).

In the present study nitrogen fixing bacteria (*Azospirillum, Azotobacter*) including *Flavobacterium sp* . (P-25) and *P. vulgaris* increased plant height, number of tillers, fresh and dry weight of shoot and root, root volume, number of spikes, grain and straw yield of wheat crop. Marginal increase was also noted due to inoculation of *G. macrocarpum* as compared to uninoculated control. Increase in these parameters could be explained on the basis of

above findings and increase in nitrogen and phosphorus uptake as observed earlier (Subba Rao *et al.*, 1982, Singh and Subba Rao, 1987,1990, Rai *et al.*, 1988, Singh, 1990, Zaghlaul *et al.*, 1996).

Flavobacterium sp. has been reported as phosphate solubilizing bacteria, which lacks nitrogenase enzyme. Increased plant parameters, were noted due to inoculation of *Flavobacterium sp.* (P-25). This bacterium was found most effective as compared to nitrogen fixing bacteria. The effect of *Flavobacterium sp.* was more pronounced in presence of *G. macrocarpum*.

Interactive effect of *Flavobacterium* and *G. macrocarpum* has been reported for the **first time**. This finding required more detail study to confirm these findings and reason for such beneficial effects. However, it could be due to the production of high amount of plant growth promoting substances and alteration in the enzymatic activity of the rhizosphere.

Proteus has been reported as a nitrogen fixing bacteria which has been found to increase crop yield of various crop like sunflower, maize, cotton etc. (Malik *et al.*, 1995, Malik, *et al.*, 1996). Since information on physiological and biochemical aspects, root colonization is not available till date, therefore, these aspects could also be taken to explain the beneficial effect of *Proteus vulgaris* on the crop.

SUMMARY

In the present investigation various isolates of *Azospirillum* were obtained from different wheat varieties *viz.*, HW-2004, HD-2669, DL, 784-3 (Vaishali), DL- 153-2 (Kundan), DL- 803-3 (Kanchan), HD-2710 and HDR-77. These isolates were identified as *Azospirillum* species based on the typical characteristic features of *Azospirillum.* These were named after their source of isolation. All the isolates of *Azospirillum* were found to be nitrogenase positive.

Seed inoculation of wheat (*Triticum aestivum,* L.) *var.* HD-2329 with *Azospirillum,* isolated from wheat, were found to increase plant growth parameters *viz.*, plant height, number of tillers, fresh and dry weight of shoot and root, root volume and grain and straw yield. Differences in increase due to inoculation of various isolates/strains were also observed.

Amongst the N_2-fixing *Azospirillum spp,* maximum increase in the above parameters were observed due to inoculation of *A. lipoferum* strain, Azo.242 and *A. brasilense* strain, *Azo. lac. lac* (isolate of actomycorrhizae). In addition *A. amazonense* was also found to increase plant height, number of tillers, fresh and dry weight of shoot and root, root volume, number of spikes and grain and straw yield of wheat *var.* HD-2329 for the **first time.**Increased in above parameters recorded with *A. brasilense* strain, *Azo. lac. lac.* and *Azosprillum* isolate, *Azo.* HW-2004-2 was found at par.

Various *Azotobacter* isolates, were also obtained from different wheat varieties *viz.*, HD-2669, DL-784-3 (Vaishali), HD-2687, HD 2643, HD-2680,

HD-2710, HD-2690 and HDR-77. These isolates were identified as *Azotobacter spp.* based on the typical characteristic features of *Azotobacter* . These isolates were also found to be nitrogenase positive. Seed inoculation of wheat (*Triticum aestivum* L.) *var.* HD-2329 with *Azotobacter* isolates, isolated from wheat and standard strains of *A. chroococcum* obtained from other sources were also found to increase plant growth parameters *viz.*, plant height, number of tillers, fresh and dry weight of shoot and root, root volume, number of spikes and grain and straw yield of wheat. Differences in increase due to inoculation of various strains/isolates were also found. Amongst the various nitrogen-fixing *Azotobacter spp.,* maximum increase in above parameters were noted due to inoculation of *A. chroococcum* strain, W-5 which is an isolate of wheat rhizosphere.

Soil inoculation of various VAM fungi resulted in increase in plant growth and yield parameters of wheat *viz.*, plant height, number of tillers, fresh and dry weight of shoot and root, root volume, number of spikes and grain and straw yield of wheat. However, amongst the eleven VA-mycorrhizae tested, *G. macrocarpum* scored the Ist rank.

Flavobacterium sp. (P-25) a phosphate solubilizing bacteria, was found to be most potent as compared to nitrogen fixing bacteria in bringing increase in various plant growth and yield parameters of wheat.

Proteus vulgaris, presently known as a nitrogen fixing bacteria still controversial was also found to increase plant growth and yield parameters of wheat *viz.*, plant height, number of tillers, fresh and dry weight of shoot and root, root volume, number of spikes and grain and straw yield of wheat.

Plant growth parameters and grain yield production were further enhanced due to interaction of *Flavobacterium sp* (P-25) in presence of *G.*

macrocarpum. N and P content in the grain and straw were further enhanced due to co-inoculation of nitrogen-fixing bacteria and *G. macrocarpum*. Co-inoculation of *Flavobacterium spp* (P-25) and *G. macrocarpum* was found to be best combination amongst other combinations (co-inoculation of nitrogen fixing bacteria and *G. macrocarpum)*.

Co-inoculation of *A. brasilense* strain, *Azo. lac. lac.* (isolated from actomycorrhizae) and *A. lipoferum* strain, Azo. 242 with *G. macrocarpum* was also found beneficial for the improvement of plant growth parameters and grain yield of wheat crop. In this investigation co-inoculation of *Azospirillum* strain, *A. amazonense* and *G. macrocarpum* was also found to increase plant growth parameters as well as grain yield of wheat for the **first time**.

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