STUDIES ON ARBUSCULAR MYCORRHIZAL (AM) FUNGI ASSOCIATED WITH DIFFERENT VARIETIES OF RICE (*ORYZA SATIVA* L.) GROWN IN *KHAZAN* LANDS OF GOA

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DECLARATION

I hereby declare that the matter embodied in this thesis entitled **"Studies on Arbuscular Mycorrhizal (AM) Fungi associated with different varieties of rice (*Oryza sativa* L.) grown in *Khazan* lands of Goa"** is the result of investigations carried out by me, under the supervision of Prof. B.F. Rodrigues and it has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or any other such similar title.

Goa University
January 2020

Ms. Wendy Francisca Xavier Martins
(Candidate)

CERTIFICATE

This is to certify that the work incorporated in this thesis entitled **"Studies on Arbuscular Mycorrhizal (AM) Fungi associated with different varieties of rice (*Oryza sativa* L.) grown in *Khazan* lands of Goa"** submitted by Ms. Wendy Francisca Xavier Martins, constitutes her independent work and the same has not been previously submitted for the award of any other degree, diploma, associate-ship, fellowship or any other such similar title.

Goa University
January 2020

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CHAPTER 1

Introduction

1.1. Rice

Rice is a principle source of food for over half of the world's population. Being a cereal, it is extensively consumed as a main source of nutrition, predominantly in Asia, where 60% of the earth's population lives. China and India itself accounts for 50% of rice cultivation and consumption. Rice has two cultivated species *viz*., *Oryza sativa* L. and *Oryza glaberrima* Steud. and about 20 wild species. *Oryza sativa* L. is grown all over the world while *O. glaberrima* is cultivated only in Africa. Presently above 1,00,000 rice varieties are available in the world for crop growing which are cultivated chiefly in China, India, Japan, Thailand and Indonesia (https://en.m.wikipedia.org/wiki/Rice).

1.1.1. Rice Description

Rice belongs to the family Poaceae (Graminae), subfamily Oryzoideae and tribe Oryzae. It is a C_3 plant (Gould and Shaw 1983), semi-aquatic, cultivated under a wide range of environmental conditions in all five continents. It is an annual grass, which grows to 0.5-2m in height.

Rice plant can be divided into two main systems *viz.* root and stem system.

1.1.1.1. Root System: when rice grain sprouts in well drained upland soils the coleorhizae appears first and if the grain grows in a lowland area submerged in water than the coleoptile develops earlier than the coleorhizae. The coleorhizae are followed by two or more secondary roots. These secondary roots develop lateral roots. The embryonic root dies and is replaced by adventitious roots which arise from the node of the culm present below the soil.

1.1.1.2. Shoot System: culm, leaves and inflorescences make the shoot system. Rice culms are made up of alternating hollow internodes and solid nodes. Every node has a leaf and a bud. Leaves are distichous, simple, and sessile held one at each node at an angle in two ranks along the stem. The leaf blade is connected at the node by a sheathing base. Ligule is present at the

junction of the leaf blade and sheath. The leaves are linear-lanceolate, serrulate along the margin, acuminate at apex with parallel venation. Buds close to the soil grow into tillers under favourable conditions. The primary tiller gives rise to the secondary and tertiary tillers. The primary tiller bears more number of leaves as compared to secondary and tertiary tillers. Rice inflorescence is called as a panicle. The topmost node of the culm bears a panicle. The panicle is made up of a number of pedicellate spikelets. The primary panicle branch subdivides into secondary which occasionally gives rise to tertiary branches. These carry spikelets. Each spiklet is zygomorphic, bisexual and are enclosed in two alternating bracts called glumes. It consists of a hard covering, known as lemma and palea. The lemma and the palea encircle six functional stamens. The stamens are with free filaments and anthers are versatile. The ovary is bicarpellary, syncarpous, superior and unilocular with a single ovule on basal placentation. Its style is short with long feathery stigma. Perianth is reduced and represented by two transparent structures called lodicules, present at the base of the flower.

Maturation of the anthers and ovules is simultaneous. Rice is a self-pollinated plant. During flowering stage, opening of the spiklet in a panicle begins from top to bottom. The lodicules become turgid and separate the lemma and palea forcefully, thus letting the stamens to appear outside the open floret. Splitting of the anthers leads to releasing of the pollen grains. Subsequently the lemma and palea close after the shedding of pollen grain.

On completion of pollination and fertilization a rice grain (caryopsis) is formed. Lemma and palea enclose the grain firmly. The dehulled rice grain is called brown rice due to the brownish pericarp, which is the outermost layer, enveloping the caryopsis. It is taken off at the time of milling and polishing of the rice grain. On the ventral side of the spikelet adjacent to the lemma is the embryo. Next to the embryo, a dot like structure called the hilum is present. The embryo contains the plumule and radicle. The plumule is called as coleoptiles and the radicle coleorhizae (https://farmer.gov.in/imagedefault/pestanddiseasescrops/rice.pdf).

1.2. Rice cultivation in India

One of the principle food crops of India is rice. It covers the maximum area under cultivation. India is the leading rice producing country after China. It is a main kharif crop. Being a

tropical plant, it thrives well in hot and humid climate. It is mainly cultivated in rain fed areas which receive heavy rainfall. It requires a temperature of 25˚C and more and rainfall more than 100cm. Rice is also cultivated in regions with less rainfall through irrigation.

Rice is cultivated in varied soil and climatic conditions due to its extensive physical adaptableness. Thus, rice is seen growing below sea-level to a height of 2000 meters. Five rice growing regions can be grouped in the country.

North–Eastern region: Assam and North eastern states are included in this region. The region receives intense rainfall and rice is cultivated mainly in rain fed conditions.

Eastern region: Bihar, Chhattisgarh, Jharkhand, Orissa, Eastern Uttar Pradesh, Madhya Pradesh and West Bengal make up this region. This region has the highest amount of rice cultivation as it receives heavy rainfall and mainly cultivated in rain fed conditions.

Northern region: Western Utter Pradesh, Haryana, Punjab, Uttrakhand, Himachal Pradesh and Jammu and Kashmir are included in this region. Single crop of rice is usually cultivated here.

Southern Region: Andhra Pradesh, Kerala, Tamil Nadu and Karnataka are included in this region. Rice is cultivated here under irrigated conditions.

Rice is cultivated through the year across diverse seasons and in varied ecologies. India is the only country in the world where rice is cultivated in different ecosystems. Rice ecosystems are grouped into four major types: Irrigated, Rainfed Upland, Rainfed lowland and Flood prone.

Irrigated rice ecosystem: states like Punjab, Haryana, Jammu and Kashmir, Andhra Pradesh, Tamil Nadu, Uttar Pradesh, Sikkim, Himachal Pradesh, Karnataka and Gujarat cultivate rice in irrigated condition. The total area under this ecosystem is about 22.00 million hectares. It accounts for about 49.5% of the total area under rice crop in the country.

Rain fed Upland Rice ecosystem: under upland rain fed rice in the country is around 6.00 million hectares which makes up for 13.5% of the total area under rice crop. Upland rice area occur in eastern zone of the country, comprising of Assam, Bihar, Orissa, Eastern Madhya Pradesh, West Bengal, Eastern Uttar Pradesh and North-eastern Hilly region.

Rain fed Lowland Rice ecosystem: in India, lowland rice is about 14.4 million hectares, which accounts 32.4% of the total area under rice crop in the country (https://farmer.gov.in/imagedefault/pestanddiseasescrops/rice.pdf).

Flood prone rice ecosystem: rice varieties cultivated in flood prone ecosystem are tolerant to submersion. These areas are narrow belt of land extending from few to around 50 km from the sea and alongside the low lying areas, estuaries and internal depressions. The coastal line of India extends for 8129 km. It includes the coast of the Bay of Bengal and Arabian Sea. These areas are known by diverse local terms such as *Khar* in Gujarat and Maharastra, *Chopan* in Karnataka, *Choddu* or *Uppu* in Andhra Pradesh (Sapkale and Rathod 2014).

In India, during the last year 2018-19, 115.6 million tonnes (MT) of rice was harvested as against 111.012 MT in the year 2017-18 (https://krishijagran.com/commodity-news/hike-in-foodgrain-production-to-281-million-tonnes-in-2018-19/) and 109.7MT in 2016-17 (https://wwwibef.org/news/total.foodgrains-production-is-estimated-at-record-27568-million-tonnes).

1.3. Rice cultivation in Goa

Goa is the smallest state of the Republic of India, its position is denoted at 15° 48' 00" N and 14° 53' 54" N Latitude and 74° 20' 13" E and 73° 40' 33" E Longitude. It has a total geographical area of 3,61,113 hectares including both north and south Goa districts (Gune 1979).

Rice is the predominant staple food crop of Goa. It is cultivated during the kharif (*Sod*) and rabi (*Vaigon*) seasons, covering an area of about 67% and 33%, respectively. Nevertheless rice production continues to be on the decline in the state. A total production of 71,279 tonnes of rice was recorded in the kharif season in Goa, while 31,718 tonnes was produced in the rabi season, thus resulted in a total of 1,02,997 tonnes in the year 2017-18. The production of rice was 1,13,227 tonnes, 1,15,068 tonnes and 1,20,505 tonnes in 2016-17, 2015-2016 and 2014-2015, respectively. The area for rice cultivation decreased from 41,970 ha in 2014-15 to 41,344 ha in 2015-16, to 40,823 ha in 2016-17. It has further reduced to 38,520 ha in 2017-18. (https://timesofindia.indiatimes.com/city/goa/less-xitt-this-yr-leaves-goa-in-a-kodi/articleshow/60250480.cms). Land holdings in Goa are small and fragmented. Socio-economic condition, high cost of labour, lack of mechanization, threshing and processing facilities including marketing infrastructure have threatened the cultivation of rice in the *Khazans.*

In Goa, the local population distinguishes different types of rice fields according to soil, rainfall conditions and nearness to the riverside (Gune 1979). The crop is cultivated in three different topographical conditions *i.e*. rainfed lateritic upland (*Morod*-8,600 ha), midland (*Ker*-16,900 ha) and low lands (*Khazans*-16,900 ha) (Manjunath *et al.* 2009).

1.4. Rice cultivation in *Khazan* fields

Khazans are traditionally community accomplished amalgamated agro-aqua ecosystems present in Goa. They are repossessed over centuries from mangrove swamps. *Khazan* knowledge in Goa safeguards agricultural fields and villages from tidal ingress due to a system of dykes (*bunds*). The outer embankments or *bunds* are defensive in nature and mostly built using locally available laterite stones, mud and clay from the *Khazan*s. Mud and straw used to build the inner embankment (*mero*) protects the fields from nutrient leaching and soil erosion. Pressure flow of water helps to control the water level with the help of wood sluice gate (*manos*). At low tide, the gates open and the water is let out from the fields. However at high tide and during monsoons, the gates close, preventing the entrance of saline water in the fields. *Adavomanos* is set up near the sluice gates, it is made up of horizontal planks to maintain and to allow water only to a canal that moves all-round the field but does not flood the field, causing inundation (Sonak *et al.* 2005) **(Plate I)**.

Khazan lands have been a resource of employment for many, as these lands were formally used for paddy cultivation, traditional farming, pisciculture and salt extraction. They were generally used as paddy fields, where *bunds* and sluice gates are used to keep the sea water away and control the inflow of saline water respectively. These ecosystems have been the rice bowls of the coastal communities of the past (Alvares 2002). Assgo, Bello, Damgo, Kalo damgo, Kalo korgut, Kalo novan, Khonchri, Korgut, Muno and Shiedi are conventionally grown rice varieties in the *Khazans* of Goa (Bhonsle 2011). However, only Korgut, Khonchri, Kalo damgo and Assgo are the main salt tolerant varieties (Bhonsle and Krishnan 2012). Cultivation of a hybrid variety of rice *i.e. Oryza sativa* L. var. Jyoti in *Khazan* has also been reported (Rodrigues and Anuradha 2009).

1.5. Occurrence of Arbuscular Mycorrhizal (AM) fungi

Existence of varied inhabitants of soil microorganism is an indication of healthy and fertile soil. An important component of which is Arbuscular Mycorrhizal fungi (AMF) (Linderman 1992). These fungi have a mutualistic symbiotic association with the roots of plants. They belong to the phylum Glomeromycota and are geographically widespread over a wide ecological range (Mosse 1973). Their presence has been registered in different plants (Sharma *et al.* 2007), in varied soils (Turner *et al.* 2000) and in diverse agro-climatic regions of India (Singh and Adholeya 2002).

1.6. AM Fungi in Agriculture

AM fungi have been regarded as a fundamental connection between plants and soil (Bethlenfalvay and Linderman 1992). Sustainable agriculture is farming using ecologically available sustainable methods that are vital in agro-ecosystem. This farming must be ecologically healthy, cost effective and socially accountable (Siddiqui and Pichtel 2008). AM fungi are ubiquitous and form symbiotic association with most of agricultural and horticultural crops making nutrient supply especially P (Abo-Rekab *et al.* 2010) available to crops. In addition they intensify soil fertility (Rillig and Mummey 2006). Generally AM fungi are responsible for plant health and development, hence attracting attention as an ecosystem engineers and biofertilizers (Fitter *et al*. 2011). In the last two decades, AM inocula has been generated and utilised in agriculture, horticulture, landscape restoration and site remediation (Hamel 1996). Field experiments disclosed that supplementation of AM fungi by inoculation can result in plant root colonization and hence increases crop productivity (Lekberg and Koide 2005, Lehmann *et al.* 2012).

1.7. AM Fungi in Rice

Rice plants promptly form AM association in upland ecosystem (Ilag *et al*. 1987). Presently, there are reports that AM fungi are able to survive in water logged paddy wetlands (Watanarojanaporn *et al.* 2013). These fungi have the potential to improve growth and productivity of plants by increasing the uptake of nutrients (Al Karaki 2000). Inoculation of rice by AM fungi has resulted in comparatively better performance in growth development and yield of lowland rice (Oladele *et al.* 2014). According to Gupta and Ali (1993),

Plate I

Khazan **ecosystem in Shiroda.**

a. Sluice gate; b. Outer *bund*; c. Agricultural field with inner *bunds*; d. Canal for water drainage.

inoculation by AM fungi in high and low fertility soil could enhance nutrient uptake in rice. Secilia and Bagyaraj (1992) have reported increase in rice yield under flooded conditions on inoculation with AM fungi.

Indigenous varieties of rice grown in the *Khazans* have to be protected. The costing and yield of the crop has to be improved to motivate the cultivation of rice in the *Khazans*. This improvement can be attained by adopting proper agronomical practices *viz.*, selection of good seeds, maintaining the plant density in the fields, timely harvest and proper storage methods. Use of AM fungi and the reduced use of agrochemicals will certainly go a long way in motivating the farmer to cultivate in the *Khazan*s. Hence there is a need to understand the diversity of AM fungi in the *Khazan*s. In the present work, an attempt was made to understand the diversity of AM fungi, recommending AM fungal inocula in different ecosystems and enhancing the productivity of the fields with the application of AM fungi which would reduce the cost and increasing the margin of profits for the farmer.

The following objectives were undertaken for the present study.

- To isolate and identify AM fungal spores from rhizosphere soils of different varieties of *O. sativa* from *Khazans* of Goa.
- To study the physico-chemical properties of rhizosphere soils of rice grown *Khazan* lands of Goa.
- To study the spatio-temporal variation of AM fungi in *O. sativa* grown in the *Khazan* lands of Goa.
- To assess AM fungal root colonization in different varieties of rice (*O. sativa* L.) grown in the *Khazan, Ker* and *Morod* lands of Goa.
- To prepare pure cultures of dominant AM fungal species and their mass multiplication.
- To study the effect of dominant AM fungal species on growth, yield and grain quality characteristics of selected rice variety grown in *Khazan* lands of Goa.

CHAPTER 2

Review of Literature

2.1. History of Arbuscular Mycorrhizal (AM) Fungi

Arbuscular Mycorrhizal (AM) fungi exemplify a monophyletic fungal ancestry (Glomeromycota) that assists terrestrial ecosystems worldwide by establishing a close association with roots of land plants and this association is called 'The mycorrhizal symbiosis' (Corradi and Bonfantante 2012). The term 'Mycorrhiza' was first coined by Frank (1885). He was perhaps the first to recognise this association between a plant root and a fungus (Frank and Trappe 2005). The spelling and name for AM fungi has changed across the years. A complete discussion of the origin of the word 'Mycorrhiza' including the amalgamation of the second 'r' was given by Kelley (1931, 1950). AM fungal symbiosis was previously frequently titled as 'Phycomycetous endo-mycorrhiza' to differentiate it from endo-mycorrhizal symbiosis formed in members of Ericaceae, Orchidaceae and higher fungi. However, the term 'Phycomycetes' no longer carrries any systematic implications (Koide and Mosse 2004). Presence of fungal structures like vesicles and arbuscules within the roots lead to the use of the term 'Vesicular Arbuscular Mycorrhiza' (Janse 1897, Gallaud 1905). But on detection that all fungi do not form vesicles lead to the renaming of this symbiosis as 'Arbuscular Mycorrhiza' which is at present widely accepted (Koide and Mosse 2004).

As early as 1842, Nägeli described AM fungi. Trappe and Berch (1985) and Rayner (1926-1927) cite early observation of AM symbiosis. Schticht (1889), Dangeard (1896), Janse (1897), Petri (1903), Gallaud (1905), Peyronel (1924), Jones (1924) and Lohman (1927) conducted extensive surveys of host plants and provided anatomical descriptions of AM fungi. The most frequently cited of all papers on root processing and staining of this symbiosis is by Phillips and Hayman (1970). Quantification of colonization has been achieved in various ways by Newman (1966), Sparling and Tinker (1975) and Read *et al*. (1976).

2.2. Classification of AM fungi

For classification of AM fungi, spore isolation from the soil is essential. The most commonly used method for extraction of spores from the soil is by Wet Sieving and Decanting Method. It is the method used for extraction of nematodes which was later adapted for AM fungi by Gerdemann (Gerdemann 1955, Gerdemann and Nicolson 1963). Several attempts of developing a classification system or method of identification of all AM spore kind have been carried out. Nicolson and Gerdermann agreed on the classical practice with Latin names. Mosse and Bowen (1968) tried a more descriptive arrangement of classification. Nicolson and Gerdemann (1968) separated fungi into two clusters of genera *Endogone*, one developing extra-radical azygospores/zygospores rising from the tip of an inflated hyphal suspensor but creating no intra-radical vesicles. Molecular data was used by Schüßler *et al.* (2001) to find a relationship among AM fungi, between AM fungi and other fungi. The cluster of AM fungi was raised to the level of Phylum Glomeromycota. This cluster was different from other fungal clusters.

Identification methods used by taxonomists have become increasingly complex. Fundamental taxonomy was centred on morphological and anatomical characters typical of fungi. They were followed by procedures centered around serology (Aldwell and Hall 1987), isoenzyme differences through gel electrophoresis (Hepper 1987) and fatty acid differences (Bentivenga and Morton 1994) were initiated. Presently, systematics depends largely on DNA-based procedures (Cummings 1990, Davidson and Geringer 1990, Simon *et al.* 1990, 1992, 1993, Redecker 2000) which are taken into consideration as the best evaluation of genealogical relationships among organisms (Koide and Mosse 2004). DNA target regions used for AM fungal identification are present on the ribosomal genes as they show deviation sufficient to differentiate between AM species (Krűger *et al.* 2012). This led to the modern era of molecular identification of AM species (Redecker *et al*. 2013). Next Generation Sequencing (NGS) tools presented a step ahead for biodiversity surveys of all organisms (Shokralle *et al*. 2012) as well as for AM fungi. The capability to correctly identify the fungi, avoid repetition of names and connect one species to another also depends largely on global culture assemblage centers like International Culture Collection of Arbuscular and Vesicular Arbuscular Mycorrhizal Fungi (INVAM) and The International Bank for Glomeromycota

(BEG/IBG) (Koide and Mosse 2004). The latest classification of Glomeromycota in Redecker *et al.* 2013 is set up on a consensus of regions spanning rRNA genes: 18S (SSU), ITSI-5.8S-ITS2(ITS) and/or 28S (LSU) **(Table 1)**.

Table 1: Consensus classification of the Glomeromycota by Redecker *et al.* (2013).

Class	**Order**	**Family**	**Genus**
Glomeromycetes	Diversisporales	Diversisporaceae	*Tricispora**
			*Otospora**
			Diversispora
			*Corymbiglomus**
			Redeckera
		Acaulosporaceae	*Acaulospora*
		Sacculosporaceae*	*Sacculospora**
		Pacisporaceae	*Pacispora*
		Gigasporaceae	*Scutellospora*
			Gigaspora
			*Intraomatospora**
			*Paradentiscutata**
			Dentiscutata
			Cetraspora
			Racocetra
		Claroideoglomeraceae	*Claroideoglomus*
	Glomerales	Glomeraceae	*Glomus*
			Funneliformis
			Septoglomus
			Rhizophagus
			Sclerocystis
		Ambisporaceae	*Ambispora*
	Archaeosporales	Geosiphonaceae	*Geosiphon*
		Archaeosporaceae	*Archacespora*
	Paraglomerales	Paraglomeraceae	*Paraglomus*

(* indicate genera of uncertain position insufficient evidence, but no formal action taken)

2.3. Morphological characters used for identification of AM fungi

Different morphological characters significant in establishing taxonomic identity and relationship of AM fungal species used for identification are described below.

2.3.1. Spore wall

Development of the spore wall occurs throughout a sporogenous hypha. It may be formed at the end of the sporogenous hypha as viewed in species belonging to *Diversispora*, *Glomus*, *Gigaspora*, *Pacispora*, *Paraglomus*, *Racocetra* and *Scutellospora*. Spore wall can grow from within the sporogenous hypha as observed in species belonging to *Acaulospora*, *Entrophospora*, *Intraspo*ra, some *Glomus* species and can develop from the margin of the sporogenous hypha as observed in species belonging to *Acaulospora*, *Ambispora*, *Archaeospora* and *Otospora*. Variations in the spore wall appear with increase in spore size *i.e.* as it grows, increase in thickness and differentiates. When the spore attains maximum size very small differences in colour, thickness and rigidity are observed in the spore wall. The spore wall layer could be permanent or impermanent structures (Blaszkowski 2012). In most young spores, the spores wall may be 1 or 2 layered. But in matured spores the spore wall distinguishes from 1-4 wall layers (Morton and Redecker 2001). Walker (1983) defined a wall group as "An aggregation of walls that are either adherent or that remain close together when a spore is crushed"

The different wall types are as follows.

2.3.1.1. Amorphous - a formless, flexible wall whose elasticity is affected by the mountant. It appears rigid in water or glycerol, in acidic mountant it is plastic and tends to collapse partially.

2.3.1.2. Coriaceous - robust, tough but flexible inner walls which turns leathery in appearance in hypertonic solution.

2.3.1.3. Germinal - innermost layer of *Gigaspora* species from which germ tube arises frequently bears papillae which project distally from innermost surface.

2.3.1.4: Evanescent - an outermost ephemeral one to multi-layered wall, which is sloughed off as the spore matures.

2.3.1.5. Laminated - generally outer many layered wall layers increasing as spore ages.

2.3.1.6. Membranous - generally inner, very thin frequently wrinkled, flexible walls that frequently collapse in hypertonic solution. Hyphal peridium is tightly adherent hyphal layers around the spore.

2.3.1.7. Unit - outer, single, rigid non-layered wall sheathings like brittle plastic on crushing.

2.3.1.8. Expanding - a unit wall which expands when placed in lactic acid.

2.3.2. Inner walls

The inner walls are colourless, permanent structures existing in AM fungi of the genera *Acaulospora*, *Entrophospora*, *Intraspora*, *Ambispora*, *Archaeospora*, *Otospora*, *Pacispora*, *Racocetra* and *Scutellospora.* Total number of inner walls may range from 1-3, and the innermost wall is called germinal wall. First inner wall develops usually after spore wall formation is complete. The subsequent inner wall layers arise only after the surrounding inner wall has completed its differentiation (Blaszkowski 2012). The germ tube comes from the pre-germination structures connected with the innermost wall. The pre-germination structure is called germination orb (seen in *Acaulospora* spp,), germination shield (seen in *Scutellospora*, *Racocetra* and *Pacispora* spp,) or germination structure (seen in *Ambispora appendicula*) (Blaszkowski 2012).

2.3.3. Pre-germination structure

2.2.3.1. Germination orb - It is developed by a centrifugally rolled hypha that is hyaline in colour. It is an impermanent structure that decays with time (Blaszkowski 2012).

2.2.3.2. Germination shield - It is developed by a coiled hypha and is normally, elliptical, irregular plate-like, more or less flexible. It may split into 1-30 compartments which include germ tube initials (Blaszkowski 2012).

2.2.3.3. Germinal layer - It is semi-flexible layer from which the germ tube emerge (Blaszkowski 2012).

2.3.4. Sporocarp

Spores develop in a very ordered or loose arrangement around a hyphal plexus (Gerdemann and Trappe 1974). The sporocarps may be surrounded by a loose or compact intermingled hyphal network called peridium.

2.3.5. Subtending hypha

The point of attachment from which the spore arises is the subtending hypha. It can be simple, recurved, constricted or swollen. The shape and width of the hypha can vary within different AM genera and species.

2.4. Life Cycle of AM fungi

Spores are considered to be the main agents of dispersal in AM fungi. During colonization, the AM fungus undergoes several developmental stages. Its life cycle is divided into three main stages: Asymbiotic, Pre-symbiotic and Symbiotic.

2.4.1. Asymbiotic Stage: this stage sometimes is referred to as the resting stage (Fitter and Garbaya 1994), and is host independent (Bago and Bécard 2002). Spore germinates and germ tube expansion occurs for approximately about 2-3 weeks without any association with roots or root exudates under appropriate water and temperature condition. During this stage, fungal growth is dependent only on spore reserves (Garg and Chandal 2010). In the absence of a host plant, the hyphal growth stops after about 2-3 weeks and before the energy reserves are depleted the cytoplasm is retracted within the spore by the formation of septa (Mosse 1988).

2.3.2. Pre-symbiotic Stage: growth of the germ tube changes dramatically including profuse hyphal branching, increased physiological activity and continued hyphal growth in the presence of signal derived from the plant root exudate (Strigolactones) (Parniske 2008). The fungal hyphae in response produces diffusible molecules identified as "Myc factors" that are recognised by the plant roots (Gutjahr and Paszkowski 2013). The sensitivity of "Myc factor" by the host cell activates swift and temporary increase in intracellular calcium ion, modification in cellular structural design and transcriptional reprogramming in the root (Gutjahr and Paszkowski 2013, Bucher *et al.* 2014). Secretion of lipochitooligosaccharides by AM fungi also stimulates development of AM symbiosis. (Herrbach *et al*. 2014). Time varies from one to several weeks for establishment of host root contact by the fungal hyphae (Declerck *et al.* 1998).

2.4.3. Symbiotic Stage: the establishment of contact of fungus and plant root results in radical changes in the fungal morphology, metabolism, marking the initiation of symbiotic phase (Maillet *et al*. 2011, Herrbach *et al.* 2014). Mycorrhizal-induced plant genes play a foremost role in the progress of the symbiotic phase (Koegel *et al.* 2013). AM fungi gives rise to a special type of appressoria called 'hyphopodium' (Bastmeyer *et al.* 2002). Fungal hyphae that emerges from the hyphopodium enters the root *via*., the pre-penetration apparatus which guides the fungal hyphae across the root cells in the direction of the cortex forming the internal hyphae.

2.4.3.1. Arbuscules: inside the cortex, hyphae appear growing along the root axis and later, penetrate into the inner cortical cells. In 'typical' AM relationship, the fungus enters the cell by small hyphal tree-shaped branched structures called arbuscules that are major sites of signal and solute transfer between the symbionts (Harrison 2005). They are formed 1-6 days after colonization and have a very short span of 8-9 days (Alexander *et al*. 1988). They grow till a certain maximum size is reached. When senescence is stimulated, the arbuscular hyphae separate by formation of a septum, after which they collapse and disappear (Javot *et al.* 2007).
AM fungi develop two morphological types of root colonization in the roots, characterized by intra-radical hyphal modification, which were first described by Gallaud (1905). Gerdemann (1965) stated that the morphology of AM fungi was influenced both by the host and the fungal

species. It is dependent on presence of intercellular spaces within the root cortex of the host plant which resulted in *Arum*-type, and in its absence formed *Paris*-type of arbuscules (Brundrett and Kendrick 1988, 1990). The *Paris*-type AM fungus expands from cell to cell and forms intra-cellular hyphal coils within host cells (Smith and Smith 1997). This type of colonization is a characteristic feature of plants growing in low nutrient and high-stress environment that show an arbuscule like branching (Brundrett and Kendrick 1990). The intra-radical hyphae of *Arum*-type spreads inter-cellularly between the root cortical cells penetrating cells to form tree-like structures (Smith and Smith 1997) and they are normally associated with fast growing plant species (Brundrett and Kendrick 1990). However roots of plants containing features of both morphological types are grouped as intermediates (Weber *et al.* 1995).

2.4.3.2. Vesicles: several fungi develop vesicles, which perform the function of lipid storage in the root (Bücking *et al*. 2012) and intra-radical spores (Dodd *et al.* 2000). They are produced by intercalary or terminal protuberance of AM fungal hyphae in inter-or intra-cellular zone of the root cortex (Javaid 2009). Vesicles are formed by members of Glomeraceae and Acaulosporaceae. But members of Gigasporaceae produce auxillary cells on the extra-radical mycelium instead of vesicles (Oehl *et al.* 2011).

2.4.3.3. AM fungal mycelium: the inter- and intra-cellular hyphae in roots contain storage materials and take part in transportation of the substances absorbed by extra-radical hyphae from the soil to arbuscules or directly to root cells of the host plant (Bieleski 1973). Intra-radical hyphae may be straight or with H or Y shaped branches, and may also form coils, whose frequency of occurrence depends on the location in the roots and the generic affiliation of the AM fungal species (Morton 2000).

The extra-radical mycelium associated with the root radiates into the soil. The AM hyphae are of two different types *viz*., runner and absorbing hyphae (Friese and Allen 1991). The runner hyphae are thicker and grow in the soil to find host roots. The hyphae that pierce the roots are initiated from the runner hyphae. The absorbing hyphae grow from the runner hyphae and form a network of thinner hyphae spreading out into the soil that absorb the nutrients and

transport to the host cell. These extra-radical hyphae significantly improve the absorptive area of roots (Bieleski 1973), create hyphal bridges transporting nutrients between co-occuring plants (Newman 1988) and bind sand grains into aggregates (Koske and Polson 1984). In certain fungi like *Gigaspora* and *Scutellospora* species, clusters of thin-walled cells developed on extra-radical hyphae are called auxillary cells. They possess a spiny surface in *Gigaspora* species (Bentivenga and Morton 1995) and knob or smooth shaped in *Scutellospora* (Morton 1995). The function of auxillary cells is to permit partitioning of nutrients and nuclei before sporulation (Morton and Benny 1990).

2.4.3.4: Spores: when a threshold level of colonization is achieved, most AM fungal species start to sporulate (Pearson and Schweiger 1993). Terminal swelling on the tip of sporogenous extra-radical hyphae produce spores, they are multinucleated single celled structures in the soil or in the roots (Koske 1985). Spores might be held singly or in groups called as sporocarps (Rodrigues and Muthukumar 2009). The quantity of spores generated depends on the AM species, competitiveness among AM fungal species, plant species, host phenology and soil fertility (Hayman 1970, Giovannetti 1985, Hetrick and Bloom 1986, Gemma *et al.* 1989, Blaszkowski 1993).

2.5. Soil

Soil is made up of minerals, soil organic matter, water and air. The composition and proportion of these components greatly influence soil physical properties which in turn affects air and water movement in the soil and thus its ability to function. Soil structure is the arrangement and binding together of soil particles into larger clusters called aggregates. Aggregation is important for increasing stability against erosion, for maintaining porosity and carbon sequestration in the soil (Nichols *et al.* 2004). Soil organic carbon (C) and nitrogen (N) are the main nutrients used for vegetation growth and are also used as indices of soil quality assessment and sustainable land use management (Liu *et al.* 2011). They reflect the soil fertility level and the ratio of C and N is an indicator of soil quality and is used for assessing C and N nutrition cycling of soil (Zhang *et al.* 2011).

2.5.1. Effect of soil parameters on AM fungi

Distribution of AM fungi has been related to soil pH, soil P level, salinity (Abbott and Robson 1991), vegetation (Johnson *et al.* 1992), hydrological condition of the soil (Escudero and Mendoza 2005) and soil gases (Khan and Belik 1994). Survival of AM fungi that colonise wetland plants is due to preferential production of vesicles which are resistant structures than arbuscules that are nutrient transfer structures (Khan 1995, Mendoza *et al.* 2005). It has been demonstrated that soil pH acts as a driver for AM fungal community not only at the local-scale level but also at the landscape level (Kidd and Proctor 2001). AM fungal diversity decreases with increasing soil acidity (Kohout *et al.* 2015). High P level in the soil not only can reduce AM spore germination and hyphal growth from the germinated spores (Miranda and Harris 1994a) but also reduce early root colonization and growth of extra-radical mycelium (Miranda and Harris 1994b).

2.6. Role of AM fungi in field crops

Agricultural practices have been reported to influence AM fungal population (Larsen *et al.* 2007). An experiment on the effect of mono- and mixed-cropping showed that mixed-cropping stimulated proliferation of AM fungi unlike mono-cropping. The result of higher propagule density under mixed cropping may be due to more intensively rooted soil in the mixed system (Schenck and Kinloch 1980). Leaving the land fallow brings down the population of AM fungi in the soil (Thompson 1987). Agricultural intensification declines AM fungal abundance and effectiveness with respect to good colonization and plant growth promotion (Bagyaraj 2014). AM inoculation is generally accepted as a substitute for chemical fertilizers and its beneficial effect on many field crops has been proved. In wheat, AM colonization was higher in well watered and well drained plants compared to stressed plants. Higher wheat plant biomass, grain yield with higher shoot P and Fe concentration were recorded with AM inoculation (Al-Karaki 1998, 2004). Aerobic rice genotypes inoculated with AM fungi showed higher root colonization (Gao *et al.* 2007). Several studies have demonstrated that agricultural management practices, such as cultivation intensity, fertilizer application and water management can severely affect AM fungal communities (Lumini *et al.* 2011, Lin *et al.* 2012). However, according to Wang *et al.* (2015), management differences alone cannot explain different results, difference in cultivars and climate may also contribute

to the inconsistency of the results. Gosling *et al.* (2013) found that different AM fungal communities in agricultural fields colonized the same host plants, depending on P concentration in the soil. According to Alguacil *et al.* (2016), pH, Zn, and Mn significantly influence the AM fungal community.

2.6.1. AM fungal colonization in rice

Rice is one of the important crops grown extensively in many countries. Generally rice is grown under shallow, flooded or wet condition. It is also cultivated where floodwater may be several meters deep.

Rice plants readily form AM association under upland conditions but under lowland condition colonization is rare due to anoxic environment (Ilag *et al.* 1987). However a positive response has been observed in lowlands rice (Sharma *et al.* 1988). Watanarojanaporn *et al.* (2013) reported that AM fungi could colonize rice roots under waterlogged conditions and there was an increase in colonization rate over the sampling times. The survival of AM fungi under anaerobic condition was due to the oxygen that is supplied from the atmosphere through the host aerenchymatous tissue. Only a few field studies documenting AM fungal colonization have been conducted in paddy fields (Lumini *et al.* 2011). Barea (1991) reported that *Glomus etunicatum* recorded fairly high colonization in rice roots and best survival under submerged conditions.

2.6.1.1. In lowland

Wang *et al.* (2015) reported that AM colonization is highly depended on the growth stages of the host. They also reported that AM fungal structures were rare at the seedling and tillering stage but were significantly higher at heading and ripening stage. This has been related to the development of aerenchyma in host plant in combination with higher mineral nutrient requirement at heading and ripening stage (Watanarojanaporn *et al.* 2013). The relatively high number of AM fungal phylotypes indicates that AM fungal diversity is not necessarily low in paddy fields and it could be due to presence of aerenchyma as well as the high ecological adaptability of some AM species.

2.6.1.2. In different ecologies

Toppo *et al*. (2012), investigated the native AM fungal diversity in rice-based cropping systems under rainfed ecology in unbunded uplands, bunded uplands and medium to low midlands. They reported increase in spore population in post monsoon season irrespective of cropping system and land situation. This increase in spore population coincides with the harvesting period and may be ascribed to fungal resource mobilization from senescing roots (Muthukumar *et al.* 2003). The Genus *Glomus* was predominant in uplands and *Gigaspora* in the midlands. The study also reports dominance of a few genera in post monsoon over shared dominance of many genera in monsoons and recommends the development of *Glomus* based inocula for uplands and *Gigaspora* based inocula for midlands (Toppo *et al*. 2012).

2.7. Mass production of native AM inocula

Advantages of native AM fungi inoculum for increased AM fungal activities in term of growth promotion of various crops are well documented (Mohammad *et al*. 2004, Douds *et al.* 2005). AM fungi are to be maintained and mass produced in pot cultures on suitable host plant (Ganesan and Veeralakshmi 2006). The host plant selected should be suitable to agro climate conditions of the area, having thick root system for sizeable sporulation and colonization, annual in growth habit and adaptable to polyhouse conditions. The host plants may stimulate selectively or limit sporulation of certain AM fungal species suggesting varied affinities between host and symbionts (Chellappan *et al.* 2005). Quantitative and qualitative population of AM fungi depends on several factors which include cultivation practises used for plant growth, environmental conditions, type of substrate and host plant. One of the most important considerations in inoculum production is the choice of fungal isolates capable of growth promotion of target host plant (Bhowmik *et al.* 2015). Selection of suitable substrate for mass production of AM fungi is also important (Kumar and Saxena 2017).

2.8. Role of AM fungi in rice improvement

Oladele and Awodun (2014) conducted field trials using *Glomus intradices* as a biofertilizer in the cultivation of rice in the lowlands of tropical rainforest agro-ecology of south-west Nigeria. The use of AM fungi did show a significant increase in vegetative and reproductive growth. Gupta and Ali (1993) reported, that inoculation of AM fungi in both high- and low-

fertility soil could promote the nutrient acquisition of rice and increase rice yield under flooded condition. According to Hajiboland *et al.* (2009), application of insoluble P to rice plants inoculated with *G. mosseae* or *G. intraradices,* recorded increased P uptake and root colonization under flooded condition.

2.9. AM fungal dependency in rice

The term 'Mycorrhizal Dependency' was put forth by Gerdemann (1975) to stress upon variation in plant response to AM formation against the main ecological variability and soil fertility. It is observed that there is variation in response to host genotype along with fungal interaction (Hong 2012).

Mycorrhizal responsiveness or level of dependence is known to vary according to rice cultivars. Slow growing varieties having high root growth compared to shoot with low nutrient demand or high supply efficiency showed negative response to AM inoculation at early stage of plant growth. On the other hand some varieties having higher shoot growth compared to root growth with high nutrient demand but low supply efficiency showed high level of responsiveness to AM at early stage of plant growth (Saha and Mondal 2016).

2.10. Role of planting density on rice

As density of plants increases the degree to which plants responds to AM colonization decreases (Koide and Dickie 2002). Crop plants depend on temperature, solar radiation, moisture and soil fertility for growth and nutritional requirement. A thick population crop may have limitations in terms of maximum availability of these factors (Baloch *et al.* 2002). Islam *et al.* (2013) reported that rice cultivated at the density of 20 cm x 10 cm with one seedling per hill produced the highest grain yield.

2.11. Effect of parboiling on rice

Rice is consumed either as parboiled or raw rice. Parboiling is a hydrothermal treatment given to rice with its husk-paddy, it changes the nutritional composition of rice and improves its quality (Chukwu 1999). It results in gelatinization of starch hence reducing the stickiness in cooked parboiled rice, as well as improves the shelf life by slowing the process of rancidity of rice grain (FAO 1990).

CHAPTER 3

Isolation and identification AM fungal spores from rhizosphere soils of different varieties of *Oryza sativa* L. from *Khazans* of Goa.

3.1. Introduction

Arbuscular mycorrhiza is a mutualistic relationship between fungi and plant roots. In this association the fungus receives photosynthetically derived C compounds from the green plants and plants have an increased access to mineral nutrients especially P (Rivera *et al.* 2005) and other minerals like K, Fe, Cu, Ca, Mg and Zn (Yaseen *et al.* 2011). The association also helps to improve the tolerance of the host plant towards biotic (Singh *et al*, 2000) and abiotic stress (Gaur and Adholeya 2004).

Rice fields in Goa are named differently, depending on soil, rainfall conditions and nearness to the riverside. They have been distinguished into *Morod* (Upland), *Ker* (Midland) and *Khazan* (Lowland). *Khazans* is the konkani term for the coastal saline lowland soils. They are integrated agro-aqua ecosystems which are traditionally managed. They have been reclaimed over centuries from marshy mangrove swamps with an intricate system of bunds and sluice gates. The gates protect the fields from inundation and control the water flow in and out of the rivulets. In Goa these lowlands were originally used for paddy cultivation, traditional farming, pisciculture and salt extraction. Paddy fields have been cultivated by using *bunds* to keep the sea water away and sluice gates to control the inflow of saline water.

Agricultural lands are artificial ecosystems and are subjected to human intervention. Nature's diversity, due to agriculture, is replaced with a small number of cultivated plants. With the change of natural ecosystem to agro-ecosystem and increase in the intensity of agricultural inputs there is a decrease in AM fungal diversity (Jefwa *et al.* 2012). Rice is grown in different ecosystem, when cultivated in the uplands readily forming AM association (Ilag *et al.* 1987). Barea (1991) has reported that AM fungi can survive in water logged condition. Wetland rice was previously considered to be non mycorrhizal but a positive response to AM fungal

inoculation has been observed (Sharma *et al.* 1988). AM fungi are important in organic and sustainable farming system that relies on biological process rather than agrochemicals (Harrier and Watson 2004), thus offering a great potential for sustainable agriculture system (Khalil *et al.* 1992). A better understanding of the field study, based on AM fungal diversity associated with agronomic crops is necessary. Hence, an effort was made to study the AM fungal association in the different varieties of rice cultivated in different *Khazan* lands of Goa.

3.2. Materials and Methods

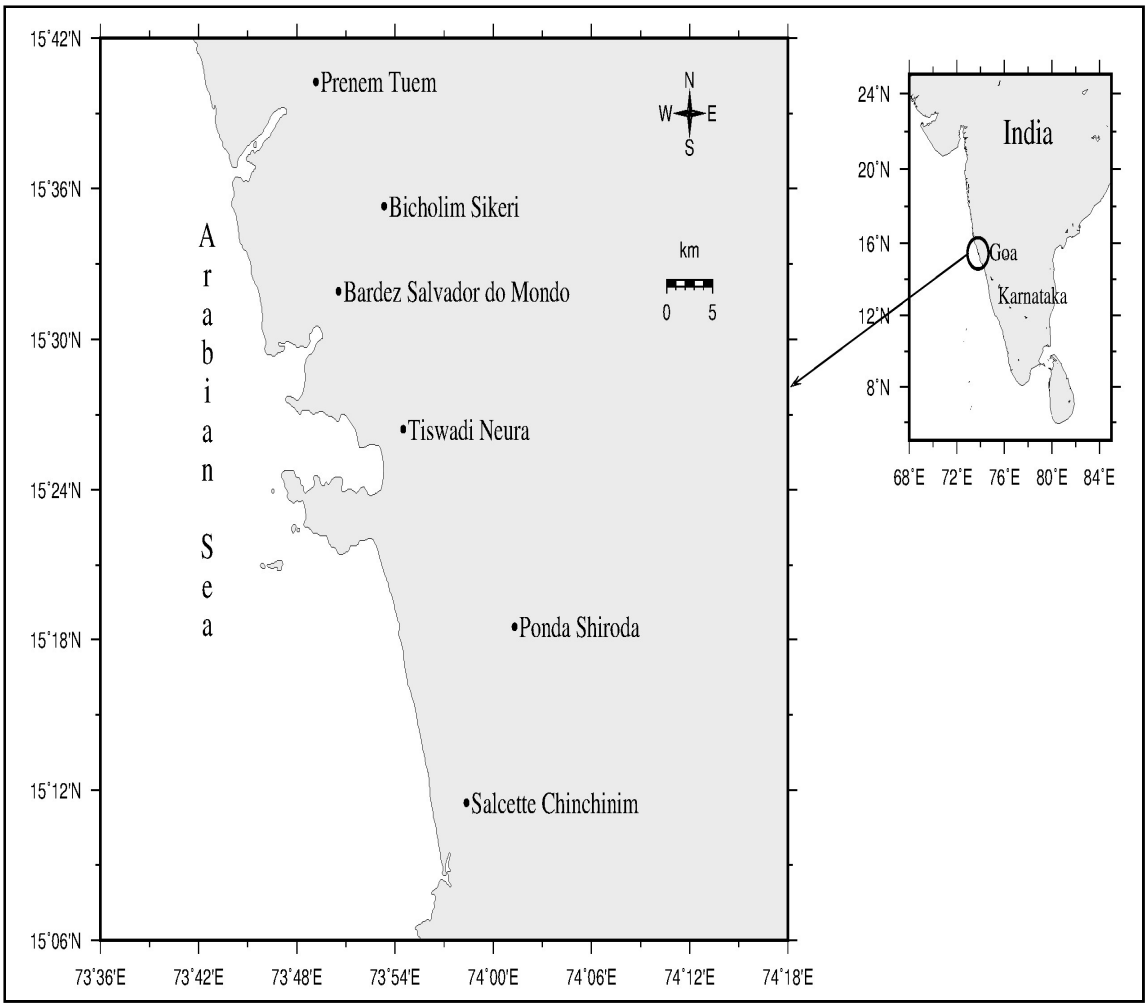
3.2.1. Collection of rhizosphere soil samples

Field visits were conducted during the flowering stage in rice dominated *Khazan* areas of six different talukas in Goa in the year 2015 **(Plate II)**. The mean maximum and minimum temperatures recorded during that period were 32.11ºC and 23.40ºC, respectively with relative humidity ranging from 46 to 95.68%, the seasonal total rainfall being 2595.1 mm as obtained from the Meteorological Department of ICAR (Central Coastal Agricultural Research Institute), Goa. Three healthy plants of each of the 11 varieties *viz.*, Jyoti, Jaya, Assgo, Bello, Damgo, Kalo korgut, Kalo novan, Khonchri, Korgut, Muno and Shiedi **(Plate III)** were collected randomly from different parts of the *Khazan* lands at each site **(Table 2)**. Rice roots along with rhizosphere soil were collected during sampling from August 2015 to September 2015. Samples were collected within 0-25cm depth. The roots were then separated from soil, mixed thoroughly to obtain a composite sample of approximately 500g of soil and brought to the laboratory for further analyses**.** Roots of each variety from each study site were divided into two subsamples, with one subsample used for estimation of AM root colonization and the other for establishing the trap cultures.

3.2.2. Root processing

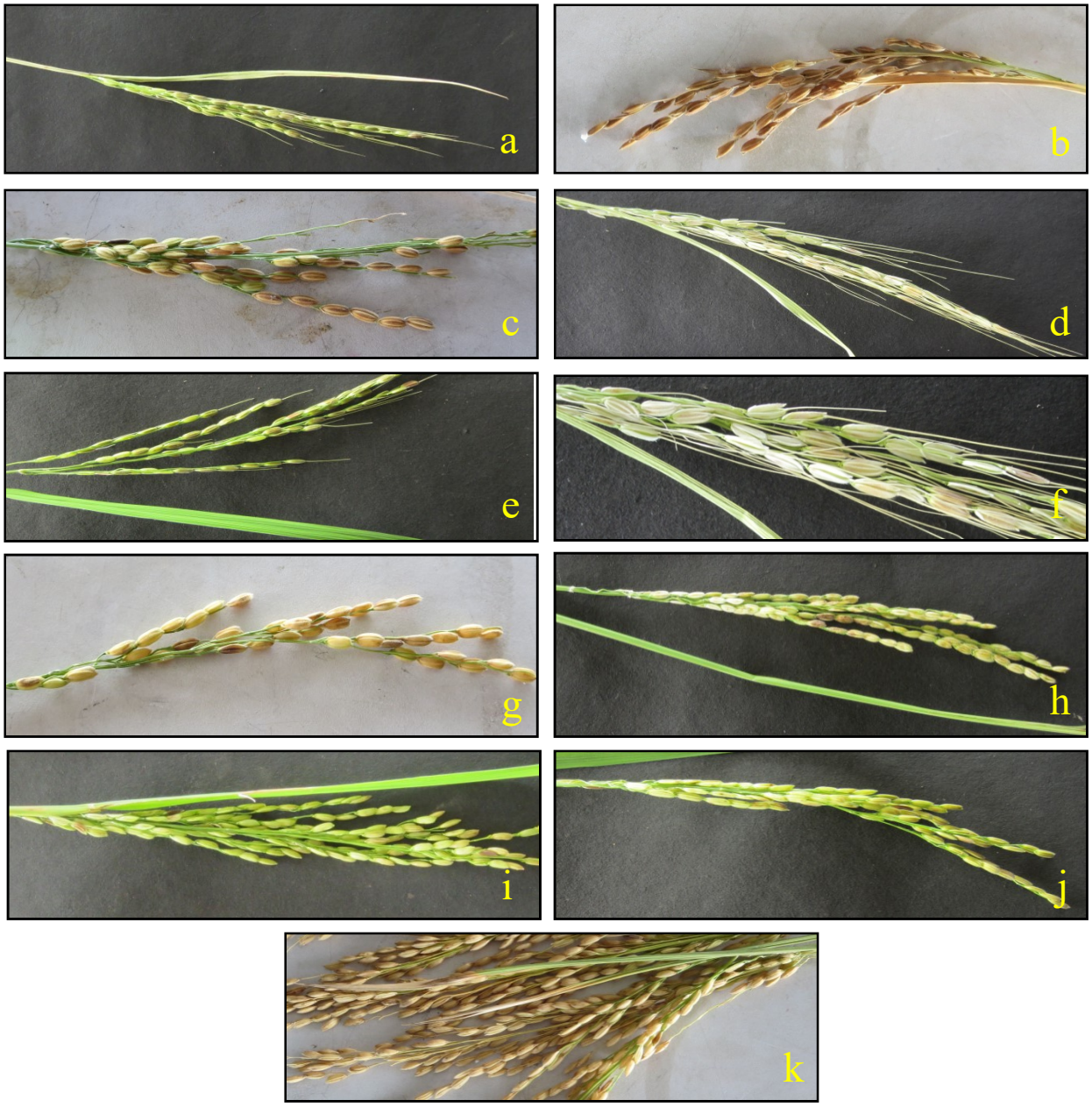
The root samples were processed for AM fungal colonization using Phillips and Hayman (1970) method. Three samples were considered for each variety per site. Roots were cleared in 10% KOH heated at 90ºC, acidified in 5N HCl and stained with Trypan blue in lactoglycerol. The stained roots were examined using an Olympus research compound microscope (20x, 40x,

Plate II



Map of Goa showing study sites.

Plate III



***Oryza sativa* L. varieties selected for study.**

a. Assgo; b. Bello; c. Damgo; d. Kalo korgut; e. Korgut; f. Kalo novan; g. Khonchri; h. Sheidi; i. Muno; j. Jyoti; k. Jaya

100x) for AM fungal structures. Microphotographs were imaged by Olympus DP12-2 and Nikon Digital Sight DS-U3 digital cameras.

Table 2: Geographical location and rice varieties cultivated at the study sites.

Taluka	Site	Rice Variety	Geographical Location		
			Latitude	Longitude	Altitude (above msl)
Pernem	Tuem	Jyoti, Jaya, Shiedi,Korgut	15° 40' 15'' N	73° 49' 10''E	3 m
Bicholim	Sikeri	Jyoti, Khonchri, Shiedi, Muno, Kalo Novan, Kalo Korgut, Bello, Damgo, Assgo	15° 35' 18'' N	73° 53' 20''E	7 m
Salcete	Chinchinim	Jyoti,Jaya, Korgut	15° 11' 29'' N	73° 58' 22''E	10 m
Ponda	Shiroda	Jyoti, Jaya, Assgo	15° 18' 31'' N	74° 01' 18''E	11 m
Tiswadi	Neura	Jyoti	15° 26' 25'' N	73° 54' 30''E	12 m
Bardez	Salvador do Mondo	Korgut	15° 31' 55'' N	73° 50' 33''E	20 m

3.2.3. Estimation of percent AM fungal root colonization

Hundred root segments of each rice variety cultivated at each sample site were analysed for percent root colonization by using the Root Slide method (Read *et al.* 1976). Trypan blue stained root fragments were mounted in polyvinyl-lacto-glycerol (PVLG). The sighting of a hypha, vesicle or arbuscule concluded that the segment was mycorrhizal.

Total root colonization was estimated using the formula,

Root colonization (%) = (Number of root segments colonized/Total number of root segments observed) x 100.

3.2.4. Establishment of trap culture

Increase in AM propagules was carried out by using trap cultures where cuttings of *Plectranthus scutellarioides* (L) Codd. was used as host. Plants were maintained in the Green house and watered adequately whenever required. Hoagland's solution minus P was provided to the plants fortnightly. Three pot cultures are maintained per rice variety and site with five cuttings maintained per pot.

A trap culture of the sample was prepared by mixing the soil sample with sterilized soil (1:1) (w:w). The roots of host species were checked for AM colonization after 45 days. Pots showing successful AM colonization were continued to be maintained for a period of 3 months, subsequently watering was lessened. At the end of three months the plants were harvested at the base and allowed to dry. The soil was then checked for the occurrence of spore.

3.2.5. Isolation of AM spores

Using wet sieving and decanting method (Gerdemann and Nicolson 1963) AM spores from the rhizosphere soil were isolated. Rhizosphere soil sample (100g) was suspended in a beaker with 1000mL of tap water. The soil suspension was stirred using a glass rod and then the sediments were permitted to settle down for 10-15 seconds. The aliquot was poured through sieves set in the descending order (250-37μm). The procedure was repeated three times for each sample. The sievates from each sieve were washed into separate beakers. Each of the collected aliquot was then sieved separately using Whatman No. 1 filter paper which was later placed in Petri plates. The filter paper was examined for the presence of spores and sporocarps under Olympus stereo microscope SZ2-ILST (10 x 4.5 zoom).

3.2.6. Estimation of AM spore density

Estimation of AM fungal spore density was carried out following the method of Gaur and Adholeya (1994). Whatman No. 1 filter paper was folded into two equal halves followed by a second fold resulting into four equal quadrats. The filter paper was opened and two perpendicular lines were drawn along the folds marking the four quadrats. Verticals lines were drawn on one half of the filter paper, dividing it into ten columns with each column about 0.5cm apart. Columns were numbered and the direction of counting was marked with arrows. The filter paper was folded in such a way that the marked portion received the aliquot during filtration and the other portion remained free of spores. The filter paper was then placed in Petri plates and observed under the stereo microscope. The spores occupying the space between the numbered columns were counted. Unbroken spores were selected and picked using a needle and mounted in polyvinyl alcohol lacto-glycerol (PVLG) for identification. Spore density was expressed as the total number of spores recorded in 100g of soil sample.

3.2.7. Taxonomic identification of AM fungi

To identify AM morphotypes, intact and unparasitized spores mounted in PVLG were used. Spores were identified to species level by the features of the spore wall *viz.*, colour, dimension, ornamentation pattern and number of wall layers. For identification of AM fungal spores using spore morphology, references were made to original species protologues described by Schenck and Perez (1990), Almeida and Schenck (1990), Rodrigues and Muthukumar (2009), Schüβler and Walker (2010), online species descriptions provided by INVAM (International Collection of Vesicular Arbuscular Mycorrhizal Fungi at the West Virginia University, USA (http://invam.caf.wuv.edu), Blaszkowski's Manual (2012) and Redecker *et al.* (2013).

3.2.8. Diversity Studies

3.2.8.1. AM species richness

Species richness (SR) is the number of AM fungal species recovered from each site per 100 grams of soil sample.

3.2.8.2. Diversity Index

Diversity Index studies were carried out for each site individually by calculating Simpson's (D) and Shannon diversity index (H) using the Multivariate Statistical Package (MVSP) program version 3.1.

3.2.8.3. Relative abundance (RA %)

Species wise relative abundance (%) for each area was calculated by using the following formula (Beena *et al.* 2000).

Relative Abundance (%) = Number of spores of particular AM species/Total spore number of all the AM species x 100.

3.2.9. Statistical Analysis

3.2.9.1. Data of AM fungal colonization and spore density for each variety of rice at each site was analysed using analysis of variance (ANOVA) IBM SPSS Statistics 20 <0.05 significance

level. Means of AM parameters of different rice varieties at each site were separated using Tukey.

3.3. Results

3.3.1: AM root colonization

All 11 rice varieties cultivated in at six different *Khazan* sites showed AM colonization (**Table 3**). Percent AM root colonization in different rice varieties varied from site to site. Rate of colonization varied between the different varieties cultivated at Tuem ($F_{(2,8)}$ = 134.43), Sikeri ($F_{(7,16)}$ = 1027.35), Chinchinim ($F_{(2,6)}$ = 55.51) and Shiroda ($F_{(2,6)}$ = 344.15) at 5% ($P < 0.05$). In Tuem, maximum root colonization was observed in variety Jyoti (88%) and minimum in variety Korgut (67%). In Sikeri, maximum root colonization was observed in variety Jyoti (98%) and minimum in variety Kalo novan (24%). In Chinchinim, maximum root colonization was observed in Jyoti (42%) and minimum in variety Korgut (18%). In Shiroda, maximum root colonization was observed in variety Jyoti (76%) and minimum in variety Jaya (27%). In Neura and Salvador do Mondo, AM root colonization was 49% and 28% respectively. Overall, maximum root colonization was observed in variety Jyoti (98%) at Sikeri and minimum was in variety Korgut (18%) at Chinchinim. The average AM fungal colonization in the different rice varieties was 45.56%. The maximum average root colonization **(Fig. 1)** was observed in Tuem (78.02%) and the minimum was observed in Salvador do Mondo (28%). In the present study, hyphal and vesicular colonization was dominant **(Plate IV)**.

3.3.2. AM Spore density

The rhizosphere soils of each variety of rice showed significant variation in AM spore number at 5% ($p< 0.05$) in Tuem ($F_{(3,8)}$ = 278.64), Sikeri ($F_{(7,16)}$ = 55.67), Chinchinim ($F_{(2,6)}$ = 95.08) and Shiroda ($F_{(2,6)}$ = 65.03) **(Table 4).** In Tuem, the maximum spore density was observed in variety Jaya (39 spores$100g^{-1}$ soil) and minimum in variety Jyoti (15 spores $100g^{-1}$ soil). In

Table 3: AM root colonization of different rice varieties cultivated in *Khazan* lands.

Variety	Study sites					
	Tuem	**Sikeri**	**Chinchinim**	**Shiroda**	**Neura**	**Salvador do Mondo**
Assgo	nd	$79.33^{b} \pm 0.33$	nd	$31.70^{a} \pm 0.89$	nd	nd
Bello	nd	$28.33^{c} \pm 0.88$	nd	nd	nd	nd
Damgo	nd	$26.00^{c} \pm 1.15$	nd	nd	nd	nd
Kalo korgut	nd	$52.00^{d} \pm 1.00$	nd	nd	nd	nd
Korgut	$66.53^{c} \pm 0.86$	nd	$18.00^{a} \pm 1.15$	nd	nd	nd
Kalo novan	nd	$24.00^{c} \pm 0.57$	nd	nd	nd	nd
Khonchri	nd	$27.33^{c} \pm 1.20$	nd	nd	nd	nd
Shiedi	$76.23^{d} \pm 0.78$	nd	nd	nd	nd	nd
Muno	nd	$28.00^{c} \pm 0.57$	nd	nd	nd	nd
Jyoti	$88.33^{b} \pm 0.80$	$98.33^{a} \pm 0.33$	$42.86^{b} \pm 1.95$	$76.33^{b} \pm 1.85$	$48.66^{a} \pm 0.88$	$28.00^{a} \pm 1.15$
Jaya	$81.00^{a} \pm 0.57$	nd	$23.33^{a} \pm 2.02$	$27.68^{a} \pm 1.45$	nd	nd
Mean	$78.02_{a} \pm 2.40$	$45.41_{b} \pm 5.59$	$28.06_{b} \pm 3.88$	$45.23_{b} \pm 7.83$	$48.66_{ab} \pm 0.88$	$28.00_{b} \pm 1.15$

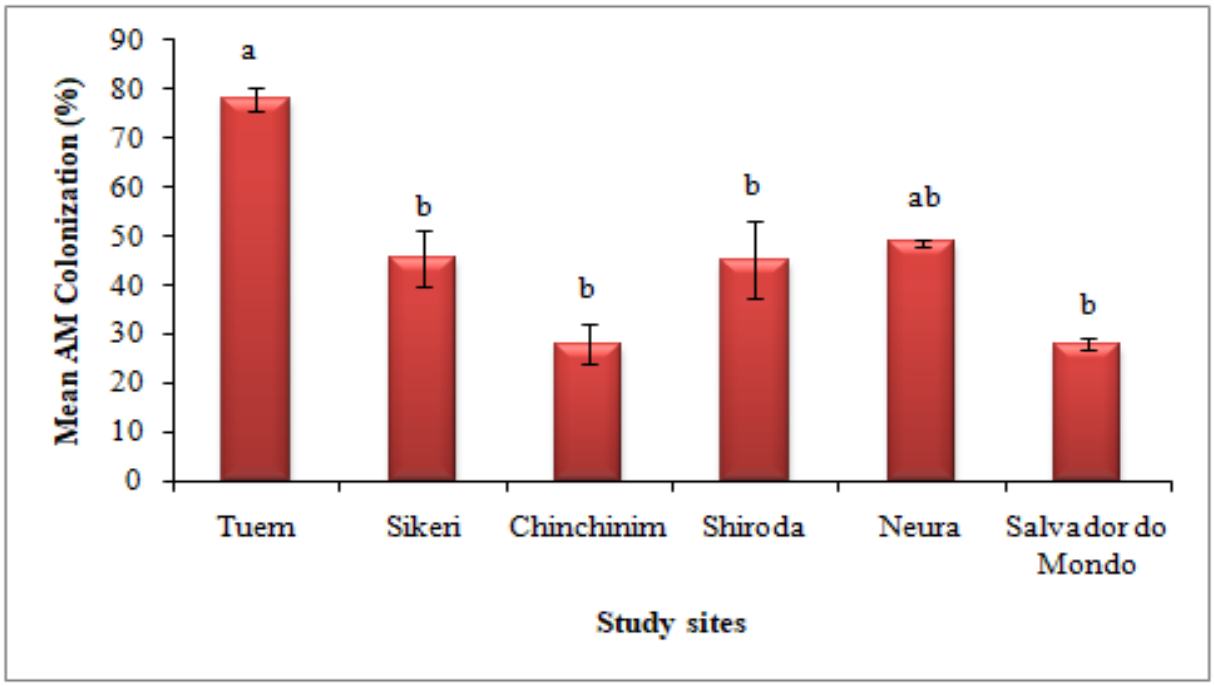
Legend: nd= Rice variety not found growing in the study site; Data presented is mean of three readings; ± indicates Standard Error; Values in a column depicted by the same letter as superscript are not significantly different between the varieties at a site, values in a row depicted by the same letter as a subscript are not significantly different the sites of study where $F_{(5,54)} = 7.24$*, $p < 0.05$. Average AM Colonization (%) = 45.56 ± 7.47.

Table 4: AM fungal species and spore density in the study sites.

Variety	Study sites					
	Tuem	**Sikeri**	**Chinchinim**	**Shiroda**	**Neura**	**Salvador do Mondo**
Assgo	nd	*A. di., A. so., A. la., F. mo.* $38.33^{b} \pm 1.66$	nd	*A. di., R. fa.* $52.33^{a} \pm 1.20$	nd	nd
Bello	nd	*A. di., A. la.* $16.67^{c} \pm 1.20$	nd	nd	nd	nd
Damgo	nd	*R. fa.* $31.33^{c} \pm 0.66$	nd	nd	nd	nd
Kalo korgut	nd	*F. mo., A. tu., T. ne.* $25.00^{df} \pm 2.88$	nd	nd	nd	nd
Korgut	*A. sc., A. de.* $20.33^{c} \pm 0.66$	nd	*A. de., A.la.* $36.67^{b} \pm 0.66$	nd	nd	nd
Kalo novan	nd	*E. in., R. fa.* $26.00^{de} \pm 1.15$	nd	nd	nd	nd
Khonchri	nd	*Ar. my., R. fa.* $35.00^{b} \pm 1.15$	nd	nd	nd	nd
Shiedi	*A. de., A. di., A. la.* $24.67^{d} \pm 0.66$	nd	nd	nd	nd	nd
Muno	nd	*G. fa.* $25.33^{d} \pm 0.33$	nd	nd	nd	nd
Jyoti	*A. sc., A. de., A. bi., T. ne.* $15.33^{b} \pm 0.33$	*R. fa., F. mo., T. ne., G. mi.* $5.33^{a} \pm 0.33$	*A. de., A. la.* $26.33^{a} \pm 0.33$	*A. di., F. mo.* $27.00^{b} \pm 1.15$	*A. bi., F. mo.* $25.33^{a} \pm 1.45$	*F. mo., A. di., A. de.* $17.66^{a} \pm 1.45$
Jaya	*A. de. , A. di., A. sc.* $38.67^{a} \pm 0.66$	nd	*A. de., G. ag.* $24.67^{a} \pm 0.88$	*A. di., F. mo., R. fa.* $48.33^{a} \pm 2.40$	nd	nd
Mean	$24.75_{a} \pm 2.63$	$25.37_{a} \pm 2.09$	$29.22_{ab} \pm 1.90$	$42.56_{b} \pm 4.02$	$25.33_{ab} \pm 1.45$	$17.66_{a} \pm 1.45$

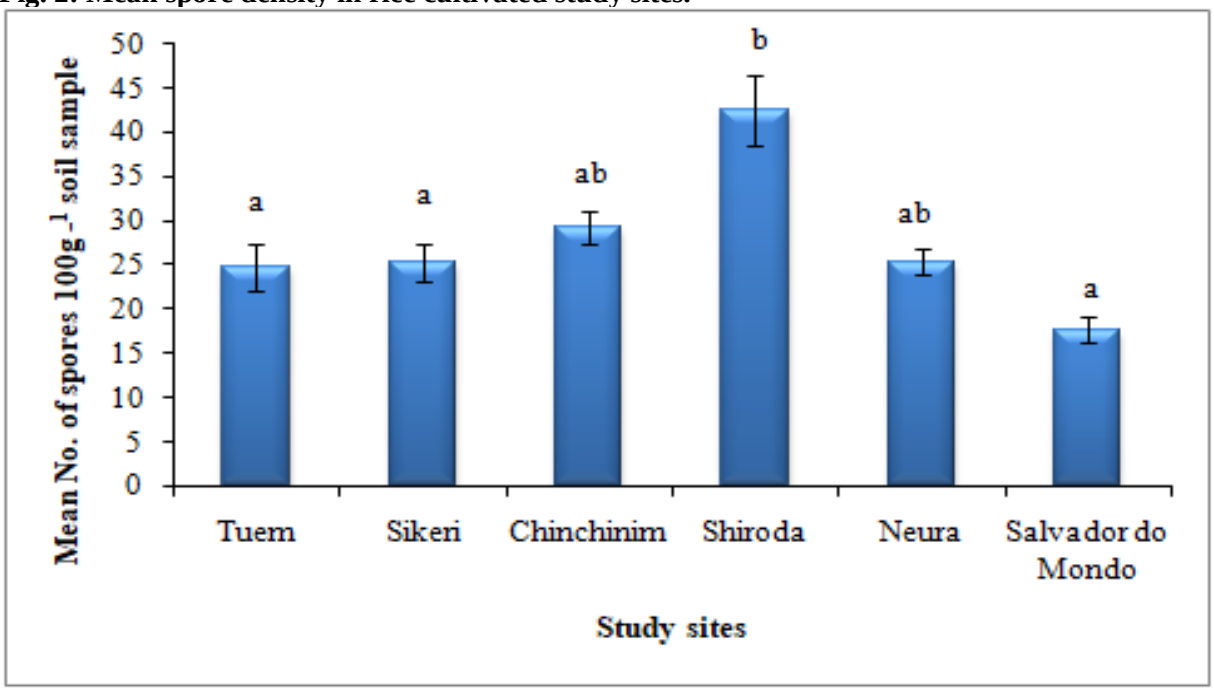
Legend: nd = Rice variety not found growing in the study site; Data presented is mean of three readings; ± indicates Standard Error; Values in a column depicted by the same letter as superscript are not significantly different between the varieties at a site, values in a row depicted by the same letter as a subscript are not significantly different. AM species: *A. bireticulata = A. bi., A. delicata = A. de., A. dilatata =A. di., A. laevis = A. la., A. scrobiculata = A.sc., A. soloidea = A. so., A. tuberculata = A. tu., Archaeospora myriocarpa = Ar. my.,Entrophospora .infrequens = E. in., Tricispora. nevadensis = T. ne., F. mosseae = F. mo., G. aggregatum = G. ag., G. microcarpum = G. mi , Rhizoglomus fasciculatum = R. fa.* Average spore density = 27.48 ± 3.38 spores 100g-1 soil.

Fig. 1: Mean AM root Colonization in rice cultivated at different study sites.



Legend: Error bars represent Standard Error; Values on the column depicted by the same letter are not significantly different between the sites.

Fig. 2: Mean spore density in rice cultivated study sites.



Legend: Error bars represent Standard Error; Values on the column depicted by the same letter are not significantly different between the sites.

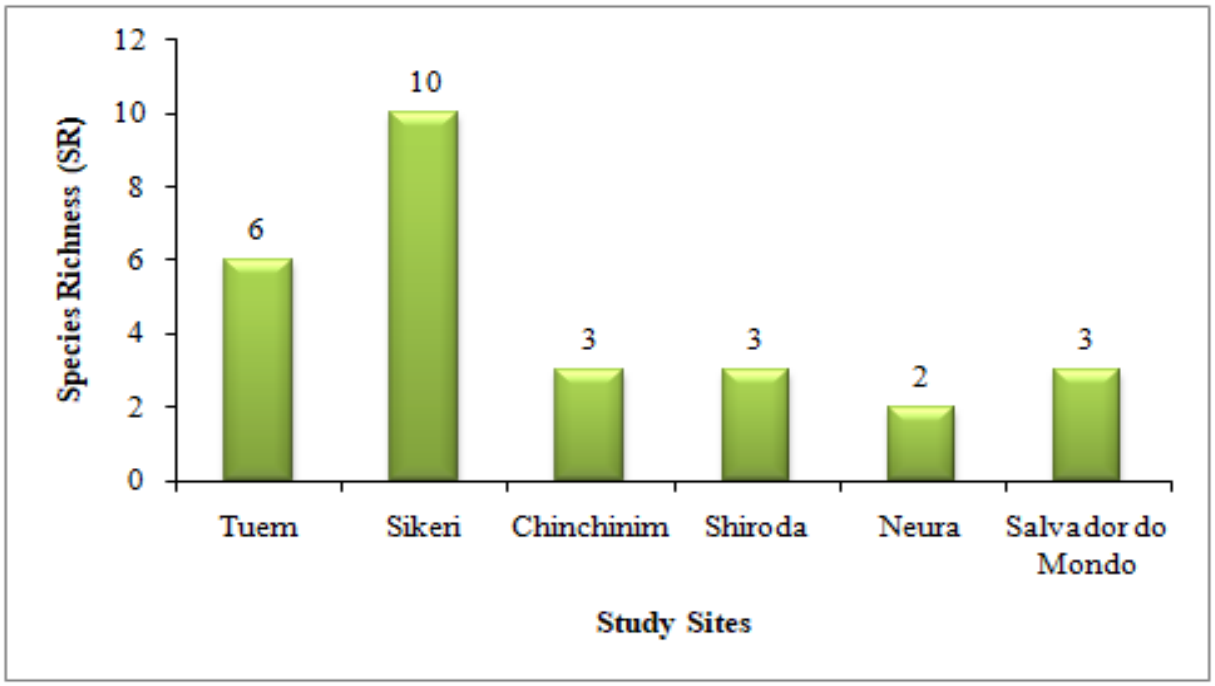
Sikeri, the maximum spore density was observed in variety Assgo (38 spores $100g^{-1}$ soil) and minimum in variety Jyoti (5 spores $100g^{-1}$ soil). In Chinchinim, the maximum spore density was observed in variety Korgut (36 spores $100g^{-1}$ soil) and minimum in variety Jaya (24 spores $100g^{-1}$ soil). In Shiroda, the maximum spore density was observed in variety Assgo (52 spores $100g^{-1}$ soil) and minimum in variety Jyoti (27 spores $100g^{-1}$ soil). In Neura and Salvador do Mondo spore density was 25 and 18 spores $100g^{-1}$ soil, respectively The highest spore number was recorded in the variety Assgo (52 spores $100g^{-1}$ soil) from Shiroda and least in variety Jyoti (5 spores $100g^{-1}$ soil) at Sikeri. The average number of spores in all the study sites was 27.48 spores $100g^{-1}$sample. The maximum average number of spores $100g^{-1}$soil sample was observed in Shiroda (42 spores $100g^{-1}$ soil) and minimum in Salvador do Mondo (18 spores $100g^{-1}$ soil) (**Fig. 2**).

3.3.3. AM spore diversity

3.3.3.1. AM species richness

In the present study, the rhizosphere soils of different varieties of rice cultivated in the *Khazans* showed variation in AM fungal diversity. In Tuem, maximum number of AM fungal species was recorded in variety Jyoti (4) and the least in variety Korgut (2), while in Sikeri, the highest number of AM species was recorded in variety Jyoti and Assgo (4 each) and the least in variety Damgo and Muno (1 each) with the spore number given in parenthesis. Two AM fungal species were recorded in the three varieties cultivated in Chinchinim. In Shiroda, maximum AM species were observed in variety Jaya (3) and in Neura (2) and Salvador do Mondo (3) with species number given in parenthesis. In all 14 AM fungal species were recorded from six study sites **(Table 4).** *Acaulospora* was the dominant genus, represented by seven species **(Plate VII a, c, d)**. The other genera recorded were *Glomus* (2) **(Plate VIII d)**, *Entrophospora* (1), *Funneliformis* (1) **(Plate VII e)**, *Rhizoglomus* (1), *Archaeospora* (1) and *Tricispora* (1) **(Plate VIII f)** while the highest number of AM fungal species recovered from all the study sites was from variety Assgo (4) in Sikeri and Jyoti (4) from Tuem and Sikeri with species number given in parenthesis. Species richness (**Fig. 3**) was maximum in Sikeri (10) and the minimum in Neura (2), with species number given in parenthesis (**Table 5**).

Fig.3: AM fungal species richness in the study sites.



Legend: Bars represents number of AM fungal species detected at each study site.

3.3.3.2. AM diversity Index

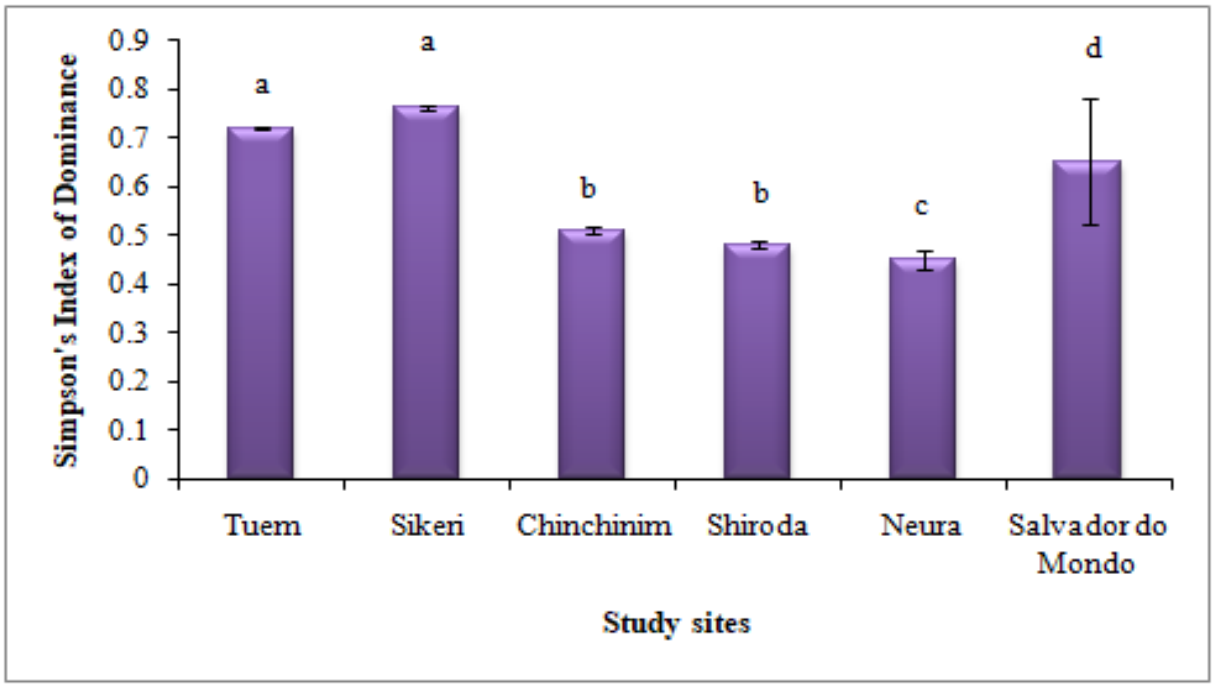
Simpson's Index of diversity (**Fig. 4**) was maximum at Sikeri (0.76) and least in Neura (0.35) which indicates shared dominance of AM fungal species. Shannon diversity Index (**Fig. 5**) was higher in Sikeri (1.78) suggesting greater diversity (**Table 5**).

Table 5: Diversity Index of AM fungal community at different study sites.

Ecological parameters	Tuem	Sikeri	Chinchinim	Shiroda	Neura	Salvador do Mondo
Simpson's Index of dominance (D)	$0.72^{a} \pm 0.002$	$0.76^{a} \pm 0.005$	$0.51^{b} \pm 0.008$	$0.48^{b} \pm 0.007$	$0.35^{c} \pm 0.019$	$0.65^{d} \pm 0.129$
Shannon Index (*H*) of diversity	$1.42^{a} \pm 0.004$	$1.78^{b} \pm 0.015$	$0.82^{c} \pm 0.021$	$0.83^{c} \pm 0.010$	$0.53^{d} \pm 0.021$	$1.07^{e} \pm 0.018$
Species Richness	06	10	03	03	02	03

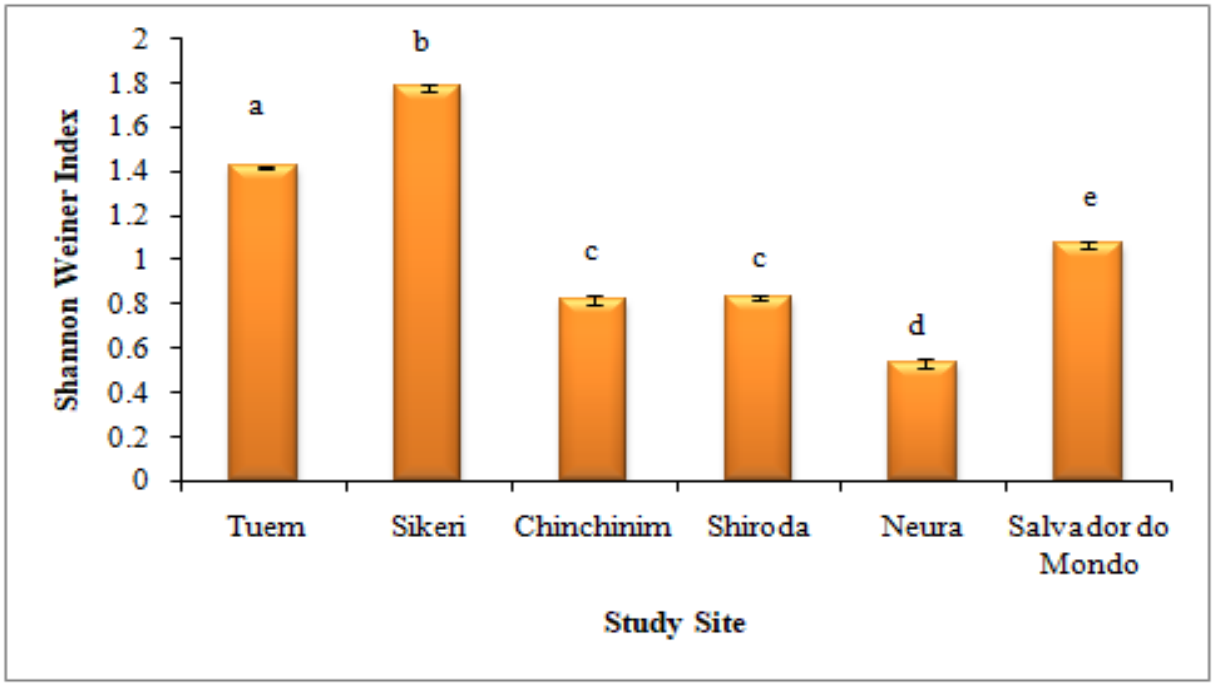
Legend: Data presented is the mean of three readings; ± indicates Standard Error; Values in the row depicted by the same letter as superscript are not significantly different between the varieties at a site.

Fig. 4: Simpson's Diversity Index at the study sites.



Legend: Error bars represent Standard Error; Values on the column depicted by the same letter are not significantly different between the varieties at a site.

Fig. 5: Shannon Diversity Index at the study sites.



Legend: Error bars represent Standard Error; Values on the column depicted by the same letter are not significantly different between the varieties at a site.

3.3.3.3. Relative Abundance of AM fungi

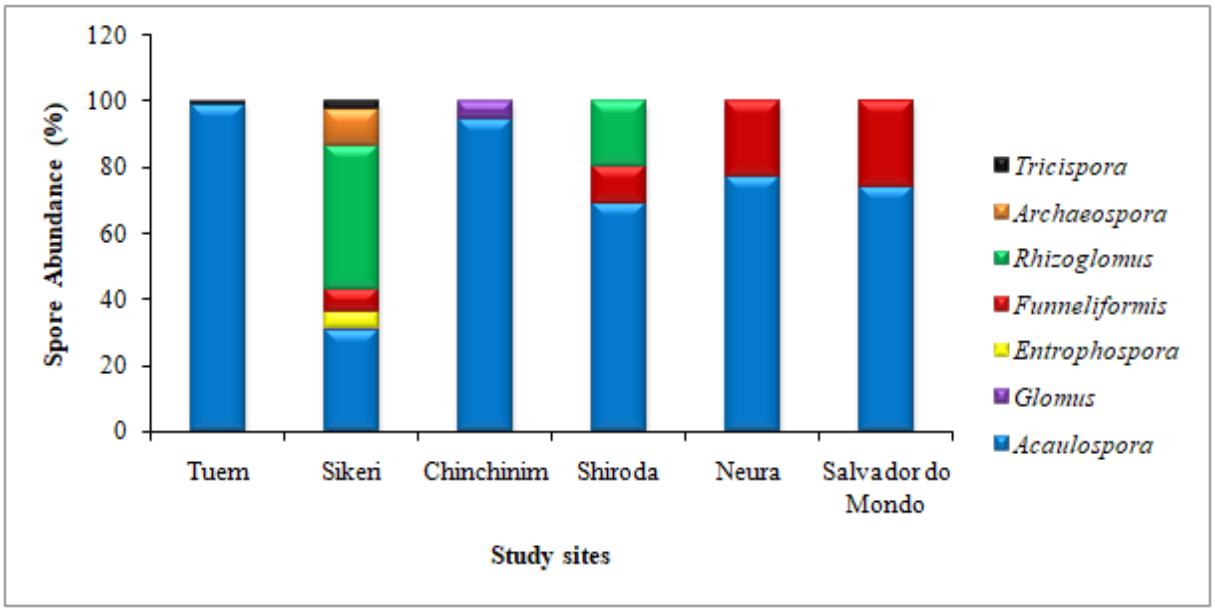
Variation in abundance of AM species was observed in all the study sites. The present study revealed that *A. delicata* was the most abundant species in Tuem and Chinchinim, *R. fasciculatum*in Sikeri, *A. bireticulata* in Neura, and *A. dilatata* in Shiroda and Salvador do Mondo **(Table 6).** *Gigaspora* and *Scutellospora* species were not recorded in any of the study sites. Genus *Acaulospora* was the most abundant in all the study except in Sikeri **(Fig. 6)**. *Acaulospora* was the most dominant genus in the studied *Khazan* lands **(Fig. 7)**.

Table 6: Spore Abundance of AM fungal species in the study sites.

AM species	Spore Abundance					
	Tuem	**Sikeri**	**Chinchinim**	**Shiroda**	**Neura**	**Salvador do Mondo**
Acaulospora bireticulata Rothwell & Trappe	05.08	nd	nd	nd	77.22	nd
Acaulospora delicata Walker Pfeffer & Bloss	36.27	nd	61.23	nd	nd	34.23
Acaulospora dilatata Morton	16.61	10.82	nd	68.70	nd	39.44
*Acaulospora laevis G*erdemann & Trappe	07.12	11.48	33.46	nd	nd	nd
Acaulospora scrobiculata Trappe	33.88	nd	nd	nd	nd	nd
Acaulospora soloidea Vaingankar & Rodrigues	nd	2.12	nd	nd	nd	nd
Acaulospora tuberculata Janos & Trappe	nd	6.37	nd	nd	nd	nd
Archaeospora myriocarpa (Sieverd & Schenck) Oehl, Silva, Golo & Sieverd. Comb. nov.	nd	10.97	nd	nd	nd	nd
Entrophospora infrequens (Hall) Ames & Sieverd	nd	4.99	nd	nd	nd	nd
Tricispora nevadensis (Palenz. Ferrol, Azćon- Aguilar &Oehl) Oehl. Palenz, Silva & Sieverd., comb. nov.	01.01	2.45	nd	nd	nd	nd
Funneliformis mosseae Gerdemann & Trappe	nd	07.03	nd	11.73	22.75	26.29
Glomus aggregatum Schenck & Smith	nd	nd	05.29	nd	nd	nd
Glomus microcarpum Tulasne & Tulasne	nd	0.16	nd	nd	nd	nd
Rhizoglomus fasciculatum (Thaxter) Sieverd, Silva & Oehl comb.nov.	nd	43.61	nd	19.55	nd	nd

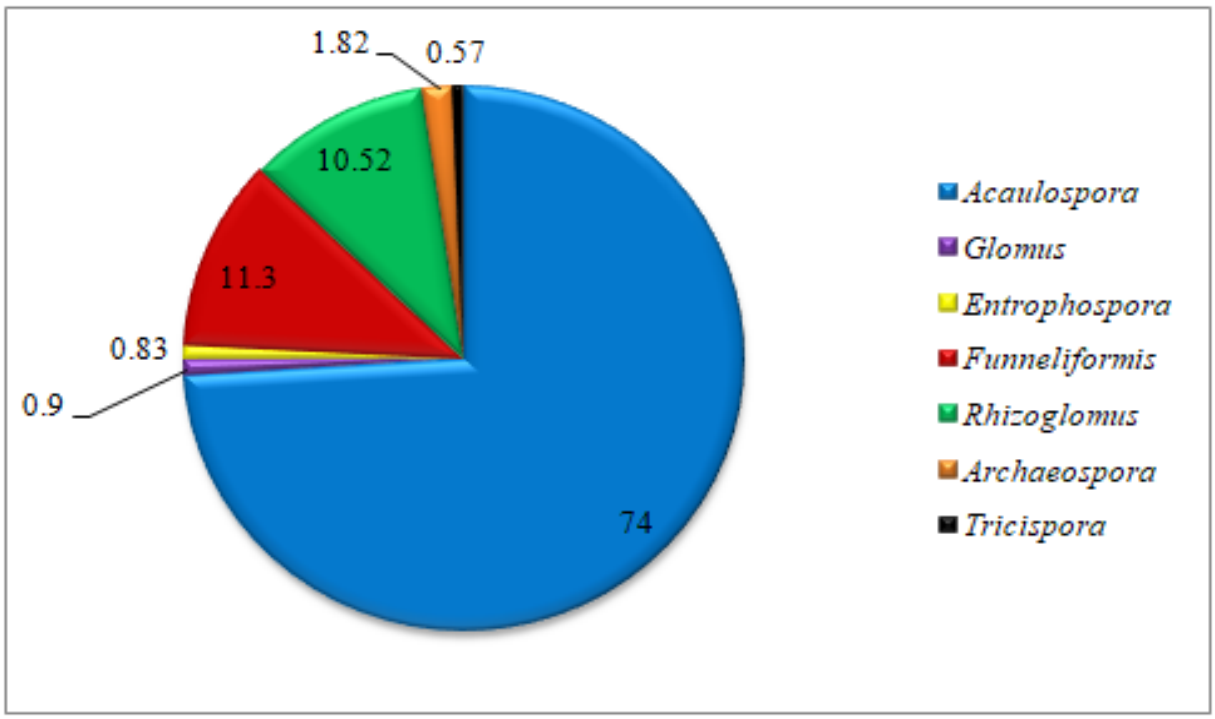
Legend: Data presented is the mean of three readings; nd = AM species not detected.

Fig. 6: Genera wise spore abundance in rice cultivated in the study sites.



Legend: Bars represents Spore Abundance of AM genera in the different study sites.

Fig.7: Genera wise Spore Abundance in the *Khazans.*



3.4. Discussion

The present study reveal that all the rice varieties cultivated in the *Khazans* were mycorrhizal with hyphal colonization being dominant as observed in an earlier study by Sengupta and Chaudhari (2002). The rice fields in the *Khazans* are always submerged under water. According to Miller and Bever (1999) AM fungi being aerobic micro-organisms that are associated with rice plants survive hypoxic condition. AM fungi may be surviving due to the oxygen provided by the aerenchyma tissue of the host. AM root colonization and spore number in the rhizosphere soils of rice showed variation and differed from site to site. None of the study sites showed significant correlation between root colonization and spore density. This finding is in agreement with earlier studies (Miller 2000, D' Souza and Rodrigues 2013). Variation in AM fungal association and spore number are known to be affected by rapid changes in soil nutrients (Abbott and Robson 1991), environmental factors, soil fertility (Brundrett 1991) or soil disturbances in the sites (Boddington and Dodd 2000). Miller *et al*. (1995) reported that spore numbers poorly reflects colonization potential of soil. He *et al*. (2002) reported increase in AM colonization when soil conditions were favourable for spore germination.

In the present study a total of 14 AM fungal species have been recorded from the six study sites. The study revealed an average of five AM species per site. AM fungal species present in the agricultural field are related to the diversity of the plant community (Bever *et al.* 2001) and in an agroecosystem, generally a single crop per field with occasional weeds is observed. Secondly, the cultural practices probably may be exerting a selective pressure on AM fungal diversity (Jansa *et al.* 2002).

The study revealed a variation in diversity of AM fungi at the different study sites. This variation may be attributed to various factors such as pH, available P or other soil nutrients (Chetan *et al.* 2008). *Acaulospora* was the dominant genus in the *Khazans*. *Acaulospora* species are often associated with acidic soils (Abbott and Robson 1991). The acidic nature of *Khazan* soils possibly explains the dominance of *Acaulospora* species at such sites. Species of *Acaulospora* occur in soils of diverse pH and nutrient accessibility. They are recognised to a

great extent in low effort farming practice fields. They are facultative symbionts acclimatized to a varied group of soils and host species (Shepherd *et al.* 1996, Straker *et al.* 2010).
Sturmer and Bellei (1994) reported that species richness is dependent on sample number. With more samples collected, more species are likely to be recovered. Maximum species richness observed at Sikeri may be correlated to diversity in rice varieties cultivated, as the number of samples examined at this site was more.

Variation in abundance of AM species was observed in all the study sites. Similar observations have been reported in earlier studies (Schenck and Kinlock 1980; Chetan *et al.* 2008). Bever *et al.* (1996) reported *Glomus* and *Acaulospora* species usually produce more spores than *Gigaspora* and *Scutellospora* species within the same environment as they have smaller spore size and require less time to sporulate (Hepper 1984).

3.5. Conclusion

The present study confirms the presence of AM colonization in rice cultivated in the *Khazans*. The presence of 14 AM fungal species, indicate rich diversity and the ability of these species to survive the various stress factors that exist in the *Khazan*s. The study reveals differences in quantitative and qualitative distribution in colonization, spore density, species richness and spore abundance of AM fungi in the *Khazan* rice fields of Goa. Additional studies are essential to deliberate on the combined effect of incidence of AM fungi at different *Khazans* in different phenological stages of rice and in different ecosystems.

CHAPTER 4

Study of the physico-chemical properties of rhizosphere soils of rice grown *Khazan* lands of Goa.

4.1. Introduction

The uppermost layer of the earth's crust in which plants grow is called soil. Soil is made up of three main components *viz.* minerals, organic matter and living organisms. Minerals are from weathered rocks or volcanic ash deposits, while remains of plants and animals form organic matter (http://www.soil.net.com). Soil provides the substratum for plant growth, home for insects and organisms, filtration for surface water, carbon store and helps in maintenance of atmospheric gases. Climatic condition, terrain, vegetation and the parent rock from which soil is formed can lead to the differences in soil at different sites (https://www.schooltoday.com:soils). Soil health is important for healthy food production as it provides nutrients, water, oxygen and support to the root. Soil supports a large community of different organisms that improves its structure, recycles essential nutrients and increase organic carbon, flood control and biological control of pests and diseases (Keesstra *et al.* 2016) and hence preservation of soil is necessary.

Mostly well drained, highly acidic (5.5 to 6.5 pH), lateritic (81%) soil with moderate organic carbon and low potassium (K) content are prevalent in Goa. About 11% of soils located along the sea coast and estuaries including midlands and beach front are sandy to sandy loams. The remaining 8% include the *Khazans* and adjoining areas which are subjected to inundation by saline water are alluvial in nature with high water table (http://www.agri.goa.gov.in).

The requisite to understand soil parameters and fertility status of soil is necessary for sustainable agriculture. For AM association the understanding of soil parameters is important, as the balance of this association can help in conservation of natural areas as well as help in management of agricultural areas. Hence the present study was carried out to investigate the variation in soil properties, and is divided into three sections *viz*., assessment of variation of

soil parameters at different *Khazan* sites, at different growth stages of rice in *Khazans* and in three different ecologies at different growth stages of rice.

4.2. Materials and Methods

4.2.1. I) Assessment of variation of soil parameters at different *Khazan* sites

4.2.1.1. Collection of rhizosphere soil samples

Field visits were conducted in the flowering stage of rice dominated *Khazan* areas of six different talukas (**Table 2**) in Goa during the year 2015. Rhizosphere soil (500g) was collected from a depth of 0-25cm, mixed thoroughly to obtain a composite sample and brought in polyethylene bags to the laboratory. The soil was then air dried at room temperature and passed through a 2mm sieve before carrying out analyses.

4.2.1.2. Soil Analyses

From the composite sample three sub-samples were drawn and analyzed separately. Using a pH meter (LI 120 Elico, India), soil pH was measured in 1:1 water solution suspension. Electrical conductivity (EC) was measured using conductivity meter (CM – 180 Elico, India). Walkley and Black (1934) rapid titration method was used to estimate organic carbon content. Nitrogen was assessed by micro-kjeldahl method (Jackson 1971). Available Phosphorus (P) was estimated using Bray and Kurtz method (1945). Potassium (K) was estimated by ammonium acetate method (Hanway and Heidal 1952). Using Atomic Absorption Spectrophotometer (AAS 4139) available iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn) were quantified by DTPA-$CaCl_2$–TEA method (Lindsay and Norvell 1978). Soil chemical analyses were carried out at Soil Analysis Laboratory, Agricultural Department, Government of Goa, Margao, Goa.

4.2.2. II) Analyses of soil at different growth stages of rice in two *Khazans*

4.2.2.1. Collection of rhizosphere soil samples

Soil was collected at three different growth stages of rice from two different *Khazans* (**Table 10**) during the year 2014 and 2015 as described under **4.2.1.1.**

4.2.2.2. Soil Analyses

Soil analyses at each growth stage in two *Khazans* studied were carried out at Soil Analysis Laboratory, Agricultural Department, Government of Goa, Margao, Goa as described under **4.2.1.2.**

4.2.3. III) Soil analyses in three different ecologies at different growth stages of rice.

4.2.3.1. Collection of rhizosphere soil samples

Rhizosphere soil was collected at three different growth stages from three different ecologies *viz.*, lowland, midland and upland (**Table 16)** as described under **4.2.1.1**.

4.2.3.2. Soil Analyses

Soil analyses at each growth stage in the three different ecologies studied were carried out at Soil Science Laboratory, ICAR-CCARI, Old Goa, as described under **4.2.1.2.**

4.2.4. Statistical Analyses:

4.2.4.1. analysis of soil data from each site and at different growth stages was carried out using analyses of variance (ANOVA) IBM SPSS Statistics 20. Means of soil parameters at sites studied were separated using Tukey at significance level <0.05.

4.2.4.2. Principal Component Analysis (PCA) is a reduction technique, was carried out to reduce the number of soil parameters by extracting the important one for each study area.

4.3. Results

4.3.1. I) Assessment of variation in soil parameters at different *Khazan* sites

4.3.1.1. Soil analyses

Results of soil analyses at different study sites in rice grown *Khazan* areas are depicted in **Table 7**. From the results, it is observed that *Khazan* soils are acidic with pH ranging from 4.8 to 6.4, and EC ranging from 0.07 to 0.50 dSm^{-1}. Available P ranged from 2.98 to 71.68 kg ha^{-1}, available K ranged from 54.90 to 269.00 $kgha^{-1}$, Fe ranged from 12.97 to 43.29 ppm, Mn ranged from 7.38 to 21.22 ppm, Cu ranged from 0.31 to 3.80 ppm and Zn ranged from 0.64 to 3.79 ppm.

Table 7: Chemical analysis of *Khazan* soils at six study sites.

Parameter	Study site						$F_{(5,12)}$, p < 0.05
	Tuem	Sikeri	Chinchinim	Shiroda	Neura	Salvador do Mondo	
pH	$5.20^{a} \pm 0.25$	$6.40^{a} \pm 0.11$	$5.30^{a} \pm 0.00$	$5.10^{a} \pm 0.17$	$5.00^{a} \pm 0.05$	$4.80^{a} \pm 0.80$	2.56
EC (dSm^{-1})	$0.19^{ac} \pm 0.02$	$0.07^{a} \pm 0.00$	$0.50^{b} \pm 0.00$	$0.30^{ab} \pm 0.05$	$0.40^{bc} \pm 0.11$	$0.30^{ab} \pm 0.05$	6.70*
OC (%)	$0.49^{a} \pm 0.08$	$1.54^{a} \pm 0.17$	$1.21^{a} \pm 0.01$	$5.11^{b} \pm 0.22$	$1.63^{a} \pm 0.20$	$1.10^{a} \pm 0.49$	42.93*
N (kg ha^{-1})	$173.25^{a} \pm 51.22$	$423.48^{a} \pm 49.20$	$352.83^{a} \pm 3.48$	$204.00^{a} \pm 2.30$	$449.16^{a} \pm 55.75$	$338.33^{a} \pm 109.6$	3.73
P (kg ha^{-1})	$22.41^{a} \pm 3.22$	$11.88^{ac} \pm 1.82$	$67.25^{b} \pm 5.85$	$2.98^{c} \pm 1.55$	$71.68^{b} \pm 4.59$	$6.82^{ac} \pm 3.55$	68.07*
K (kg ha^{-1})	$150.30^{a} \pm 0.22$	$156.80^{a} \pm 0.64$	$213.00^{b} \pm 6.92$	$269.00^{c} \pm 3.27$	$123.00^{a} \pm 2.30$	$54.90^{d} \pm 5.56$	98.27*
Fe (ppm)	$12.97^{a} \pm 1.76$	$43.29^{b} \pm 0.37$	$41.33^{b} \pm 0.19$	$41.77^{b} \pm 0.21$	$39.02^{b} \pm 3.52$	$23.93^{c} \pm 1.31$	52.77*
Mn (ppm)	$7.38^{a} \pm 0.62$	$9.39^{ab} \pm 0.12$	$14.43^{ab} \pm 3.99$	$9.48^{ab} \pm 3.44$	$21.22^{b} \pm 2.80$	$20.81^{bc} \pm 0.98$	5.95*
Cu (ppm)	$0.31^{a} \pm 0.03$	$1.71^{b} \pm 0.07$	$1.76^{b} \pm 0.10$	$3.80^{c} \pm 0.13$	$1.59^{b} \pm 0.32$	$3.54^{c} \pm 0.24$	51.20*
Zn (ppm)	$0.64^{a} \pm 0.98$	$3.79^{b} \pm 0.15$	$3.14^{bd} \pm 0.25$	$2.07^{ce} \pm 0.30$	$2.62^{cd} \pm 0.19$	$1.51^{ae} \pm 0.15$	30.91*

Legend: Values are mean of three readings; ± indicates Standard Error; Values in a row depicted by the same letter as a superscript are not significantly different at the level of probability indicated; OC = Organic Carbon; N = Nitrogen; P = Phosphorus; K = Potassium; Fe = Iron; Mn = Manganese; Cu = Copper; Zn = Zinc.

4.3.1.2. Influence of soil parameters on study sites

Principle Component Analysis (PCA) was carried out to understand the influence of soil parameters on the study sites. Eigen values are used to determine the number of possible principal components. The principal components with eigen value greater than one were retained for further study (Brejda *et al.* 2000). Of the 10 possible factors only 4 had eigen values > 1 accounting for 98.23% cumulative variance (**Table 8; Fig. 8**). Having eigen value less than one implies that the factor explains less variance than could individually contribute (Shukla *et al.* 2006). PC_1 suggests 31.14% variance showing high loading of N, Zn and Fe with negative effect and Neura showing the highest rating on PC_1. PC_2 shows 29.15% variance which was positively influenced by K, OC and pH. Mn rate was negatively loaded with Shiroda and positively loaded with Salvador do Mundo. The highest rating on PC_3 was at Sikeri with 23.35% variance and was positively influenced by pH, N and Zn (**Tables 8 and 9**).

Table 8: Factor co-ordinates of variables based on correlation at different *Khazans* sites.

Variables	PC_1	PC_2	PC_3	PC_4	PC_5
pH	-0.23	0.60	0.75	0.07	0.07
EC	-0.45	-0.40	-0.63	-0.42	0.19
OC	-0.01	0.70	-0.66	0.07	-0.25
N	-0.87	-0.20	0.29	0.31	-0.09
P	-0.65	-0.46	0.01	-0.59	-0.10
K	-0.04	0.76	-0.31	-0.56	0.03
Fe	-0.81	0.55	-0.15	0.02	-0.04
Mn	-0.50	-0.70	-0.33	0.36	-0.06
Cu	-0.08	0.27	-0.72	0.59	0.16
Zn	-0.85	0.42	0.26	0.06	0.11
Eigen value	3.11	2.91	2.33	1.45	0.17
Total variance (%)	31.14	29.15	23.35	14.58	1.76
Cumulative variance (%)	31.14	60.30	83.65	98.23	100.00

Legend: OC = Organic Carbon; N = Nitrogen; P = Phosphorus; K = Potassium; Fe = Iron; Mn = Manganese; Cu = Copper; Zn = Zinc; PC Principle Component.

Table 9: Factor co-ordinates of six *Khazan* sites based on correlation.

Site	Factor 1	Factor2	Factor 3	Factor4	Factor 5
Tuem	2.81	-0.76	1.12	-1.08	-0.10
Sikeri	-0.86	1.81	2.28	0.89	0.01
Chinchinim	-1.59	-0.22	-0.31	-1.39	0.58
Shiroda	0.77	2.35	-2.11	-0.01	-0.16
Neura	-1.81	-1.49	-0.23	-0.21	-0.63
Salvador do Mondo	0.68	-1.69	-0.74	1.81	0.30

4.2.2. II) Analyses of soil at different growth stages of rice in two *Khazans* sites

4.2.2.1. Soil analyses

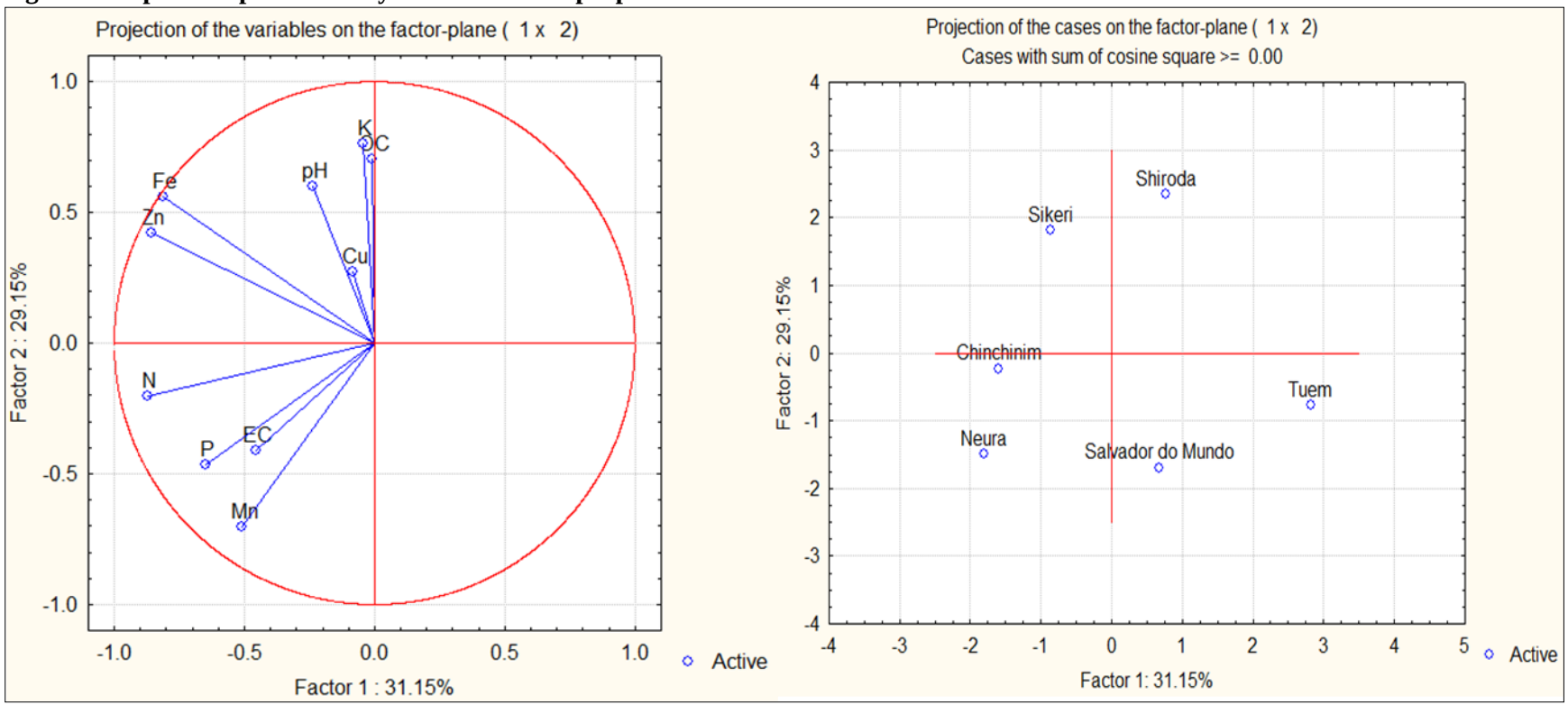
The results of physico-chemical analyses of soil from the two study sites *viz*., Tuem and Sikeri (**Table 10**) for 2014 and 2015 are presented in **Table 11** and **Table 12.**

Table 10: Geographical location of soil collections for spatio-temporal variation.

Taluka	Site	Geographical Location		
		Latitude	Longitude	Altitude (above msl)
Pernem	Tuem	15° 40' 15'' N	73° 49' 10''E	3 m
Bicholim	Sikeri	15° 35' 18'' N	73° 53' 20''E	7 m

The soil pH at both the sites varied significantly for both the years. However no specific pattern common to the two sites could be established. The soil pH was acidic except during the vegetative stage in 2015 at Sikeri where the pH was 7.03. The EC value was less than one at both the sites in both years except in 2014 in Tuem during the harvesting stage. Available P was limiting in both the years at the two sites except during the harvesting stage at Sikeri in The soil pH at both the sites varied significantly for both the years. However no specific pattern common to the two sites could be established. The soil pH was acidic except during the vegetative stage in 2015 at Sikeri where the pH was 7.03. The EC value was less than one

Fig.8: Principal Component Analysis based on soil properties at six *Khazan* sites.



Legend: OC = Organic Carbon; N = Nitrogen; P = Phosphorus; Fe = Iron; Mn = Manganese; Cu = Copper; Zn = Zinc

at both the sites in both years except in 2014 in Tuem during the harvesting stage. Available P was limiting in both the years at the two sites except during the harvesting stage at Sikeri in 2015, however decreased with plant maturity at both sites in 2014, while it increased in 2015. Organic carbon increased significantly at both the sites in 2014. However there was no significant difference at both the study sites in the subsequent year. Organic carbon ranged from 0.37 to 1.36% at Tuem and 1.23 to 1.68% at Sikeri for both the years. There was a significant difference in the micronutrient levels at various growth stages for both the years (**Tables 11 and 12**).

With reference to study sites, significant differences was observed for pH (5.72 at Tuem and 5.04 at Sikeri), EC (0.57 dSm^{-1}at Tuem and 0.12 dSm^{-1}at Sikeri), OC (0.72 % at Tuem and 1.50% at Sikeri), N (242.04 kg ha^{-1}at Tuem and 384.79 kg ha^{-1} at Sikeri), Fe (23.77 ppm at Tuem and 47.47 ppm at Sikeri) and Zn (1.17 ppm at Tuem and 2.40 ppm at Sikeri). No significant difference was observed for P (25.93 kg ha^{-1}at Tuem and 40.24 kg ha^{-1} at Sikeri), K (179.18 kg ha^{-1} at Tuem and 199.91 kg ha^{-1} at Sikeri), Mn (18.59 ppm at Tuem and 15.95 ppm at Sikeri) and Cu (2.75 ppm at Tuem and 1.92 ppm at Sikeri) **(Table 13)**.

4.2.2.2: Influence of soil parameters at different growth stages in two *Khazan* areas.

PCA studies of the two different *Khazan* sites obtained are depicted in **Fig. 9. T**he four PCs studied further indicated 84.67% cumulative variance. PC_1 suggests 34.46% of variance which was influenced positively by EC while all the other nine factors were negatively loaded. Sikeri showed high rating on PC_1 and was influenced by pH, OC, N, P, K, Fe, Mn and Zn while Tuem showed high rating on PC_2 and was influenced by EC, K, Fe, Cu and Zn **(Tables 14 and 15)**. Hence it can be conclude that pH, OC, N, P, Zn, Cu, Mn and EC bring about a difference in the soil characters in the two *Khazans* sites.

Table 11: Physico-chemical properties of the soil from Tuem site.

Year	2014				2015			
Parameter	Growth stage			$F_{(2,6)}$, p<0.05	Growth stage			$F_{(2,6)}$, p<0.05
	Vegetative	Flowering	Harvesting		Vegetative	Flowering	Harvesting	
pH	$04.64^{a} \pm 0.04$	$05.56^{b} \pm 0.33$	$05.36^{c} \pm 0.33$	180.34*	$07.03^{a} \pm 0.46$	$05.20^{b} \pm 0.25$	$06.50^{ab} \pm 0.05$	9.37*
EC (dSm^{-1})	$00.78^{a} \pm 0.03$	$00.21^{b} \pm 0.01$	$02.10^{c} \pm 0.13$	158.64*	$00.06^{a} \pm 0.03$	$00.19^{b} \pm 0.02$	$00.70^{a} \pm 0.00$	28.00*
OC (%)	$00.37^{a} \pm 0.02$	$00.47^{b} \pm 0.01$	$00.50^{b} \pm 0.00$	31.47*	$01.36^{a} \pm 0.90$	$00.50^{a} \pm 0.09$	$01.13^{a} \pm 0.05$	0.72
N ($kg\ ha^{-1}$)	$120.27^{a} \pm 9.32$	$200.00^{b} \pm 2.00$	$204.00^{b} \pm 0.00$	73.54*	$417.5^{a} \pm 225.94$	$173.25^{a} \pm 51.22$	$337.21^{a} \pm 14.03$	0.86
P ($kg\ ha^{-1}$)	$06.53^{a} \pm 0.27$	$21.26^{b} \pm 0.57$	$05.21^{a} \pm 2.01$	49.80*	$32.85^{a} \pm 22.59$	$22.41^{a} \pm 3.22$	$66.32^{a} \pm 0.87$	3.02
K ($kg\ ha^{-1}$)	$110.09^{a} \pm 5.22$	$143.73^{a} \pm 23.33$	$313.60^{b} \pm 19.39$	37.66*	$261.00^{a} \pm 36.75$	$150.30^{ab} \pm 5.22$	$96.33^{b} \pm 31.27$	8.97*
Fe (ppm)	$14.04^{a} \pm 2.41$	$20.25^{ab} \pm 8.71$	$42.15^{b} \pm 0.59$	07.97*	$29.56^{a} \pm 5.06$	$12.97^{b} \pm 1.76$	$23.62^{ab} \pm 0.77$	7.22*
Mn (ppm)	$23.16^{a} \pm 2.58$	$13.37^{b} \pm 0.25$	$13.06^{b} \pm 1.07$	12.50*	$26.12^{a} \pm 1.75$	$07.38^{b} \pm 0.62$	$28.42^{a} \pm 0.67$	101.40*
Cu (ppm)	$00.25^{a} \pm 0.08$	$04.02^{b} \pm 0.13$	$05.25^{c} \pm 0.36$	137.03*	$02.85^{a} \pm 0.44$	$00.31^{b} \pm 0.03$	$03.78^{a} \pm 0.20$	40.94*
Zn (ppm)	$00.23^{a} \pm 0.05$	$00.67^{b} \pm 0.02$	$03.14^{c} \pm 0.01$	1708.9*	$01.04^{a} \pm 0.21$	$00.64^{a} \pm 0.59$	$01.26^{a} \pm 0.02$	05.39

Legend: Values are mean of three readings, ± indicates Standard Error; Different letters between the soil collected at the different growth stages of rice of each year of study indicate significant differences at $P < 0.05$; OC = Organic Carbon; N = Nitrogen; P = Phosphorus; K = Potassium; Fe = Iron; Mn = Manganese; Cu = Copper; Zn = Zinc.

Table 12: Physico-chemical properties of the soil from Sikeri site.

Year	2014				2015			
Parameter	Growth phase			$F_{(2,6)}$, p<0.05	Growth phase			$F_{(2,6)}$, p<0.05
	Vegetative	Flowering	Harvesting		Vegetative	Flowering	Harvesting	
pH	$4.57^{a} \pm 0.03$	$4.83^{b} \pm 0.03$	$4.70^{c} \pm 0.00$	24.00*	$4.83^{a} \pm 0.33$	$6.40^{b} \pm 0.12$	$4.90^{a} \pm 0.05$	96.32*
EC(dSm^{-1})	$0.19^{a} \pm 0.01$	$0.08^{b} \pm 0.00$	$0.11^{b} \pm 0.01$	50.14*	$0.08^{a} \pm 0.01$	$0.07^{a} \pm 0.00$	$0.17^{b} \pm 0.01$	58.99*
OC (%)	$1.23^{a} \pm 0.02$	$1.54^{ab} \pm 0.14$	$1.68^{b} \pm 0.00$	08.02*	$1.54^{a} \pm 0.14$	$1.54^{a} \pm 0.17$	$1.47^{a} \pm 0.11$	0.07
N (kg ha^{-1})	$370.16^{a} \pm 24.37$	$382.87^{a} \pm 8.56$	$374.31^{a} \pm 0.00$	0.89	$382.87^{a} \pm 8.56$	$423.48^{a} \pm 49.20$	$375.02^{a} \pm 10.32$	0.78
P (kg ha^{-1})	$22.40^{a} \pm 1.16$	$8.53^{b} \pm 2.58$	$4.72^{b} \pm 1.47$	25.41*	$8.53^{a} \pm 2.58$	$11.89^{a} \pm 1.83$	$185.35^{b} \pm 7.55$	39.84*
K(kg ha^{-1})	$150.30^{a} \pm 1.05$	$156.80^{a} \pm 00.00$	$350.93^{b} \pm 7.46$	685.81*	$156.80^{a} \pm 00.00$	$156.80^{a} \pm 6.65$	$227.80^{a} \pm 65.38$	1.17
Fe (ppm)	$36.31^{a} \pm 0.88$	$39.42^{a} \pm 0.90$	$72.98^{b} \pm 0.59$	620.21*	$39.42^{a} \pm 0.09$	$43.29^{a} \pm 1.76$	$53.38^{a} \pm 10.43$	1.42
Mn (ppm)	$15.20^{a} \pm 1.32$	$15.08^{a} \pm 0.55$	$31.65^{b} \pm 1.07$	96.94*	$15.08^{a} \pm 0.55$	$9.39^{b} \pm 0.13$	$9.39^{a} \pm 0.07$	98.87*
Cu (ppm)	$0.69^{a} \pm 0.11$	$2.64^{b} \pm 0.06$	$2.05^{c} \pm 0.13$	82.13*	$2.65^{a} \pm 0.06$	$1.71^{b} \pm 0.08$	$1.76^{b} \pm 0.09$	48.40*
Zn (ppm)	$0.84^{a} \pm 0.06$	$1.89^{b} \pm 0.06$	$2.10^{b} \pm 0.04$	132.86*	$1.89^{a} \pm 0.06$	$3.79^{b} \pm 0.15$	$3.89^{a} \pm 0.54$	129.8*

Legend: Values are mean of three readings, ± indicates Standard Error.; Different letters between the soil collected at the different growth stages of rice of each year of study indicate significant differences at $P < 0.05$; OC = Organic Carbon; N = Nitrogen; P = Phosphorus; K = Potassium; Fe = Iron; Mn = Manganese; Cu = Copper; Zn = Zinc.

Table 13: Average Physico-chemical properties of soil at two *Khazan* sites.

Parameters	Study sites		$F_{(1,34)}$, p<0.05
	Tuem	Sikeri	
pH	$5.72^{a} \pm 0.20$	$5.04^{b} \pm 0.15$	6.87*
EC (dSm^{-1})	$0.57^{a} \pm 0.17$	$0.12^{b} \pm 0.01$	6.45*
OC (%)	$0.72^{a} \pm 0.15$	$1.50^{b} \pm 0.05$	22.14*
N ($kg\ ha^{-1}$)	$242.04^{a} \pm 40.88$	$384.79^{b} \pm 9.10$	11.61*
P ($Kg\ ha^{-1}$)	$25.93^{a} \pm 5.88$	$40.24^{a} \pm 16.27$	0.68
K ($Kg\ ha^{-1}$)	$179.18^{a} \pm 21.02$	$199.91^{a} \pm 19.89$	0.57
Fe (ppm)	$23.77^{a} \pm 2.83$	$47.47^{b} \pm 3.40$	28.67*
Mn (ppm)	$18.59^{a} \pm 1.93$	$15.95^{a} \pm 1.83$	0.97
Cu (ppm)	$2.75^{a} \pm 0.46$	$1.92^{a} \pm 0.16$	2.83
Zn (ppm)	$1.17^{a} \pm 0.23$	$2.40^{b} \pm 0.26$	12.24*

Legend: Data presented is mean of at each ecosystem, ± indicates Standard Error, for each ecosystem, values in the row affected by the same letter are not significantly different at P< 0.05 level of probability.

Table 14: Factor co-ordinates of variables based on correlation of soil at Tuem and Sikeri at different growth stages.

Variables	PC_1	PC_2	PC_3	PC_4	PC_5
pH	-0.10	-0.07	0.61	-0.69	-0.02
EC	0.29	0.90	-0.05	0.01	-0.01
OC	-0.90	-0.36	0.05	0.09	-0.14
N	-0.87	-0.35	0.14	-0.10	-0.18
P	-0.34	-0.10	-0.44	-0.44	0.68
K	-0.54	0.63	0.13	0.31	0.15
Fe	-0.88	0.26	-0.10	0.34	0.01
Mn	-0.14	-0.09	0.76	0.40	0.40
Cu	-0.16	0.61	0.45	-0.40	-0.06
Zn	-0.72	0.38	-0.37	-0.31	-0.12
Eigenvalue	3.44	2.10	1.57	1.34	0.72
Total variance (%)	34.46	21.06	15.72	13.41	7.27
Cumulative variance (%)	34.46	55.53	71.25	84.67	91.95

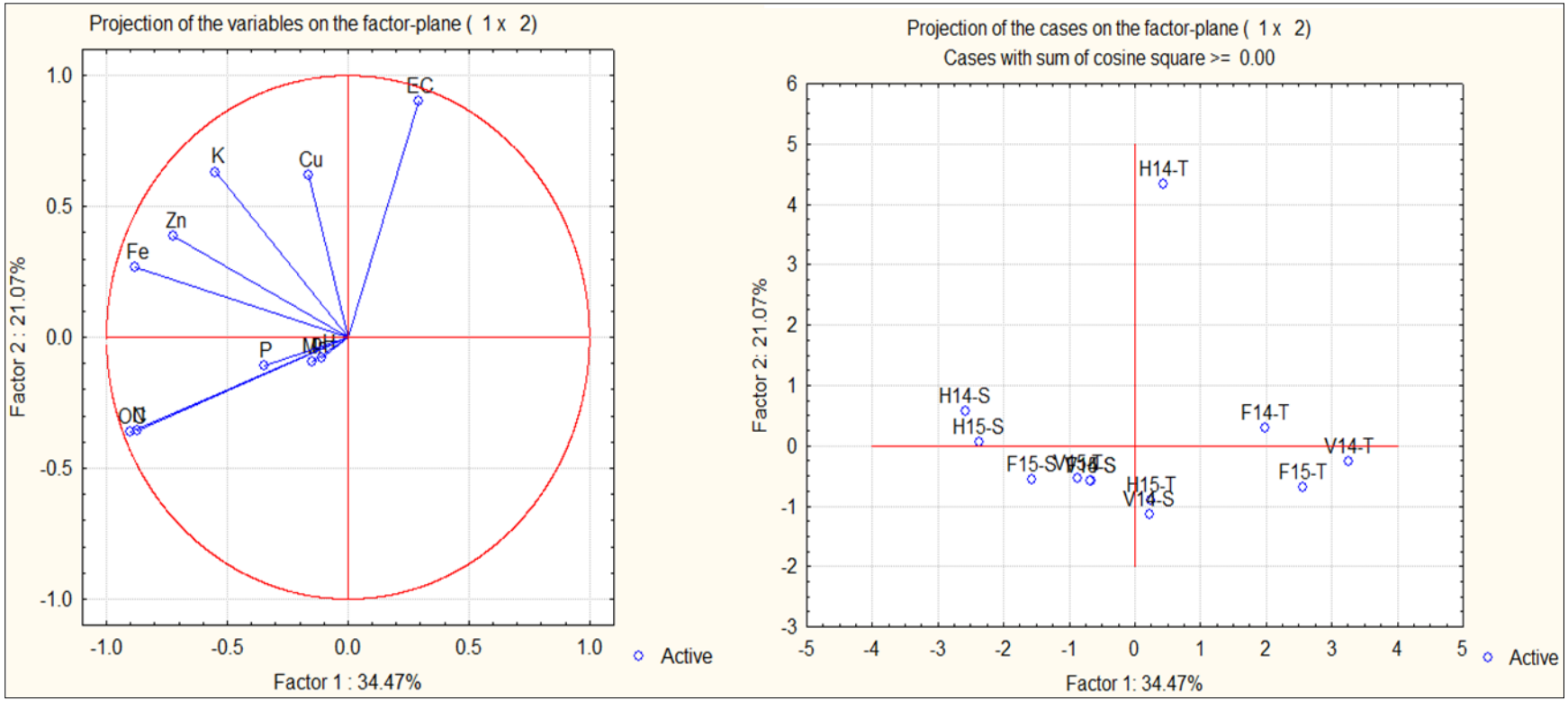
Legend: OC = Organic Carbon; N = Nitrogen; P = Phosphorus; K = Potassium; Fe = Iron; Mn = Manganese; Cu = Copper; Zn = Zinc; PC = Principal Component.

Table 15: Factor co-ordinates of Tuem and Sikeri at different growth stages.

StudySite	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
V14-T	3.26	-0.25	-0.23	1.21	0.68
F14-T	1.99	0.29	0.41	-0.79	-0.03
H14-T	0.44	4.33	0.04	-0.36	-0.33
V15-T	-0.86	-0.53	2.23	-0.69	0.39
F15-T	2.55	-0.69	-1.12	-0.02	-0.12
H15-T	0.24	-0.90	1.73	-1.34	0.88
V14-S	0.22	-1.13	-0.70	0.99	-0.29
F14-S	-0.66	-0.58	-0.18	0.37	-0.90
H14-S	-2.57	0.56	0.89	2.53	0.49
V15-S	-0.67	-0.58	-0.20	0.34	-0.85
F15-S	-1.57	-0.56	-0.37	-1.21	-1.46
H15-S	-2.37	0.05	-2.50	-1.03	1.56

Legend: V14 = Vegetative stage of 2014; F14 = Flowering stage of 2014; H14 = Harvesting stage of 2014, V15 = Vegetative stage of 2015; F 15- Flowering stage of 2015; H15- Harvesting stage of 2015; T = Tuem; S= Sikeri

Fig.9: Component Analysis based on soil properties and study sites (Tuem and Sikeri).



Legend: OC = Organic Carbon; N = Nitrogen; P = Phosphorus; K = Potassium; Fe = Iron; Mn = Manganese; Cu = Copper; Zn = Zinc; V14 = Vegetative stage of 2014; F14 = Flowering stage of 2014; H14 = Harvesting stage of 2014, V15 = Vegetative stage of 2015; F 15- Flowering stage of 2015; H15- Harvesting stage of 2015; T = T uem; S = Sikeri

4.2.3. III) Soil analyses in three different ecologies at different growth stages of rice.

4.2.3.1. Soil analyses

Results of the physico-chemical analyses of soil of three ecosystems (**Table 16**) are depicted **in Table 17, Table 18** and **Table 19**. In lowland, there was no significant difference for pH, EC, OC, N, K, Fe, Cu and Zn at the different growth stages for both the years of study. Even though a significant difference did exist in the P content for the year 2015 and Mn content for the year 2016, no common pattern could be established for both the years (**Table 17**). In midlands, there was no significant difference for pH, EC, N, P, K, Mn and Cu at the different growth stages for both the years except for Fe and Zn for the year 2015 and OC in 2016 (**Table 18**).In uplands, there was no significant difference in any of the edaphic factors at the different growth stages during the year 2015, while, there was a significant difference in pH, EC, OC, P and Fe for the year 2016 (**Table 19**).

Table 16: Geographical location of soil collection from different ecologies.

Ecosystem	Site	Geographical Location		
		Latitude	Longitude	Altitude (m) (above msl)
Lowland (*Khazan*)	Sikeri	15° 35' 18'' N	73° 53' 20''E	7
	Tuem	15° 40' 15'' N	73° 49' 10''E	3
Midland (*Ker*)	Saligao	15° 33' 07'' N	73° 47' 01''E	29
	Velim	15° 09' 39'' N	73° 58' 45''E	21
Upland (*Morod*)	Morpilla	15° 06' 53'' N	73° 59' 54''E	378
	Quitolla	15° 07' 58'' N	73° 57' 36''E	204

In relation to the different ecological sites, there was no significant difference in pH, EC, P, Fe, Cu and Zn content in the three different ecologies (**Table 20**). The soil pH was acidic and ranged from 5.4 to 5.6, while the EC ranged from 0.14 to 0.29 dSm^{-1}. Mean available P was lower in lowlands (46.94 kg ha^{-1}) and upland (49.34 kg ha^{-1}) and higher in midlands (82.18 kg ha^{-1}). However, a significant difference in OC content was recorded. Maximum OC content was recorded in uplands (3.19%), followed by lowland (1.29%) and least in midland (0.89%). There was a significant difference in N content at the three ecosystems. Maximum mean N

Table 17: Physico-chemical properties of the soil from lowlands.

Year	2015				2016			
Parmeter	Growth stage			$F_{(2,6)}$, p<0.05	Growth stage			$F_{(2,6)}$,p<0.05
	Vegetative	Flowering	Harvesting		Vegetative	Flowering	Harvesting	
pH	05.93^{a} ± 0.63	05.80^{a} ± 0.34	05.70^{a} ± 0.33	00.05	04.34^{a}± 0.37	05.17^{a} ± 0.37	05.55^{a}±0.06	4.01
EC (dSm^{-1})	00.06^{a} ± 0.00	00.13^{a} ± 0.03	00.12^{a} ± 0.13	01.83	00.29^{a}± 0.02	00.46^{a}± 0.08	00.68^{a}±0.32	1.05
OC (%)	01.44^{a}± 0.05	01.01^{a} ± 0.30	01.30^{a} ± 0.09	01.37	01.22^{a}± 0.06	01.02^{a}± 0.32	01.76^{a}±22.63	0.61
N ($kg\ ha^{-1}$)	400.18^{a} ± 9.99	298.36^{a}±72.23	365.11^{a}±10.91	01.43	245.77^{a}± 6.7	260.18^{a}± 5.78	210.63^{a}±7.52	0.18
P ($kg\ ha^{-1}$)	28.90^{ab}± 11.76	17.14^{a}± 3.03	125.83^{b} ± 2.01	08.03*	34.27^{a}± 3.66	36.30^{a}± 3.22	66.32^{a}± 0.87	3.02
K ($kg\ ha^{-1}$)	208.90^{a} ±30.07	153.55^{a} ± 1.87	162.06^{a}±37.95	01.13	200.31^{a}±55.54	227.93^{a}±42.08	380.72^{a}±50.74	3.81
Fe (ppm)	34.49^{a} ± 2.84	28.12^{a} ± 8.74	38.50^{a} ± 8.59	00.51	50.36^{a}± 6.70	36.34^{a}± 13.49	26.66^{a}± 7.77	1.47
Mn (ppm)	20.60^{a} ± 3.18	08.38^{a}±0.58	18.87^{a} ± 5.51	03.20	05.71^{a}± 0.82	08.26^{ab}± 0.50	14.30^{b}± 2.07	11.13*
Cu (ppm)	02.74^{a} ± 0.06	1.01^{a} ± 0.40	2.76^{a}±0.58	05.95	00.29^{a}± 0.09	00.29^{a}± 0.01	00.33^{a}± 0.03	0.16
Zn (ppm)	01.46^{a} ±0.24	2.21^{a} ±0.90	02.57^{a} ±0.75	00.65	01.57^{a}±0.46	01.18^{a}±0.31	00.86^{a}±0.08	01.18

Legend: Values are mean of three readings, ± indicates Standard .Error; Different letters between the soil collected at the different growth stages of rice of each year of study indicate significant differences at P< 0.05; OC = Organic Carbon; N = Nitrogen; P = Phosphorus; K = Potassium; Fe = Iron; Mn = Manganese; Cu = Copper; Zn = Zinc.

Table 18: Physico-chemical properties of the soil from midlands.

Year	2015				2016			
Parameter	Growth stage			$F_{(2,6),}$ p<0.05	Growth stage			$F_{(2,6),}$ p<0.05
	Vegetative	Flowering	Harvesting		Vegetative	Flowering	Harvesting	
pH	$06.05^{a} \pm 0.25$	$05.78^{a} \pm 0.04$	$05.91^{a} \pm 0.10$	00.67	$05.55^{a} \pm 0.15$	$05.07^{a} \pm 0.17$	$04.96^{a} \pm 0.23$	2.67
EC (dSm^{-1})	$00.18^{a} \pm 0.07$	$00.10^{a} \pm 0.02$	$00.18^{a} \pm 0.07$	00.57	$00.09^{a} \pm 0.04$	$00.21^{a} \pm 0.09$	$00.38^{a} \pm 0.14$	2.11
OC (%)	$00.65^{a} \pm 0.11$	$00.98^{a} \pm 0.26$	$00.62^{a} \pm 0.08$	01.30	$00.92^{ab} \pm 0.21$	$01.59^{a} \pm 0.28$	$00.59^{b} \pm 0.11$	5.65*
N($kg\ ha^{-1}$)	$253.96^{a} \pm 34.71$	$152.10^{a} \pm 27.34$	$212.20^{a} \pm 10.91$	03.93	$127.52^{a} \pm 20.52$	$294.44^{a} \pm 90.33$	$165.16^{a} \pm 18.10$	2.58
P($kg\ ha^{-1}$)	$95.93^{a} \pm 04.07$	$117.69^{a} \pm 21.88$	$87.31^{a} \pm 00.77$	01.48	$38.45^{a} \pm 01.30$	$31.76^{a} \pm 1.91$	$32.23^{a} \pm 7.78$	0.63
K($kg\ ha^{-1}$)	$208.90^{a} \pm 0.07$	$153.55^{a} \pm 1.87$	$162.06^{a} \pm 37.95$	01.13	$109.84^{a} \pm 9.86$	$95.62^{a} \pm 7.45$	$86.39^{a} \pm 2.43$	2.63
Fe (ppm)	$31.65^{a} \pm 2.35$	$40.86^{b} \pm 1.64$	$35.35^{ab} \pm 0.74$	07.31*	$24.40^{a} \pm 6.90$	$19.04^{a} \pm 2.67$	$32.83^{a} \pm 9.38$	1.01
Mn (ppm)	$14.57^{a} \pm 07.00$	$15.75^{a} \pm 05.75$	$06.01^{a} \pm 1.40$	01.00	$04.60^{a} \pm 2.39$	$07.62^{a} \pm 4.29$	$02.34^{a} \pm 0.20$	0.86
Cu (ppm)	$02.30^{a} \pm 0.23$	$2.15^{a} \pm 0.61$	$1.03^{a} \pm 0.12$	03.17	$00.21^{a} \pm 0.05$	$00.08^{a} \pm 0.00$	$00.12^{a} \pm 0.05$	2.19
Zn (ppm)	$02.07^{ab} \pm 0.43$	$2.76^{a} \pm 0.02$	$00.91^{b} \pm 0.20$	11.28*	$06.44^{a} \pm 3.47$	$00.45^{a} \pm 0.01$	$00.84^{a} \pm 0.16$	01.78

Legend: Values are mean of three readings, ± indicates Standard .Error.; Different letters between the soil collected at the different growth stages of rice of each year of study indicate significant differences at P< 0.05; OC = Organic Carbon; N = Nitrogen; P = Phosphorus; K = Potassium; Fe = Iron; Mn = Manganese; Cu = Copper; Zn = Zinc.

Table 19: Physico-chemical properties of the soil from uplands.

Year	2015				2016			
Parameter	Growth stage			$F_{(2,6)}$, p<0.05	Growth stage			$F_{(2,6)}$, p<0.05
	Vegetative	Flowering	Harvesting		Vegetative	Flowering	Harvesting	
pH	06.39^{a} ± 0.15	05.95^{a} ± 0.14	05.84^{a} ± 0.16	03.57	05.62^{a} ± 0.08	04.71^{b} ± 0.16	05.19^{ab} ± 0.07	18.37*
EC (dSm^{-1})	00.17^{a} ± 0.07	00.06^{a} ± 0.02	00.22^{a} ± 0.06	01.78	00.09^{a} ± 0.01	00.25^{b} ± 0.04	00.08^{a}± 0.02	9.86*
OC (%)	03.64^{a} ± 0.51	04.53^{a} ± 0.27	04.46^{a} ± 0.37	01.53	01.60^{a} ± 0.13	02.32^{b} ± 0.03	02.59^{b}± 0.13	19.97*
N (kg ha^{-1})	1115.93^{a} ± 24.24	1000.67^{a} ± 210.10	864.78^{a} ±115.86	00.44	499.74^{a} ±18.06	435.57^{a} ±55.11	369.56^{a}±61.71	1.77
P (kg ha^{-1})	85.17^{a} ± 02.28	55.37^{a} ± 26.59	69.08^{a} ± 2.01	00.86	20.36^{a} ± 2.95	26.03^{a} ± 0.08	40.04^{b}± 3.78	13.35*
K (kg ha^{-1})	205.19^{a} ± 29.64	155.66^{a} ± 12.31	117.33^{a} ± 09.23	05.21	262.52^{a} ± 89.77	172.22^{a} ±52.92	76.84^{a}± 00.13	2.11
Fe (ppm)	38.32^{a} ± 00.38	37.85^{a} ± 01.36	36.67^{a} ± 0.49	00.95	37.76^{a} ± 0.20	28.11^{a} ± 2.19	37.97^{a}± 3.48	5.60*
Mn (ppm)	23.27^{a} ± 2.85	20.48^{a} ± 4.31	22.98^{a} ± 0.64	00.26	017.71^{a} ± 2.82	07.21^{a} ± 1.41	13.73^{a}± 2.69	04.88
Cu (ppm)	01.69^{a} ± 0.54	1.36^{a} ± 0.53	1.66^{a} ± 0.53	00.11	00.16^{a} ± 0.03	00.14^{a} ± 0.06	00.21^{a}± 0.06	0.41
Zn (ppm)	01.126^{a} ± 0.24	0.68^{a} ± 0.19	00.58^{a} ± 0.30	02.44	00.81^{a} ± 0.14	01.12^{a} ± 0.17	00.51^{a}±0.04	05.25

Legend: Values are mean of three readings, ± indicates Standard .Error; Different letters between the soil collected at the different growth stages of rice of each year of study indicate significant differences at P< 0.05; OC = Organic Carbon; N = Nitrogen; P = Phosphorus; K = Potassium; Fe = Iron; Mn = Manganese; Cu = Copper; Zn = Zinc.

content was recorded in upland (714.37 kg ha^{-1}) followed by lowland (295.05 kg ha^{-1}) and midland (200.89 kg ha^{-1}). A significant difference in K content was recorded between the three ecologies. The highest mean K content was observed in lowland (222.24 kg ha^{-1}) followed by upland (163.29 kg ha^{-1}) and least in midland (98.77 kg ha^{-1}). Micronutrients showed variation in their distribution in the different ecologies. Mean Mn content showed significant difference in its distribution, maximum being in upland (17.56 ppm), followed by lowland (12.68 ppm) and least in midland (8.31 ppm).

Table 20: Average Physico-chemical properties of soil at the different ecosystem.

Parameters	Ecosystem			$F_{(2,6)}$, p<0.05
	LowLand	Midland	Upland	
pH	5.4^{a}±0.24	5.5^{a}±0.18	5.6^{a}±0.24	0.2
EC (dSm^{-1})	0.29^{a}±0.09	0.19^{a}±0.04	0.14^{a}±0.03	1.32
OC (%)	1.29^{b}±0.11	0.89^{b}±0.15	3.19^{a}±0.49	16.20*
N(kg ha^{-1})	295.05^{b}±29.49	200.89^{b}±26.29	714.37^{a}±130.19	12.11*
P (Kg ha^{-1})	46.94^{a}±16.09	82.18^{a}±28.82	49.34^{a}±10.29	0.97
K(Kg ha^{-1})	222.24^{a}±33.73	98.77^{b}±5.09	163.29^{ab}±25.45	6.31*
Fe (ppm)	35.74^{a}±3.48	30.02^{a}±3.50	34.44^{a}±1.72	1.35
Mn (ppm)	12.68^{ab}±2.51	8.31^{b}±2.12	17.56^{a}±2.53	3.71*
Cu (ppm)	1.23^{a}±0.49	0.98^{a}±0.41	0.87^{a}±0.31	0.20
Zn (ppm)	1.64^{a}±0.26	2.24^{a}±0.91	0.80^{a}±0.10	1.73

Legend: Data presented is mean of nine readings at each ecosystem, ± indicates S.E., for each ecosystem, values in the row affected by the same letter are not significantly different at P< 0.05 level of probability.

4.2.3.2. Influence of soil parameters in different growth stages at different ecologies.

Further study of four PCs indicated 82.31% cumulative variance. PC_1 suggests 35.85% of variance which was positively influenced by EC and Zn while all the other eight factors were negatively loaded. Lowlands showed high rating on PC_2 for the year 2015 depicting positive loading for pH, P, Cu and Zn, while for the year 2016, EC, OC, N, K, Fe and Mn influenced the soil characteristics. In midland, high rating on PC_2 was noted for pH, P, Cu and Zn, while in uplands, PC_1 showed high loading which was influenced by pH, N, P, OC, Fe, Mn and Cu **(Tables 21 and 22; Fig. 10)** Therefore can conclude that pH, P and Cu content are having

common distribution and the distribution of N, OC, K and Mn content brings about the variation in soil properties at the different studied ecologies.

Table 21: Factor co-ordinates of variables based on correlation at different ecologies.

Variables	PC_1	PC_2	PC_3	PC_4	PC_5
pH	-0.73	0.34	-0.25	0.36	0.22
EC	0.47	-0.57	-0.51	0.01	0.27
OC	-0.70	-0.49	0.38	0.07	0.10
N	-0.82	-0.38	0.28	0.07	0.08
P	-0.31	0.53	-0.34	-0.43	0.44
K	-0.00	-0.63	-0.62	0.29	-0.22
Fe	-0.38	-0.11	-0.16	-0.74	-0.46
Mn	-0.92	-0.16	-0.15	0.15	-0.13
Cu	-0.69	0.46	-0.34	0.02	-0.14
Zn	0.23	0.64	-0.00	0.46	-0.35
Eigen value	3.58	2.19	1.23	1.21	0.77
Total variance (%)	35.85	21.99	12.35	12.10	7.73
Cumulative variance (%)	35.85	57.85	70.20	82.31	90.05

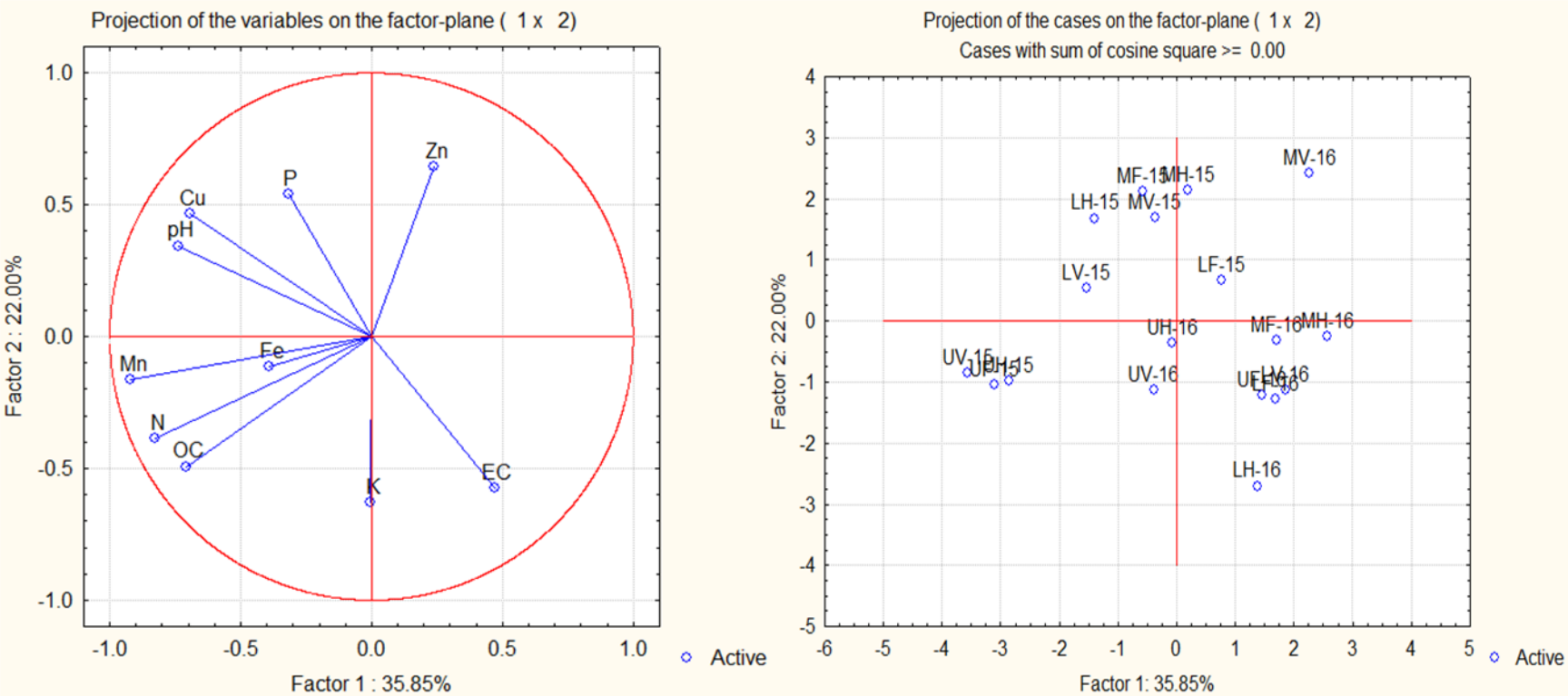
Legend: OC = Organic Carbon; N = Nitrogen; P = Phosphorus; K = Potassium; Fe = Iron; Mn = Manganese; Cu = Copper; Zn = Zinc. PC = Principle Component.

Table 22: Factor co-ordinates of sites based on correlation at different ecologies.

StudySite	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
LV-15	-1.53	0.53	-0.72	0.76	-1.06
LF-15	0.76	0.66	0.33	1.04	-0.20
LH-15	-1.39	1.68	-1.19	-0.39	-0.48
MV-15	-0.37	1.69	-0.53	0.40	0.07
MF-15	-0.57	2.11	-0.81	-0.75	-0.54
MH-15	0.19	2.14	-0.86	-1.79	2.02
UV-15	-3.56	-0.84	-0.13	0.47	0.35
UF-15	-3.09	-1.04	1.24	0.12	0.11
UH-15	-2.86	-0.98	0.76	-0.08	0.59
LV-16	1.85	-1.12	-0.06	-2.09	-1.78
LF-16	1.69	-1.27	-0.95	-0.29	-0.09
LH-16	1.37	-2.70	-2.68	1.40	0.72
MV-16	2.26	2.41	1.05	2.37	-0.54
MF-16	1.70	-0.31	1.41	0.54	1.29
MH-16	2.56	-0.25	0.47	-0.85	0.51
UV-16	-0.39	-1.13	0.02	0.22	-0.89
UF-16	1.44	-1.21	1.06	0.08	0.17
UH-16	-0.07	-0.36	1.58	-1.16	-0.26

Legend: LV15- Lowland Vegetative stage of 2015; LF 15- Lowland Flowering stage of 2015; LH15- Lowland Harvesting stage of 2015; MV15- Midland Vegetative stage of 2015; MF 15- Midland Flowering stage of 2015; MH15- Midland Harvesting stage of 2015; UV15;Upland Vegetative stage of 2015; UF 15- Upland Flowering stage of 2015; UH15- Upland Harvesting stage of 2015; LV16- Lowland Vegetative stage of 2016; LF 16- Lowland Flowering stage of 2016; LH16- Lowland Harvesting stage of 2016; UV15-Upland Vegetative stage of 2016; UF 16- Upland Flowering stage of 2016; UH16- Upland Harvesting stage of 2016

Fig. 10: Principal Component Analysis based on soil properties at different ecologies.



Legend: OC = Organic Carbon; N = Nitrogen; P = Phosphorus; K = Potassium; Fe = Iron; Mn = Manganese; Cu = Copper; Zn = Zinc. LV15- Lowland Vegetative stage of 2015; LF 15- Lowland Flowering stage of 2015; LH15- Lowland Harvesting stage of 2015; MV15- Midland Vegetative stage of 2015; MF 15- Midland Flowering stage of 2015; MH15- Midland Harvesting stage of 2015; UV15; Upland Vegetative stage of 2015; UF 15- Upland Flowering stage of 2015; UH15- Upland Harvesting stage of 2015; LV16- Lowland Vegetative stage of 2016; LF 16- Lowland Flowering stage of 2016; LH16- Lowland Harvesting stage of 2016; UV15-Upland Vegetative stage of 2016; UF 16- Upland Flowering stage of 2016; UH16- Upland Harvesting stage of 2016.

4.4. Discussion

The state of Goa has three main physical divisions *viz*., the mountainous region of the Sahyadris in the east, the middle level plateaus in the center and the low-lying river basins with the coastal plains. The portion of the Sahyadris lying in Goa has an area of about 600 sq. km. The central portion consisting of plateaus at varying levels and the coastal region is divided into two interrelated ecozones *viz.*, the coastal region exposed directly to the Arabian Sea, and the alluvial plains, mud flats and estuarine flood plains. In Goa, the saline flood plains are called as *Khazans.* The principle geological feature of the land is extensive laterization which occurs because of its position in the tropical moist climate and exposure to vast seasonal changes. These lateritic soils show association with iron (Fe) and manganese (Mn) ore deposits. Lateritic rocks also store water. The plains are formed by the rivers which deposit eroded material from the Sahyadris along their banks (Alvares 2002).

In the present study, soil analysis of rice cultivated rhizosphere *Khazan* soils was carried out during the flowering stage. The soil collected from different sites showed non-significant variation in pH. The soil was acidic at all sites as reported in earlier study by Mahajan *et al.* (2015a). Variations in soil physico-chemical characteristics were observed at different *Khazan* sites. These variations possibly could be because the study sites are situated at different river basins and each of the rivers originate at different region, pass through different villages having different soil properties before merging with the Arabian Sea.

Soil analyses at Tuem and Sikeri at the different growth stages indicate that there were variations in soil properties between the different growth stages at each site and this could be attributed to the constant flushing of water leading to deposition of salts (Rodrigues and Anuradha 2009) and rainfall.

Studies on soils collected at different growth stages and at different ecological sites revealed that there was significant variation for P and Mn in the lowland, Fe, Zn and OC in the midland and pH, EC, OC, P and Fe in the upland. In relation to the different ecological sites studied, there was no significant difference in pH, EC, P, Fe, Cu and Zn in the three different

ecologies, while there was significant difference in OC, N, K and Mn levels. The variation in OC content at the different ecologies could be attributed to the deposition of aquatic and vegetative residue into the soil during their formation (Joshi and Kadrekar 1987). In the present study, midland with excessively drained soil recorded the least levels of N. This could be attributed to loss by leaching influenced by soil type, and is more pronounced in sandy soils rather loam soils (Tandon 1994). Higher K level in the lowland can be attributed to salinity indicating that K from the sea water might be the major source contributing to higher soil K (Joshi and Kadrekar 1987). Soil available micronutrients were high in the lowlands, midlands and uplands, which could be due to high acidic soil reaction (Mahajan *et al.* 2015b). Variations in soil parameters could also be due to vegetation, cropping history, temperature, type of ecosystem and fertilizer application (Mathimaran *et al*. 2007).

4.5. Conclusion

Variability in soil properties at different sites was observed in relation to time and space. The difference in soil characteristics could not be attributed to a single soil character but revealed that up to four soil characters contributed to the difference. Natural variability results from interaction between geology, topography, climate, cultivation, land use and soil erosion (Quine and Zhang 2002). Variability in soil properties within a field can be due to water and nutrient movement and supply to plants (Shukla *et al*. 2004). From previous studies, there are clear evidences that AM fungal community composition and distribution at different habitats are mainly affected by host plant species and environmental factors such as soil type (Jansa *et al.* 2014, Oehl *et al.* 2010). Hence from the present study it can be concluded that variations in soil characteristics at the different *Khazan* sites, in the different growth stages of rice and different ecologies will result in variation in the distribution of AM fungal community.

CHAPTER 5

Spatio-temporal variation of AM fungi in *O*ryza *sativa* L var. Jyoti grown in the *Khazan* lands of Goa.

5.1. Introduction

Mycorrhiza is a type of mutualistic association between plants and fungus. This association assists, in nutrient cycling and shielding the host against environmental stress in the ecosystem. In natural condition, presence of mycorrhizae is a normal phenomenon and AM association is the commonest type (Zhang *et al.* 2003). However in agricultural system, edaphic factors, crops and management practices interact to influence AM fungal species composition and spore population (Kurle and Pfleger 1994).

Hetrick *et al.* (1990) observed a close relationship between the degree of association with AM fungi and the metabolic pathway in ten species of Poaceae. The fungal symbiont influence different developmental stages of grasses depending on host type, favouring seedlings in C_3 and flowering in C_4 hosts (Hartnett *et al.* 1993, Wilson and Hartnett 1997). Finally, host plant growth and colonization by AM fungi are a function of plant/fungus combinations (Sanders and Fitter 1992).

Monitoring of AM symbiotic association during the different phenological growth stages of the plant is necessary to understand the role of AM fungi in increasing plant growth and nutrient uptake. Patterns and timing of AM colonization within the plant roots vary depending on edaphic factors and nutrient levels (Sanders 1999).

Due to paucity of information on spatio-temporal variation of AM fungi in rice cultivated in the *Khazan*, the present work was initiated to understand the variation of AM fungi in relation to the different growth stages or phenology of rice plant in two selected *Khazans* of Goa.

5.2. Materials and Methods

5.2.1. Study Site

Samples of *Oryza sativa* var. Jyoti were collected two geographic locations in North Goa *viz.*, Tuem in Pernem (15° 40' 15'' N and 73° 49' 10''E) and Sikeri in Bicholim (15° 35' 18'' N and 73° 53' 20''E). The study was conducted from June to October for two successive years (2014 and 2015). In both the sites, the fields were in lowlands that were conventionally managed. The bunds and sluice gates were maintained and the flow of water was controlled by opening and closing of the gates. The fields were sown by broadcasting the seeds in Tuem while transplantation method was employed in Sikeri. Fields in Tuem are on a flat low lying area, three meters above the mean sea level and have been cultivated continuously over the years. The fields in Sikeri are located on a slope, seven meters above the mean sea level and these fields were cultivated for the first time in 2013.

5.1.2. Collection of rhizosphere soil samples

Roots and rhizosphere soil samples were collected three times, during the different growth stages of rice plant *viz.*, vegetative (August), flowering (September) and harvesting (October) during 2014 and 2015 from both the study sites, with the month of sampling given in parenthesis. During sampling, three sub-samples from the rhizosphere were collected in polyethylene bags and brought to the laboratory. The roots were then separated from the soil. The soil was than pooled together to form a composite soil sample and divided into three parts, one part for AM spore isolation, one part for preparation of trap culture and one for soil analyses.

5.1.3. Root processing

Root processing was carried out as described under **3.2.2.**

5.2.4. Estimation of AM fungal root colonization percentage

Estimation of AM fungal root colonization was carried out as described under **3.2.3.**

5.2.5. Isolation of AM spores

Isolation of AM spores was carried out as described under **3.2.5.**

5.2.6. Estimation of AM spore density

Estimation of AM spore density was carried out as described under **3.2.6**

5.2.7. Taxonomic identification of AM fungi

Taxonomic identification of AM fungi was carried out as described under **3.2.7.**

5.2.8. Diversity Studies

5.2.8.1. AM species richness

AM species richness at each growth stage was carried out as described under **3.2.8.1.**

5.2.8.2. Diversity Index

AM diversity index at both the study sites at the different growth stages was carried out as described under **3.2.8.2.**

5.2.8.3. Relative abundance (RA %)

Relative abundance of AM fungi species at different growth stages was carried out as described under **3.2.8.3.**

5.2.8.4. Frequency of occurrence (%)

Species wise frequency of occurrence (%) for each growth stage at the two study sites was calculated by using the following formula (Beena *et al.* 2000).

Frequency of occurrence (%) = Number of soil samples that possess spores of particular AM species/Total number of all the AM species x 100.

5.2.9. Statistical Analyses

5.2.9.1. Analysis of AM fungi colonization and spore density at each growth stage at the two study sites were carried out as described under **3.2.9.1.**

5.2.9.2. Relationship of AM fungal root colonization, spore density and edaphic factors was determined by Pearson's correlation coefficient using IBM SPSS Statistics 20 $P< 0.05$ significance level.

5.2.9.3. Canonical correspondence analysis (CCA) was carried out using Multivariate Statistical Package (MVSP) program version 3.1 to understand the correlation between relative abundance of AM fungi and the physico-chemical properties of soil at the two study sites.

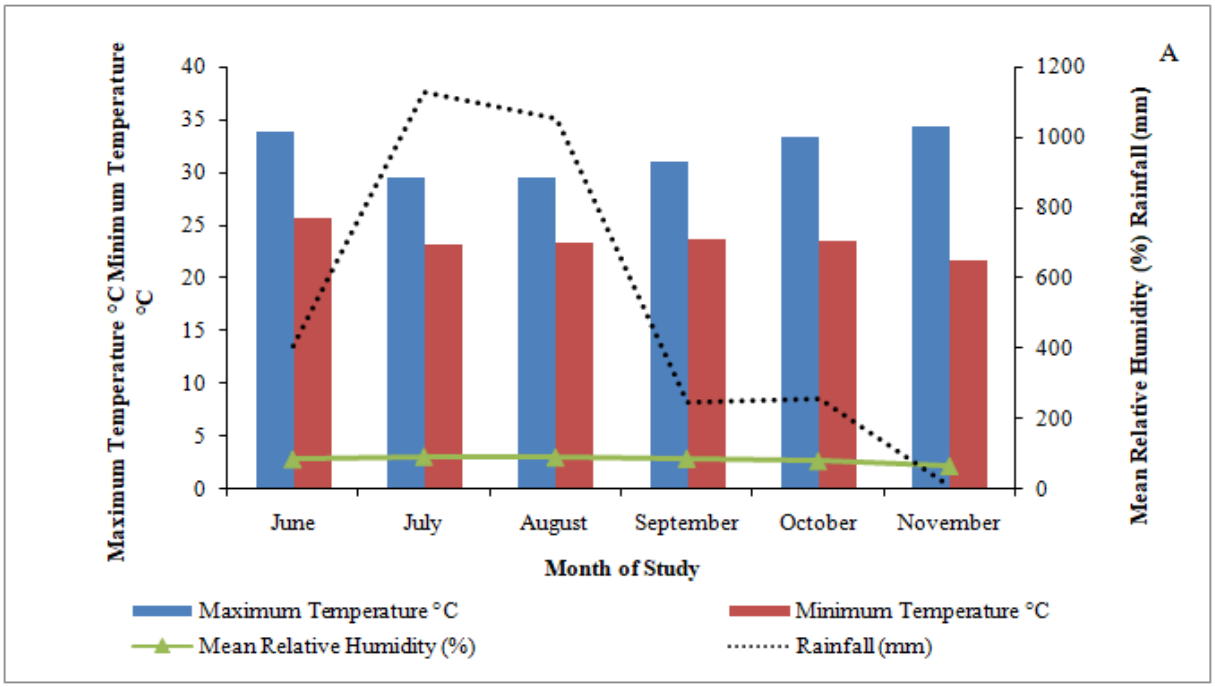
5.2.9.4. Univariate analysis of variance (ANOVA) was carried out to understand if there is an interaction between two independent variables on a dependent variable.

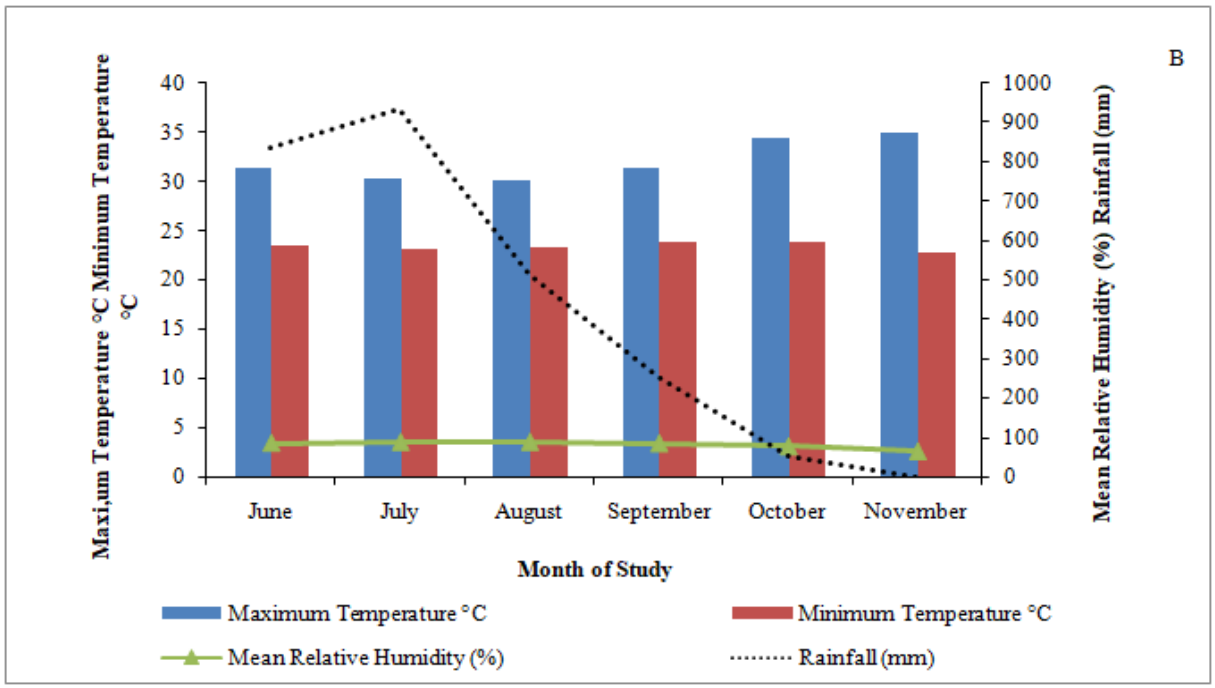
5.3. Results

5.3.1. Climate Data

Climate data recorded in both the sites during the study period is depicted in **Fig. 11.** There was no significant difference in the climatic conditions for the two years. However the average relative humidity and rainfall was higher in the year 2014 and as compared to 2015. The average temperature was higher in the year 2015. In the year 2014, the average relative humidity was 82.57 %, rainfall was 516.40mm and maximum and minimum temperature was 31.91°C and 23.48°C, respectively. In the year 2015, the average relative humidity was 81.30%, rainfall was 432.51mm and maximum and minimum temperature was 32.11°C and 23.40°C, respectively.

Fig. 11: Climate data recorded during the study period (2014 and 2015).





Legend: Sites: A -2014, B –2015.

5.3.2. AM root colonization

Oryza sativa var. Jyoti recorded AM fungal colonization at both the sites. Hyphal colonization was common and recorded in all growth stages. Numerous hyphal coils were also observed during vegetative stage, while vesicles were predominant during the flowering stage. Few arbuscules were also recorded. Year-wise results of AM colonization in variety Jyoti at both the study sites are depicted in **Table 23**. Colonization pattern varied significantly between the different growth stages during the two years of study period **(Fig. 12)**. The present study, suggests that AM colonization is influenced by phenology of the plant. In Tuem and Sikeri, the mean root colonization was significantly higher during flowering stage and least during the harvesting stage in both years. Mean colonization percentage was 67.69, 76.77 and 54.76 during the vegetative, flowering and harvesting stage respectively. Mean AM colonization was significantly higher in 2014 (76.23%) compared to 2015 (56.58%). Mean AM colonization percentage during 2014 and 2015 was significantly higher at Tuem (76.17) as compared to Sikeri (56.65).

Table 23: Influence of phenology on root colonization.

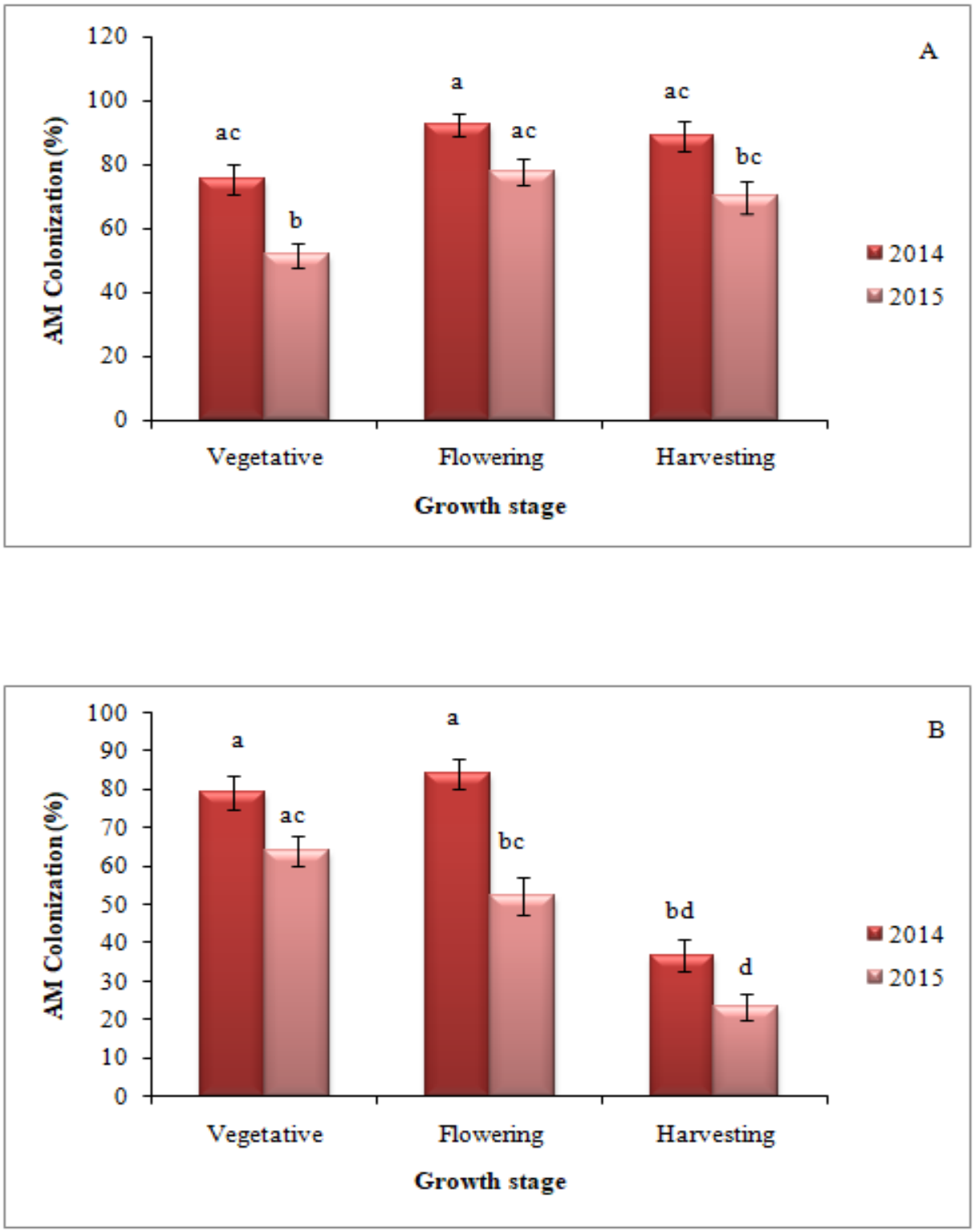
Site	Vegetative stage		Flowering stage		Harvesting stage		$F_{(5,12)}$
	2014	2015	2014	2015	2014	2015	p<0.05
Tuem	75.59^{ac}± 4.65	51.85^{b}± 3.70	92.55^{a}± 3.72	77.97^{ac}± 4.00	89.04^{ac}± 4.52	70.00^{bc}± 5.00	11.55*
Sikeri	79.33^{a}± 4.33	64.00^{ac}± 4.00	84.19^{a}± 4.12	52.38^{bc}± 4.76	36.67^{bd}± 4.40	23.33^{d}± 3.33	32.62*

Legend: Data presented is mean of three readings at each growth stage; Different letters between the years of study at different growth stage indicate significant differences at P < 0.05; ± indicates Standard Error. Mean percent colonizationwas 76.23^{a}± 4.74 and 56.58^{b}± 4.47 for the year 2014 and 2015 respectively at $F_{(1,34)}$=9.07*, p<0.05; Mean percent colonization was 76.13^{a}± 3.55 and 56.65^{b}± 5.48 at Tuem and Sikeri respectively at $F_{(1,34)}$=8.92*, p<0.05; Mean percent colonization was 67.69^{ab} ± 3.70, 76.77^{a} ± 4.86 and 54.76^{b} ± 8.08 at Vegetative, flowering and harvesting stage respectively at $F_{(2,33)}$=3.577*, p<0.05.

5.3.3. Correlation between adaphic factors and root colonization

A significant correlation has been observed between root colonization and pH, N, K, Mn and Cu at Tuem. However, no significant correlation existed between root colonization and spore density, EC, OC, P, Fe and Zn at Tuem. At Sikeri, a significant negative correlation was

Fig. 12: Influence of phenology on mean root colonization at the study sites.



Legend: Sites: A - Tuem, B – Sikeri; Error bars represent Standard Error; Values on the column affected by the same letter are not significantly different between the growth stage of the two years of study.

observed between root colonization and spore density, EC, P, K, Mn and Zn and no significant correlation was observed between root colonization and pH, OC, N, Fe and Cu **(Table 24)**.

Table 24: Pearson Correlation coefficient (r value) between spore density (SD) and root colonization (RC), and between root colonization and pH, EC, N, P, K, Fe, Mn, Cu, and Zn.

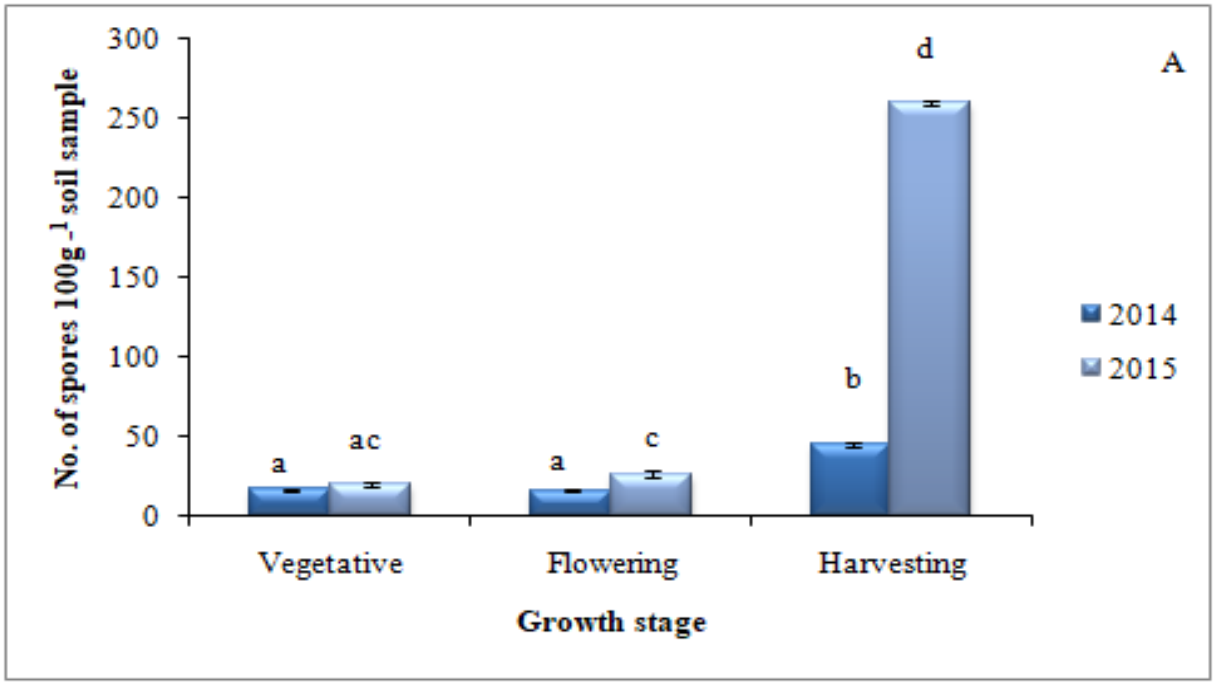
Parameter	Tuem		Sikeri	
	2014	2015	2014	2015
RC v/s SD	0.218	0.204	-0.747*	-0.934**
RC v/s pH	0.818**	- 0.775*	0.074	0.219
RC v/s EC	-0.042	0.604	0.087	-0.876**
RC v/s OC	0.645	- 0.243	-0.489	0.244
RC v/s N	0.813**	- 0.266	-0.124	0.253
RC v/s P	0.533	0.026	0.534	-0.879**
RC v/s K	0.380	- 0.748*	-0.946**	-0.556
RC v/s Fe	0.371	- 0.630	-0.960	-0.597
RC v/s Mn	-0.882**	- 0.590	-0.958**	0.677*
RC v/s Cu	0.694*	- 0.469	-0.110	0.622
RC v/s Zn	0.330	- 0.384	-0.494	-0.714*

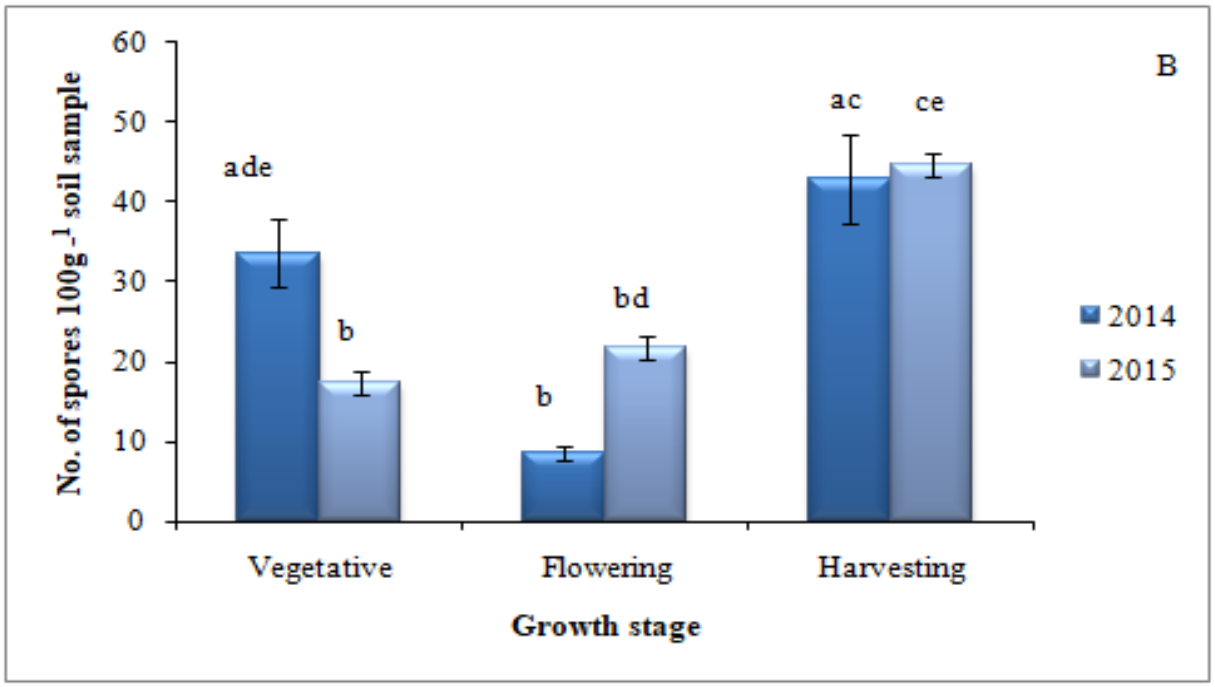
Legend: * = significant at P< 0.05; ** = significant at P< 0.01. OC = Organic Carbon; N = Nitrogen; P = Phosphorus; K = Potassium; Fe = Iron; Mn = Manganese; Cu = Copper; Zn = Zinc

5.3.4. AM Spore density

Maximum spore density was recorded during the harvesting stage in both years at both the sites **(Fig.13)**. At Tuem, spore density varied from 16 to 260 spores$100g^{-1}$ of soil sample while in Sikeri it ranged from 8 to 44 spores$100g^{-1}$ of soil sample for the two years. Spore density varied significantly between the growing stages in the two years. At Tuem, the mean spore density was significantly higher during harvesting stage and least during the vegetative stage in both years. While at Sikeri, spore density was significantly higher at harvesting stage for both years and minimum during flowering stage in 2014 and vegetative stage in 2015 (**Table 25)**. Mean Spore density was higher in 2015 (65.22 spores$100g^{-1}$ of soil sample) compared to 2014 (27.22 spores$100g^{-1}$ of soil sample). Mean Spore density was higher at Tuem (64.28 spores$100g^{-1}$ of soil sample) compared to Sikeri (28.17 spores$100g^{-1}$ of soil sample).

Fig. 13: Influence of phenology on AM spore density at the study sites.





Legend: Sites: A - Tuem, B – Sikeri; Error Bars represent Standard Error; Values on the column affected by the same letter are not significantly different between the growth stages of the two years of study.

Table 25: Influence of phenology on AM fungal spore density.

Site	Vegetative stage		Flowering stage		Harvesting stage		$F_{(5,12)}$ p<0.05
	2014	2015	2014	2015	2014	2015	
Tuem	17.00^{a}± 0.57	20.33^{ac}± 2.02	16.00^{a}± 1.15	26.67^{c}± 1.76	45.00^{b}± 1.73	260.00^{d}± 1.85	3922.48*
Sikeri	33.67^{ade}± 4.17	17.33^{b} ± 1.45	8.67^{b}± 0.88	21.67^{bd} ± 1.44	43.00^{ac} ± 5.56	44.67^{ce} ± 1.46	22.94*

Legend: Data presented is mean of three readings at each growth stage; different letters within the years of study indicate significant differences at P < 0.05; ± indicates Standard Error. Mean spore density was 27.22^{a}±3.55 and 65.22^{a}±21.31 for the year 2014 and 2015 respectively at $F_{(1,34)} = 3.09$, p<0.05;df(1,34) p<0.05, Mean spore density was 64.28^{a}±21.43 and 28.17^{b}±3.39 at Tuem and Sikeri respectively at $F_{(1,34)} = 2.76$*, p<0.05; Mean spore density was 22.08^{a} ± 2.30, 18.25^{a} ± 2.08 and 98.33^{b} ± 28.29 at vegetative, flowering and harvesting stage respectively at $F_{(2,33)} = 7.56$*, p<0.05.

Table 26: Pearson Correlation coefficient (r value) between spore density (SD) and pH, EC, N, P, K, Fe, Mn, Cu, and Zn.

Parameter	Tuem		Sikeri	
	2014	2015	2014	2015
SD v/s pH	0.394	0.186	-0.593	-0.318
SD v/s EC	- 0.216	-0.418	0.495	0.946**
SD v/s OC	0.274	0.107	0.78	-0.140
SD v/s N	0.245	0.079	-0.097	-0.175
SD v/s P	0.716**	0.679*	0.047	0.953**
SD v/s K	- 0.353	-0.654	0.63	0.575
SD v/s Fe	0.067	0.115	0.609	0.625
SD v/s Mn	0.512*	0.55	0.690*	-0.616
SD v/s Cu	0.296	0.667*	-0.463	-0.533
SD v/s Zn	0.149	0.610	-0.093	0.628

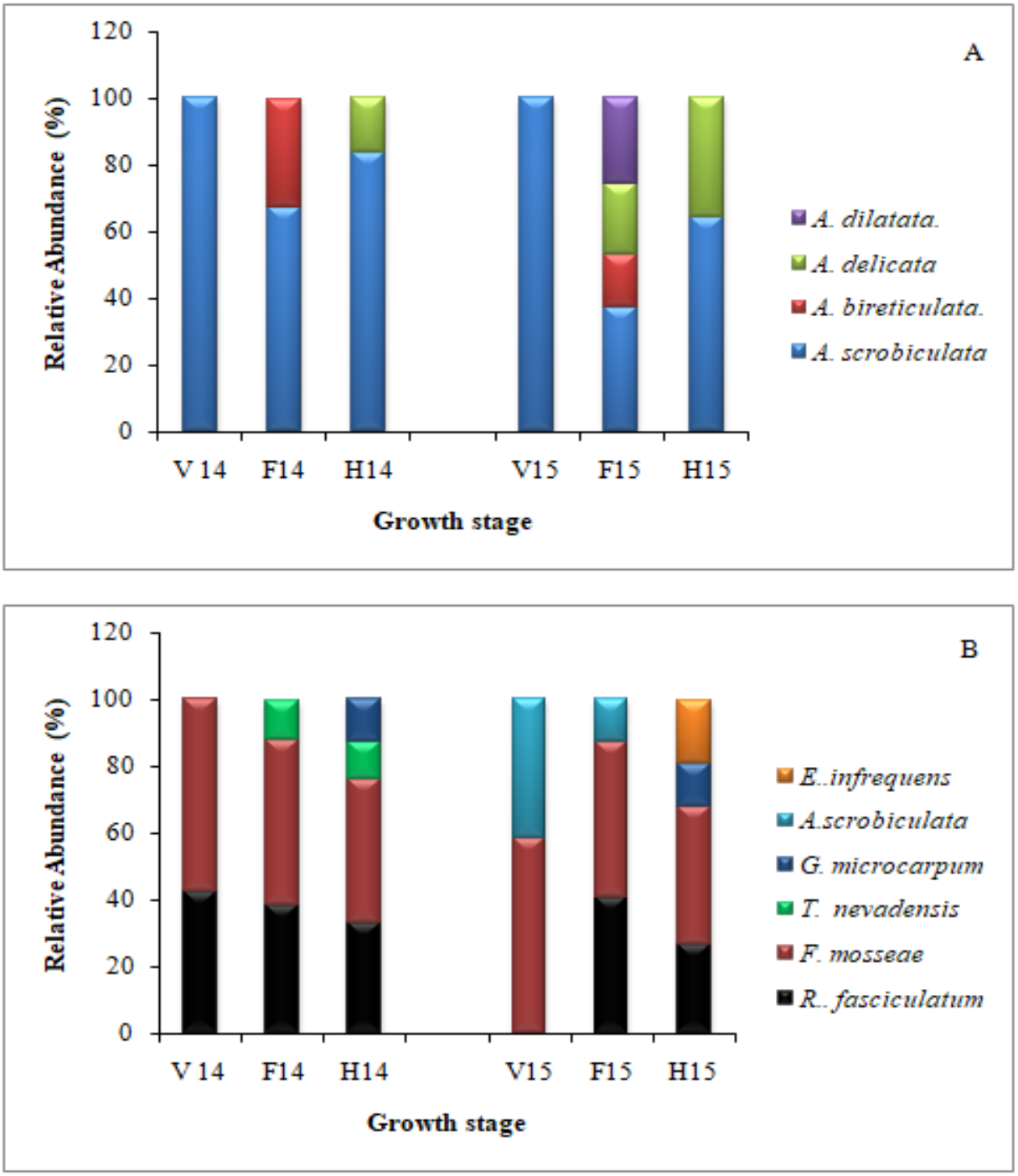
Legend: * = Significant at P< 0.05; ** = significant at P< 0.01, OC = Organic Carbon; N = Nitrogen; P = Phosphorus; K = Potassium; Fe = Iron; Mn = Manganese; Cu = Copper; Zn = Zinc.

5.3.5. Correlation between adaphic factors and spore density

At Tuem, significant correlation has been observed between spore density and P, Mn and Cu. However, no significant correlation existed between spore density and EC, OC, N, K, Fe and Zn. At Sikeri, a significant correlation was observed between spore density and EC, P and Mn,

while no significant correlation was observed between spore density and pH, OC, N, K, Fe, Cu and Zn **(Table 26)**.

Fig.14: Species wise spore abundance in rice cultivated at the study sites.



Legend: Sites: A - Tuem, B – Sikeri; Bars represents Relative Abundance AM species in different growth stages during 2014 and 2015, V14- Vegetative stage of 2014; F 14- Flowering stage of 2014; H14- Harvesting stage of 2014, V15- Vegetative stage of 2015; F 15- Flowering stage of 2015; H15- Harvesting stage of 2015.

5.3.6.1. AM species richness

Six AM fungal genera *viz., Acaulospora* (**Plate VII a, c, d)**, *Glomus* **(Plate VIII d)**, *Funneliformis* **(Plate VIII e)**, *Rhizoglomus, Tricispora* **(Plate VIII f)** and *Entrophospora* were recovered from rhizosphere soils of the two study sites. *Acaulospora* was the most dominant genera. Maximum AM species were recovered from Sikeri (6 spp.) compared to Tuem (4 spp.). *Acaulospora scrobiculata* was common in both the sites. In Tuem, *A. scrobiculata, A. bireticulata* and *A. delicata* were recorded in the year 2014. In addition to the above species, *A. dilatata* was recorded in 2015. In Sikeri, *Rhizoglomus fasciculatum, Funneliformis mosseae, Tricispora nevadensis* and *Glomus microcarpum* were recovered in 2014. While in 2015 two additional AM species *viz., A. scrobiculata and Entrophospora infrequens* were recorded **(Fig.14)**.

5.3.6.2. AM diversity Index

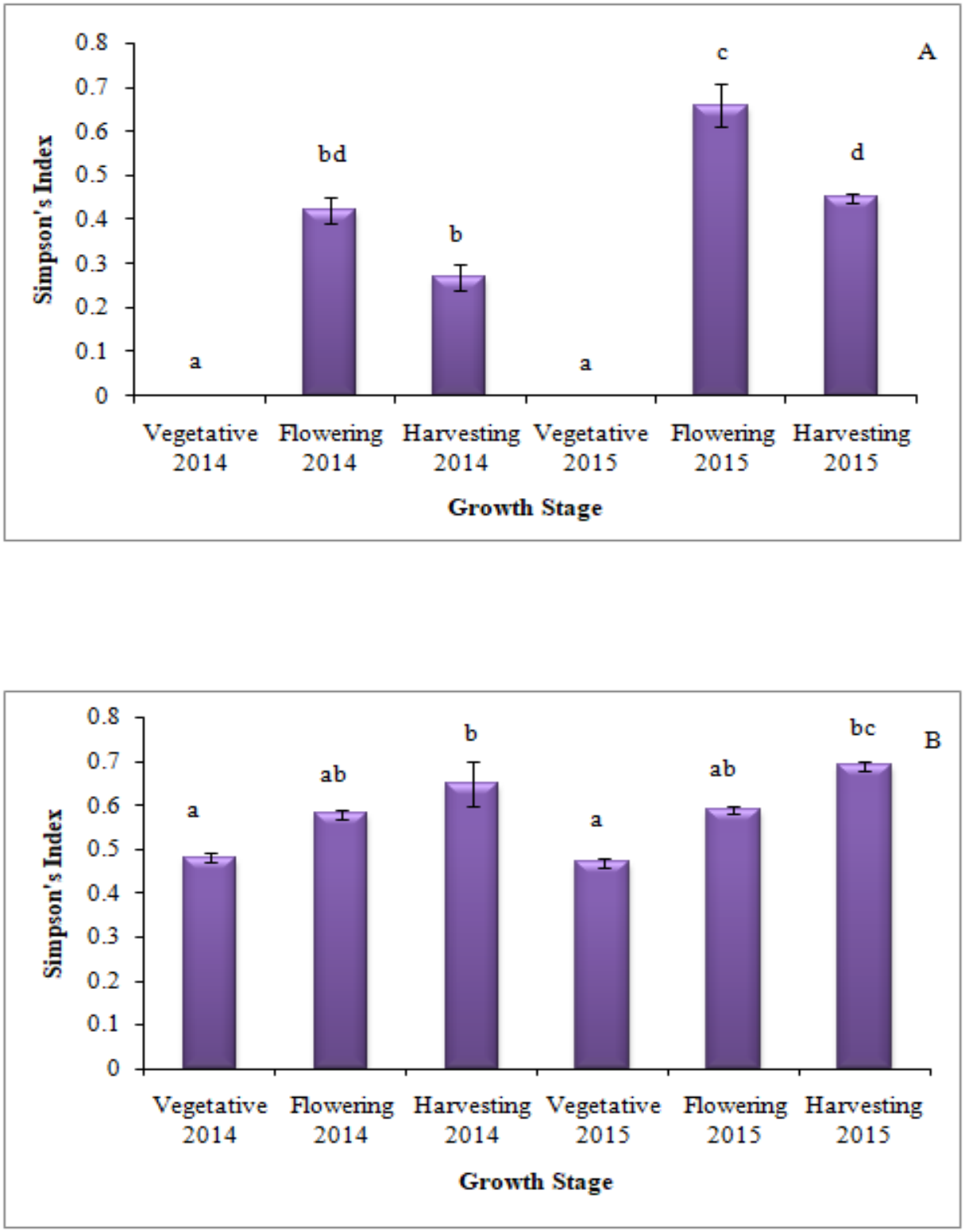
Simpson's and Shannon's Index **(Table 27)**, showed variation between the different growth stages for both the years of study. In Tuem maximum AM species diversity index was recorded at the flowering stage while at Sikeri maximum was recorded at the harvesting stage for both the years **(Fig. 15 and 16)**.

Table 27: Diversity Measurement of AM fungal communities at the study sites.

Site	Ecological Parameters	Vegetative stage		Flowering stage		Harvesting stage		$F_{(5,12)}$, p<0.05
		2014	2015	2014	2015	2014	2015	
Tuem	Shannon - Index (*H*)	0.00^{a}± 0.00	0.00^{a}± 0.00	0.55^{ab}± 0.03	1.07^{b}± 0.26	0.38^{ac}± 0.03	0.58^{bc} ± 0.05	13.45*
	Simpsons Index (*D*)	0.00^{a}± 0.00	0.00^{a} ± 0.00	0.43^{bd}± 0.04	0.66^{c}± 0.05	0.27^{b}± 0 .03	0.45^{d} ± 0.02	69.25*
Sikeri	Shannon - Index (*H*)	0.67^{a} ± 0.01	0.67^{a} ± 0.02	0.96^{b} ± 0.21	0.97^{b} ± 0.02	1.19^{bc} ± 0.10	1.28^{c} ± 0.04	26.24*
	Simpsons Index (*D*)	0.48^{a} ± 0.01	0.47^{a} ± 0.01	0.59^{ab} ± 0.01	0.59^{ab} ± 0.01	0.65^{b} ± 0.05	0.69^{bc} ± 0.02	12.13*

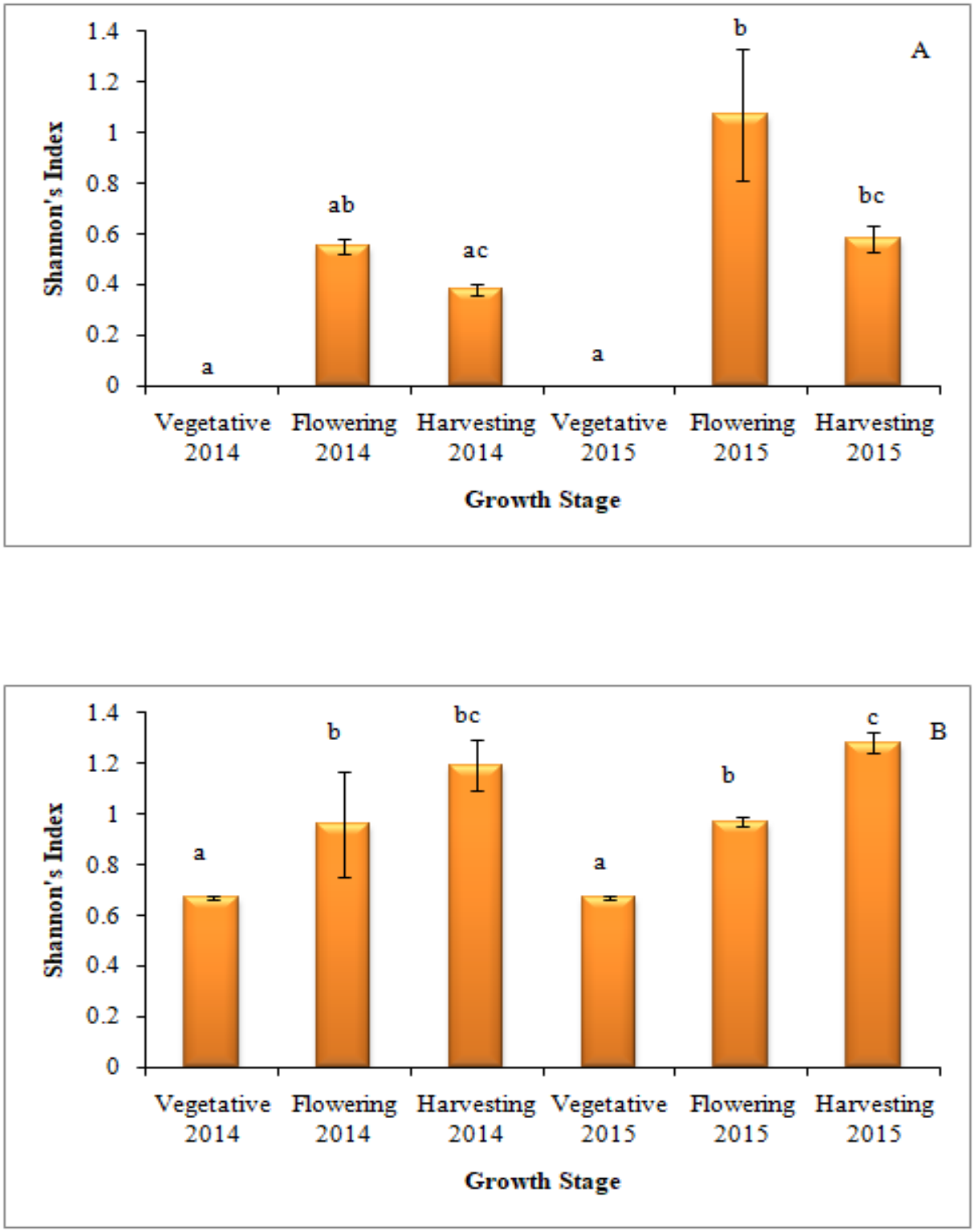
Legend: Values are mean of three readings at each growth phase; different letters within a site of study between the growth stages indicates significant differences; ± indicates Standard Error.

Fig. 15: Simpson's Index at the study sites.



Legend: Sites: A - Tuem, B – Sikeri; Error bars represents Standard Error; Values on the column affected by the same letter are not significantly different between the growth stages of the two years of study.

Fig. 16: Shannon Index of Diversity at the study sites.



Legend: Sites: A - Tuem, B – Sikeri; Error bars represents Standard Error; Values on the column affected by the same letter are not significantly different between the growth stages of the two year of study.

5.3.6.3. Relative Abundance (RA) of AM fungi

In Tuem, maximum RA was observed in *A. scrobiculata* during all the growth stages for both the years with the highest RA recorded in the vegetative stage **(Table 28; Fig. 14)**. Canonical correspondence analysis (CCA) was performed to understand the correlation of AM fungal abundance and physico-chemical properties of soil in Tuem. The length of arrow in the CCA plot illustrates the relative importance affecting the community, while the angle between the variables denotes the degree to which factors are correlated **(Fig. 17)**. The resulting ordination is presented in **Tables 29 and 30.** The eigen values of the first and second axis was 0.31 and 0.21 respectively. The cumulative percentage variance of species data showed 51.49% and 86.07% of variability on the first and second axis, respectively showing a high RA of *A. scrobiculata* during the vegetative stage for both the years of study which was closely related to Cu content in the soil. *A. bireticulata* showed high RA during the flowering stage which is closely related to Cu content. High RA of *A. delicata* and *A. dilatata* was observed during the flowering stage which was closely related to P level.

Table 28: RA and frequency of occurrence of AM fungi at different growth stages of *O. sativa* var. Jyoti in Tuem.

Sr. No.	AM species	Relative Abundance (%)						Frequency of occurrence (%)
		Vegetative stage		Flowering stage		Harvesting stage		
		2014	2015	2014	2015	2014	2015	
1.	*Acaulospora scrobiculata* Trappe	100	100	67.13	37.34	83.50	64.42	100
2.	*Acaulospora bireticulata* Rothwell & Trappe	-	-	32.52	15.75	-	-	33.33
3.	*Acaulospora delicate* Walker Pfeffer & Bloss	-	-	-	21.24	16.50	35.56	50.00
4.	*Acaulospora dilatata* Morton	-	-	-	25.63	-	-	16.66
	Species Richness (SR)	01	01	02	04	02	02	-

Legend: Data presented is the means of three readings at each growth phases

Table 29: CCA variable scores at Tuem.

Biological variables	Axis 1	Axis 2
A. sc.	-0.27	0.03
A. bi.	0.86	1.19
A. de	0.44	-0.87
A. di	2.03	-0.32
Eigen value	0.31	0.21
Variation %	51.49	34.57
Cummulative %	51.49	86.07

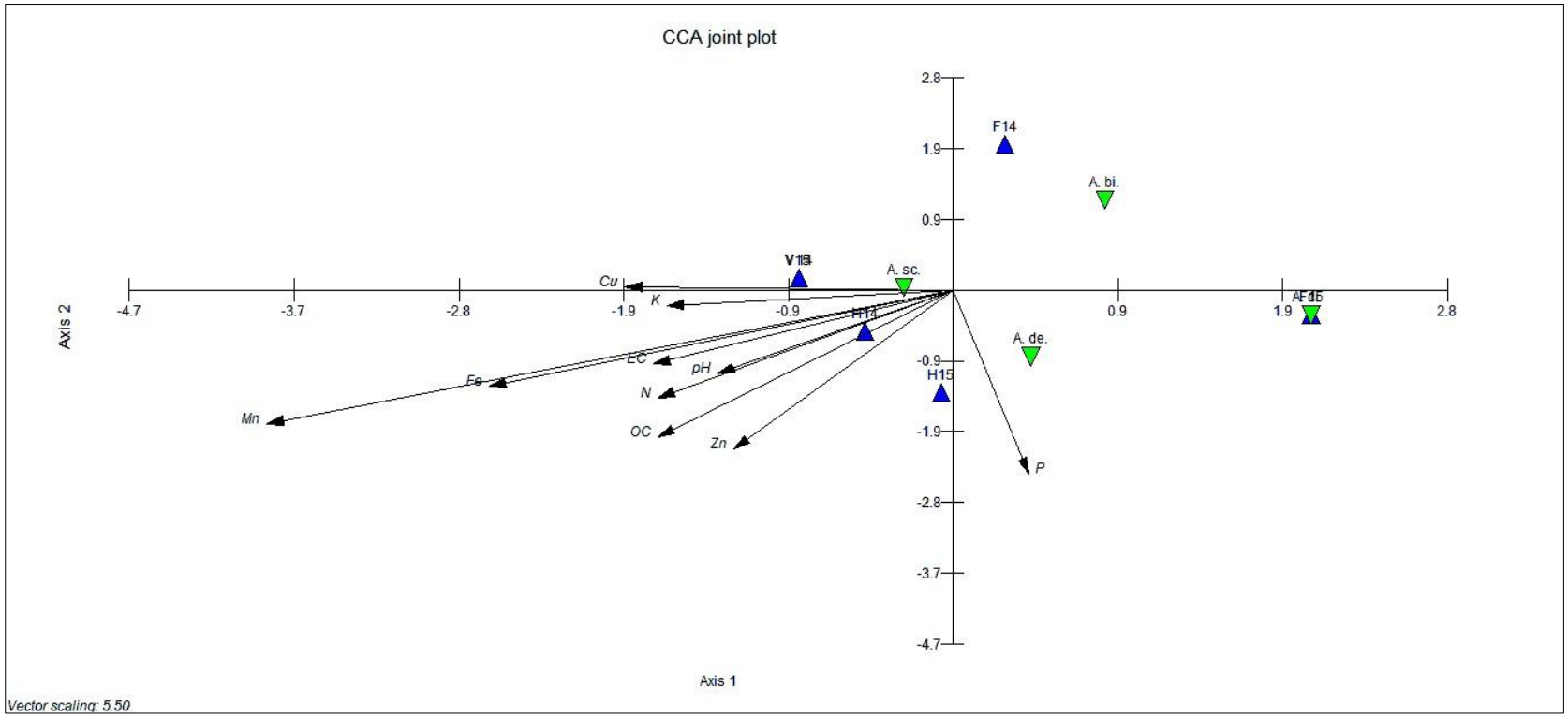
Legend: *A. sc.= Acaulospora scrobiculata; A. bi.= A.bireticulata; A.de.= A. delicata; A. di= A. dilatata*

Table 30: Biplot scores for soil variables at Tuem.

Soil variable	Axis 1	Axis 2
pH	-0.24	-0.19
EC	-0.30	-0.17
OC	-0.30	-0.35
N	-0.30	-0.25
P	0.07	-0.44
K	-0.29	-0.03
Fe	-0.47	-0.23
Mn	-0.70	-0.32
Cu	-0.33	0.00
Zn	-0.22	-0.38

Legend: OC = Organic Carbon; N = Nitrogen; P = Phosphorus; K = Potassium; Fe = Iron, Mn = Manganese; Cu = Copper; Zn = Zinc.

Fig.17: CCA of soil physico-chemical properties and relative spore abundance percent of different AM species in Tuem.



Legend: Diagram of CCA of soil properties pH, EC, OC, N, P, K, Fe, Mn, Cu, Zn and species wise relative abundance distribution; V14- Vegetative stage of 2014; F 14- Flowering stage of 2014; H14- Harvesting stage of 2014, V15- Vegetative stage of 2015; F 15- Flowering stage of 2015; H15- Harvesting stage of 2015, OC = Organic Carbon; N = Nitrogen; P = Phosphorus; K = Potassium; Fe = Iron, Mn = Manganese; Cu = Copper; Zn = Zinc; *A. sc.=Acaulospora scrobiculata; A. bi.= A. bireticulata; A.de.=A. delicata; A. di= A. dilatata*.

At Sikeri, maximum RA was observed in *F. mosseae* during all the growth stages for both the years with the highest RA recorded in the vegetative stage **(Table 31; Fig. 14)**. The potential correlation of AM fungal abundance and physico-chemical properties was performed by CCA **(Fig. 18)**. The resulting ordination is presented in **Tables 32 and 33.** The eigen values on the first and second axis was 0.36 and 0.20, respectively. The cumulative percentage variance of species data showed 54.57% and 84.88% of variability on the first and second axis respectively. Even though, *A. scrobiculata* was not recovered in the year 2014, it showed higher RA during the vegetative stage in the year 2015 and showed strong correlation to Cu, followed by N. *Rhizoglomus fasciculatum* showed high RA in the vegetative stage during 2014 and showed a correlation with EC, K, P and Fe in the soil. *Funneliformis mosseae* showed higher RA in 2015 at the vegetative stage and showed correlation with Cu and N content.

Table 31: RA and frequency of occurrence of AM fungi at different growth stages of *O. sativa* var. Jyoti in Sikeri.

Sr. No.	AM species	Relative Abundance (%)						Frequency of occurrence %
		Vegetative stage		Flowering stage		Harvesting stage		
		2014	2015	2014	2015	2014	2015	
1	*Acaulospora scrobiculata* Trappe	-	41.50	-	12.58	-	-	33.33
2	*Rhizoglomus fasciculatum* (Thaxt.) Sieverd, Silva & Oehl comb. nov.	42.60	-	38.72	40.82	33.09	27.00	83.33
3	*Funneliformis mosseae* (Nicolson & Gerd.) Walker & Schüssler	57.39	58.49	49.46	46.58	42.94	40.99	100
4	*Glomus microcarpum* Tulasne & Tulasne	-	-	-	-	12.62	12.67	33.33
5	*Tricispora nevadensis* (Palenz., Ferrol, Azćon-Aguilar & Oehl) Oehl. Palenz.	-	-	11.79	-	11.33	-	33.33
6	*Entrophospora infrequens* (Hall) Ames & Schneid	-	-	-	-	-	19.31	16.66
Species Richness		02	02	03	03	04	04	-

Legend: Values are mean of three readings.

Table 32: CCA variable scores at Sikeri.

Biological variables	Axis 1	Axis 2
R. fe	-0.35	-0.24
F. mo	0.09	-0.04
T. ne	-0.55	-0.73
G. mi	-0.82	0.77
A. sc	1.65	0.32
E. in	-0.97	1.97
Eigen value	0.36	0.20
Variation %	54.57	30.31
Cummulative %	54.57	84.88

Legend: *A. sc =Acaulospora scrobiculata; R. fa = Rhizoglomus fasciculatum; F. mo = Funneliformis mosseae; T. ne = Tricispora nevadensis; G. mi = Glomus microcarpum*; *E. in = Entrophospora infrequens.*

Table 33: Biplot scores of soil variables at Sikeri.

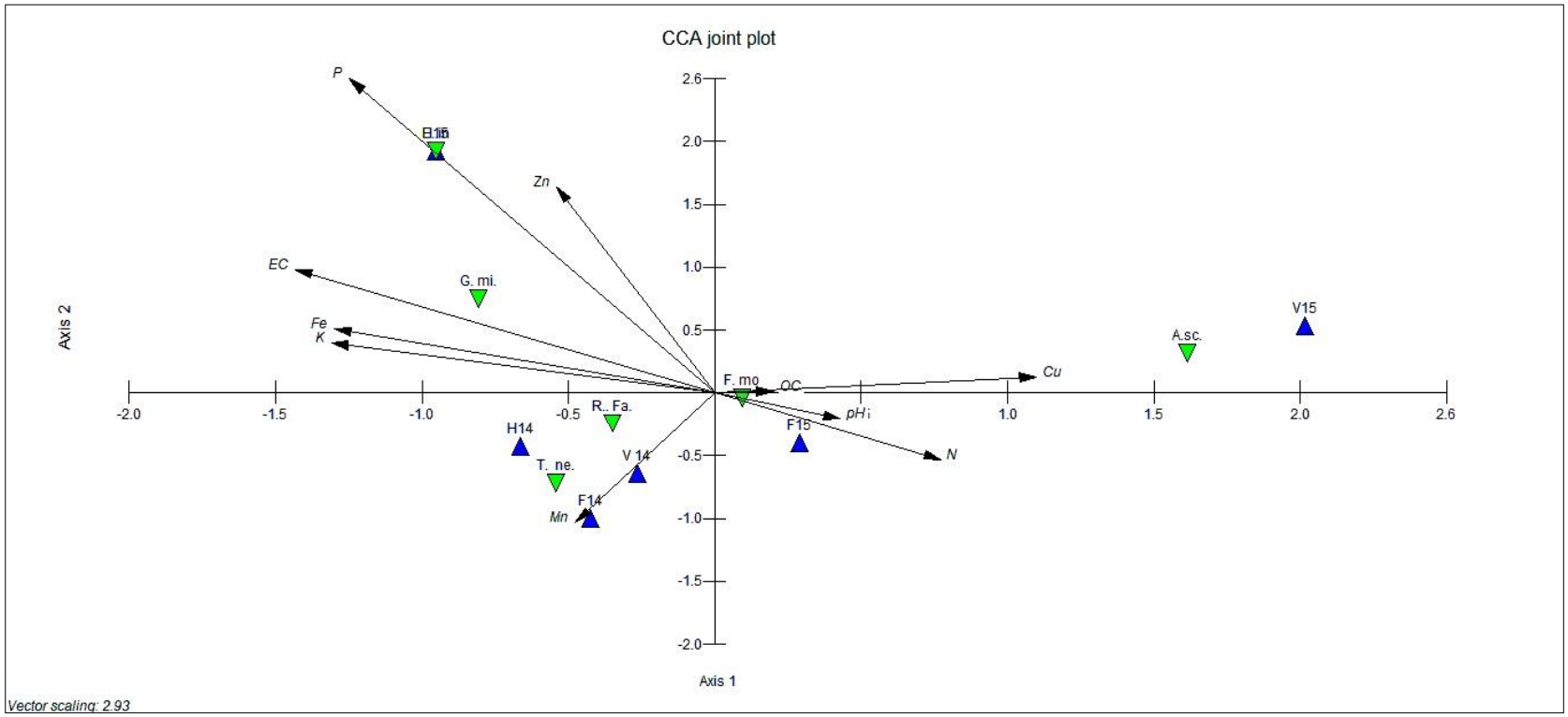
Soil variable	Axis 1	Axis 2
pH	0.15	-0.07
EC	-0.50	0.34
OC	0.07	0.00
N	0.26	-0.18
P	-0.43	0.87
K	-0.45	0.13
Fe	-0.45	0.17
Mn	-0.16	-0.36
Cu	0.38	0.04
Zn	-0.18	0.57

Legend: OC = Organic Carbon; N = Nitrogen; P = Phosphorus; K = Potassium; Fe = Iron, Mn = Manganese; Cu = Copper; Zn = Zinc.

5.3.6.3. Frequency of occurrence of AM fungi

In Tuem, frequency of occurrence was maximum for *A. scrobiculata*, as it was recorded in all the growth stages in both the years **(Table 28)**. In Sikeri, maximum frequency of occurrence was observed for *F. mosseae* **(Table 31)**.

Fig. 18: CCA of soil physico-chemical properties and relative spore abundance percent of different AM species in Sikeri.



Legend: Diagram of CCA of soil properties pH, EC, OC, N, P, K, Fe, Mn, Cu, Zn and species wise relative abundance distribution;V14- Vegetative stage of 2014; F14-Flowering stage of 2014; H14- Harvesting stage of 2014; V15- Vegetative stage of 2015;F15- Flowering stage of 2015; H15-Harvesting stage of 2015, OC = Organic Carbon; N = Nitrogen; P = Phosphorus; K = Potassium; Fe = Iron, Mn = Manganese; Cu = Copper; Zn = Zinc; *A. sc = Acaulospora scrobiculata; R. fa. = Rhizoglomus fasciculatum; F. mo. = Funneliformis mosseae; T. ne. = Tricispora nevadensis; G. mi. = Glomus microcarpum*; *E. in. = Entrophospora infrequens*.

Table 34: Analysis of variance for AM colonization at Tuem and Sikeri for the years of study and phenology of rice plant.

	Tuem					Sikeri				
Source	***df***	***SS***	***MS***	***F***	***sig.***	***df***	***SS***	***MS***	***F***	***sig.***
Year	1	1645.65	1645.65	29.70*	0.00	1	1829.11	1829.11	34.84*	0.00
Phenology	2	1493.18	746.59	13.47*	0.00	2	6426.57	3213.28	61.20*	0.00
Year x Phenology	2	63.03	31.51	0.56	0.56	2	308.66	156.33	2.94	0.09
Error	12	664.80				12	630.00			
Total	18	108292					66961.50			

Legend: *df* – Degree of freedom; *SS* - Sum of Squares; *MS*- Mean Square; *F*- Fisher's value; * significant at P < 0.05

Table 35: Analysis of variance of AM spore density at Tuem and Sikeri for the years of study and phenology of rice plant.

	Tuem					Sikeri				
Source	***df***	***SS***	***MS***	***F***	***sig.***	***df***	***SS***	***MS***	***F***	***sig.***
Year	1	26373.38	26373.38	3680.00*	0.00	1	1.38	1.38	0.05	0.80
Phenology	2	70600.11	35300.05	4925.58*	0.00	2	2529.33	1264.66	45.52*	0.00
Year x Phenology	2	43582.11	21791.05	3040.61*	0.00	2	656.44	328.22	11.81*	0.00
Error	12	86.00				12	333.33	27.77		
Total	18	275011					17801.00	27.77		

Legend: *df* – Degree of freedom; *SS* - Sum of Squares; *M S*- Mean Square; *F*- Fisher's value; * significant at P < 0.05

5.3.6.5. Effect of period of study and plant phenology on AM colonization and Spore density

Univariate analysis of variance (Two way ANOVA) was calculated to compare the effect of year of study and plant phenology on percent AM colonization at Tuem and Sikeri. Higher the Fisher value greater is the influence. Both the year and plant phenology had an significant effect in Tuem at $F_{(1,12)} = 29.70$; $p < 0.05$ and $F_{(2,12)} = 13.47$; $p < 0.05$ as well as in Sikeri at $F_{(1,12)} = 34.84$; $p < 0.05$ and $F_{(2,12)} = 61.20$; $p < 0.05$, respectively **(Table 34)**.

For spore density, univariate analysis of variance (Two way ANOVA), revealed that it was significant for year ($F_{(1,12)} = 3680.00$; $P < 0.05$) and plant phenology ($F_{(2,12)} = 4925.58$; $P < 0.05$) at Tuem. At Sikeri, spore density showed nonsignificant effect on year ($F_{(1,12)} = 0.05$; $p < 0.05$), but a significant effect on plant phenology ($F_{(2,12)} = 45.52$; $p < 0.001$) **(Table 35)**.

5.4. Discussion

In the present study, AM colonization was recorded in all the three growth stages. Similar observations were reported earlier by Watanarojanaporn *et al.* (2013), who reported that AM fungi are present at early growth stages in rice roots in conventional rice fields (paddy wetlands). However, Vallino *et al.* (2009) and Lumini *et al.* (2011) reported low or lack of AM fungal colonization in rice roots grown under conventional system. Average root colonization varied in different growth stages at the two sites for both the years of study. Mean Root colonization gradually increased from vegetative to flowering stage and then decreased at the harvesting stage. This variation is attributed to dependence of AM fungi on plant growth and turn over (Lugo *et al.* 2005). According to Kaschuk *et al.* (2009), lower rate of AM colonization at fruiting stage may be due to the decrease in the rate of photosynthesis leading to lower photosynthate supply to the roots.

Mean spore density reduced from vegetative to flowering stage and then increased during the harvesting stage in the year 2014 at both the study sites. However, in the year 2015 there was a gradual increase in the mean spore density from vegetative to flowering to harvesting stage. This variation in spore density may be due to the interspecific competition and difference in the timing of spore production in the associated host plant (Brundrett and Kendrick 1990), fungal dormancy and its distribution patterns (Zhao 1999). There was no significant correlation between spore density and root colonization at Tuem but a significant negative correlation existed at Sikeri. Miller *et al.* (1995) reported that spore number poorly reflects the colonization potential of soil and it may not always relate to rate and extent of AM colonization (Abbott and Robson 1982). Germination potential of AM fungi vary at different times of the year (Gemma and Koske 1988) as well as its adaptability to particular soil condition may result in absence of correlation (Dhar and Mridha 2003). He *et al.* (2002)

observed that during favourable soil condition there exist an increase in AM colonization and decrease in spore number resulting in negative correlation between AM colonization and spore density.

Composition and richness of AM fungal species showed variation at different phenological stages at the two study sites. However *A. scrobiculata* was common in both the sites. According to Nemec *et al.* (1981) the occurrence of certain AM fungal species in an area depicts specificity of certain species to that area. In Tuem, four different species of genus *Acaulospora* were observed with *A. scrobiculata* being the most frequently occurring and showed the highest relative abundance at the different growth stages. The acidic nature of the rice fields may explain the dominance of *A. scrobiculata* (Stutz *et al.* 2000). In the vegetative stage, only *A. scrobiculata* was detected. However, during flowering stage maximum number of AM species was detected. In Sikeri, *F. mosseae* was the most frequently occurring species and showed highest relative abundance at different growth stages. Maximum species richness of AM fungi was recorded during the harvesting stage. Bever *et al.* (1996) suggested that inter specific fungal interaction and host preference may play a role in determining AM fungal composition and distribution during various growth stages. The present study is in agreement with earlier study by Khade and Rodrigues (2010), who reported temporal variation in spore density, spore type and AM colonization occurring in each site and were differing among sites. Maximum Shannon's and Simpson's Diversity Index was recorded in the flowering stage at Tuem while it was maximum in the harvesting stage at Sikeri. Rosendahl and Stukenbrock (2004) suggested that change in functional role of symbiotic organism in the plant cycle lead to variation in diversity. In the present study, difference in AM species and diversity index between sites could be due to variance in abundance and its geographic distribution, difference in soil fertility, and environmental conditions as observed in earlier studies (Gosling *et al.* 2006, Lumini *et al*. 2011, Barber *et al*. 2013).

Univariate analyses revealed that time of study (year) and phenology (growth stages) co-affected AM colonization and spore density. It was observed that phenology of the plant has shown to have greater influence than the time of study. According to Su *et al*. (2011) time and host phenology are important features stimulating AM colonization and spore density in an

ecosystem. As the host controls C supply to roots, supply secondary metabolites and modifies the soil condition with time.

5.5: Conclusion

From the present study, it is concluded that the composition and association between AM fungi and a rice variety does vary with space and time. It is also observed that the phenology of the plant also influences the extent of AM association in rice. However, further work needs to be carried out to understand whether AM species show preference to any particular ecology.

CHAPTER 6

Assessment of AM fungal root colonization in different varieties of rice (*Oryza sativa* L.) grown in the *Khazan*, *Ker* and *Morod* lands of Goa.

6.1. Introduction

Rice is cultivated in various ecosystems. Based on hydrology, it is roughly classified as irrigated, rain fed lowland, upland and flood prone. Rainfall patterns, its intensity, land topography, soil properties and drainage determine the degree of flooding (Ito *et al.* 1999). Approximately half of the world rice area is irrigated and the remaining, is distributed among rain fed lowland (25%), uplands (13%) and flood-prone (9%) (Herdt and Palacpac 1983). In Goa too rice is cultivated in three different topographical situations *i.e.* upland (*Morod*), midland (*Ker*) and lowland (*Khazans*) from June to October. Rice cultivation in uplands accounts for 16.4%, in midland and lowlands 32% each of the total rice area in the state (Manjunath *et al*. 2011).

Rice plants readily form AM association in upland (Maiti *et al*. 1995) and midland condition (Toppo *et al.* 2012), but under submerged condition colonization is rare due to anoxic condition (Ilag *et al.* 1987). Solaiman and Hirata (1998) observed a drop in the rate of AM fungal colonization in rice roots in wet as compared to dry areas. They also observed that with the growth of rice plant there was a fall in the rate of AM colonization. The growing understanding of existence of AM fungi in wetland ecosystem (Ipsilantis and Sylvia 2007, Wang *et al*. 2011) lead to the deduction that soil conditions control the AM status of the host and that these fungi are obligate aerobes in nature (Barea 1991).

In the recent past, attempts have been made to obtain suitable formulation for AM fungal inocula and appropriate ways for their application in the field (Gianinazzi and Vosatka 2004). The development of AM inoculum, one has to take into account the indigenous AM fungal population. Hence the aim of the present work was to study the distribution of native AM fungi and with an objective to formulate AM inocula for different ecosystem.

6.2. Materials and Methods

6.2.1. Study Site

Rhizosphere soil samples of three rice varieties *viz.*, Jyoti, Khonchri and Jaya were collected from lowland, midland and upland during vegetative, flowering and harvesting stages for two consecutive years *viz.*, 2015 and 2016 **(Table 16)** and brought to the laboratory for further processing.

In Goa, depending upon where rice is cultivated, the cultivation process varies. When rice is cultivated in uplands, the growing period is approximately 115-120 days. Land is prepared by ploughing early in the season followed by leveling so that the field is ready for sowing before the regular onset of monsoon. Plantlets are transplanted by planting uniformly in lines spaced by 20cm. Transplanting is carried out with a thin film of water. The crop grown in the midland has relatively longer growing period (approx. 130-135 days). Seedlings are raised in wet or dry nurseries after germination. The seedlings are ready for transplanting after 21-24 days. Three to four seedlings are planted per hill at a distance of 20 x 10 cm. Seedlings are transplanted in fields that are ploughed and leveled at the first shower. In both uplands and midlands, fertilizers are used and as weed infestation are high, weedicides or hand weeding is carried out. Rice cultivation in the lowland occupies an area of 32% of the rice area in the state, with varieties having growth duration of approximately 105-115 days. Fields are ploughed in the summer. Seeds are either broadcasted or transplanted by raising a nursery. However in the lowland, it is essential to sow at regular onset of monsoon after ensuring flushing of salts from the fields. Besides the weed infestation is relatively low (Manjunath *et al*. 2011).

6.2.2. Collection of rhizosphere soil samples

Three healthy plants were randomly selected at three different growth stages from three different ecologies. Rhizosphere soil and root samples were collected and further processed as described under **5.2.2.**

6.2.3. Root processing

Root processing was carried out as described under **3.2.2.**

6.2.4. Estimation of percent root colonization

Estimation of AM root colonization was carried out as described under **3.2.3.**

6.2.5. Isolation of AM spores

Isolation of AM spores was carried out as described under **3.2.5.**

6.2.6. Estimation of AM spore density

Estimation of AM spore density was carried out as described under **3.2.6.**

6.2.7. Taxonomic identification of AM fungi

Taxonomic identification of AM fungi was carried out as described under **3.2.7.**

6.2.8. Diversity Studies

6.2.8.1. Diversity Index

AM species diversity index at the three studies ecologies was carried out as described under **3.2.8.2.**

6.2.8.2. AM species richness

AM species richness at each growth stage in each ecological site was carried out as described under **3.2.8.1.**

6.2.8.3. Relative abundance (RA %)

Relative abundance of AM fungal species at different growth stages in the three ecologies was carried out as described under **3.1.8.3.**

6.2.8.4. Frequency of occurrence (%)

Species wise frequency of occurrence (%) at each ecological was carried out as describes under **5.2.8.4.**

6.2.9. Statistical Analysis

6.2.9.1. Analysis of data of AM fungi colonization and spore density at each growth stage at the three ecological sites were carried out as described under **3.2.9.1.**

6.2.9.2. Relationship of AM fungal root colonization, spore density and edaphic factors at each ecology were carried out as described under **5.2.9.2.**

6.2.9.3. Relationship between relative abundance of AM fungi and physico-chemical properties of soil at the different ecologies two study sites was carried out as described under **5.2.9.3.**

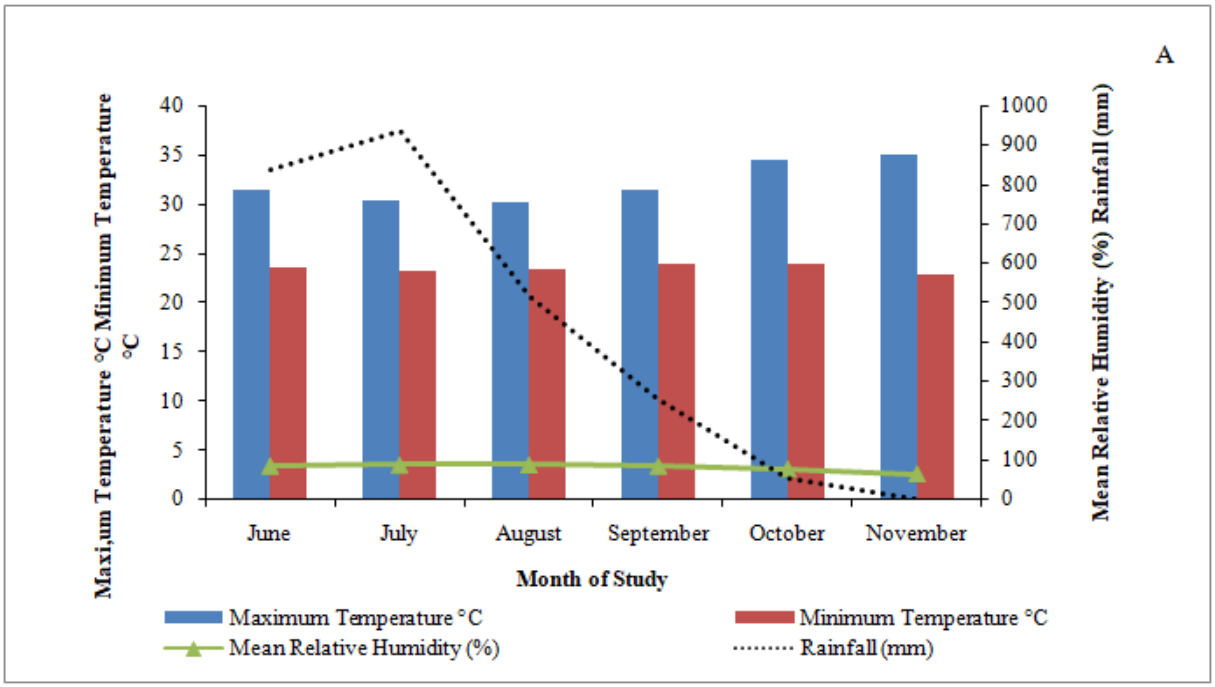
6.3. Results

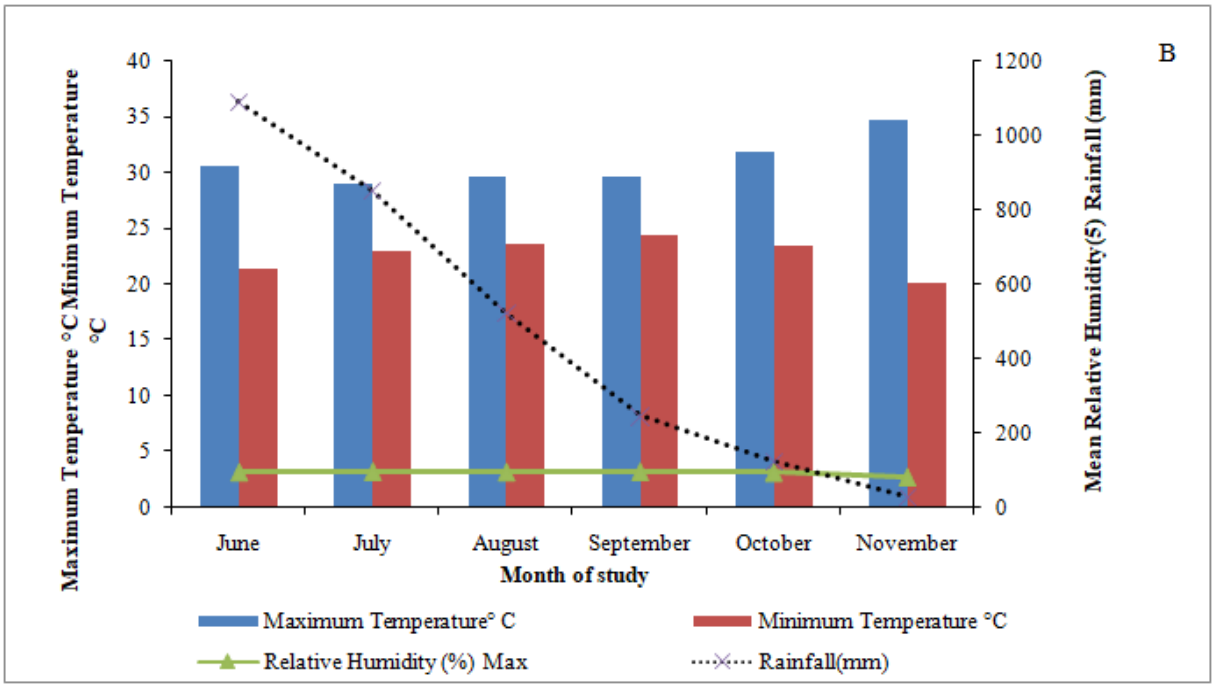
6.3.1. Climate Data

Climate data during the study period is depicted in **Fig. 19**. There were no significant differences in the climatic conditions in the two year of study period except for relative humidity $F_{(1,10)} = 5.81$ ($p < 0.05$). However the average temperature was higher in the year 2015 whereas relative humidity and rainfall was higher in the year 2016.

In the year 2015, the average relative humidity was 81.31%, rainfall was 432.52mm and maximum and minimum temperature was 32.12 and 23.40°C, respectively. While in the year 2016, the average relative humidity was 91.73%, rainfall was 476.95mm, maximum and minimum temperature was 30.95 and 22.68°C, respectively.

Fig. 19: Climate data recorded during the study period (2015 and 2016).





Legend: A-2015; B-2016.

6.3.2. AM root colonization

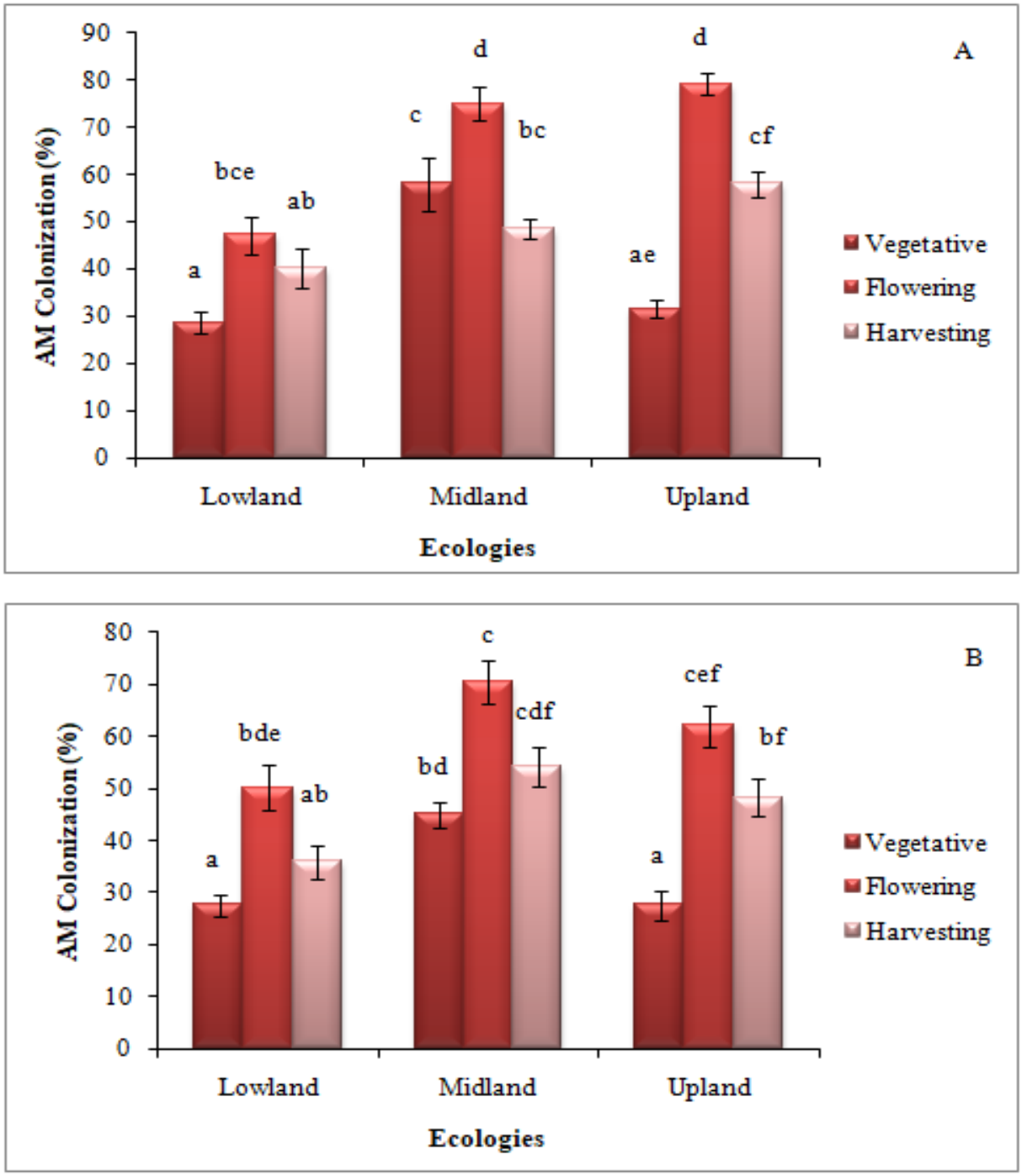
In all the three rice varieties the different land types and growth stages had significant effect on AM colonization for both years of study **(Fig. 20)**. All the three rice varieties *viz*., Jyoti, Khonchri and Jaya showed maximum colonization during the flowering stage in the three studied ecologies **(Tables 36 and 37; Fig. 20)**. No significant difference in AM colonization was observed for both the years of study at $p < 0.05$ when, $F_{(1,160)} = 2.97$. Maximum colonization was observed in the year 2015 (51.90%) compared to 2016 (46.98%). In relation to ecology, mean percent colonization showed a significant difference at $p < 0.05$, $F_{(2,159)} = 20.15$. Maximum root colonization was recorded in the midland (58.55%) **(Plate 5 a, b, c d)** followed by upland (51.09%) **(Plate 6 a, b, c, d)** and minimum in the lowland (38.67%) **(Plate 4 c, d, f)**. In relation to phenology, a significant difference in mean root colonization was observed at the different growth stages *viz*., vegetative (36.39%), flowering (64.87%) and harvesting (47.05%) stage at $p < 0.05$, $F_{(2,159)} = 56.41$.

6.3.3. Correlation between adaphic factors and AM colonization

In the year 2015, a non-significant correlation was observed between AM colonization and adaphic factors in lowlands and midlands. But in uplands a significant negative correlation was observed between colonization and pH ($r = -0.72$, $p = 0.05$) and with Zn ($r = -0.68$, $p = 0.05$) **(Table 38)**.

In the year 2016, a non-significant correlation was observed between AM fungal colonization and adaphic factors in the lowland **(Table 38**). However in midland a significant positive correlation was observed for OC ($r = 0.78$, $p = 0.05$) and N ($r = 0.79$, $p = 0.05$). Similarly a significant negative correlation did exist for Cu ($r = -0.74$, $p = 0.05$). In uplands a significant negative correlation was observed with pH ($r = -0.80$, $p = 0.01$) and a positive correlation with OC ($r = 0.69$, $p = 0.05$). Irrespective of year or ecology there was no significant correlation between AM colonization and spore density **(Table 38)**.

Fig. 20: Influence of varied ecology on root colonization.



Legend: Year of study: A - 2015, B –2016; Error bars represents Standard Error; Values on the column affected by the same letter are not significantly different between the growth stage of each years of study.

Table 36: Influence of plant phenology on root colonization at different ecological sites in 2015.

Rice variety	Lowland (*Khazan*)			Midland (*Ker)*			Upland (*Morod*)			$F_{(8,18)}$, p < 0.05
	Vegetative	Flowering	Harvesting	Vegetative	Flowering	Harvesting	Vegetative	Flowering	Harvesting	
Jyoti	22.33^{a} ±1.20	44.00^{bg} ±2.08	33.33^{c} ±0.88	43.33^{bg} ±0.88	79.00^{d} ±2.08	53.00^{e} ±0.49	39.00^{bc} ±0.58	86.66^{f} ±2.03	49.00^{eg} ±0.58	231.95*
Khonchri	26.00^{a} ±1.52	38.33^{b} ±1.20	30.33^{a} ±0.88	50.00^{c} ±1.15	62.00^{d} ±1.15	52.50^{ef} ±1.05	27.33^{a} ±1.20	70.67^{e} ±0.88	57.67^{df} ±1.45	187.93*
Jaya	37.67^{a} ±1.45	64.00^{b} ±1.52	54.00^{c} ±0.57	80.67^{d} ±1.2	84.00^{d} ±1.15	40.33^{a} ±0.88	28.33^{e} ±1.86	80.33^{d} ±0.88	67.33^{b} ±1.20	273.97*

Legend: Data presented is the mean of three readings at each growth phase; different letters within the variety indicate significant differences at P< 0.05; ± indicates Standard Error.

Table 37: Influence of plant phenology on root colonization at different ecological sites in 2016.

Rice variety	Lowland (*Khazan*)			Midland (*Ker)*			Upland (*Morod*)			$F_{(8,18)}$, p < 0.05
	Vegetative	Flowering	Harvesting	Vegetative	Flowering	Harvesting	Vegetative	Flowering	Harvesting	
Jyoti	26.00^{ac} ± 2.31	53.33^{bei} ±1.33	20.67^{a} ±1.33	36.33^{cf} ± 2.19	60.77^{bd} ± 0.67	42.00^{efg} ± 3.46	37.33^{cg} ± 3.71	74.67^{h} ± 1.33	56.67^{di} ± 2.40	57.53*
Khonchri	22.67^{a} ± 1.77	46.00^{bd} ± 2.00	38.67^{ab} ± 1.76	46.67^{bd} ± 2.40	84.00^{c} ± 6.92	58.67^{d} ± 4.37	21.33^{a} ± 2.40	62.00d^{c} ± 2.00	50.67^{bd} ± 4.06	31.47*
Jaya	34.00^{aef} ± 2.00	62.00^{b} ± 4.16	42.67^{acd} ± 0.67	52.00^{bdg} ± 2.00	66.67^{b} ± 1.76	62.00^{b} ± 4.00	24.00^{e} ± 3.06	49.33^{bcfg} ± 3.52	37.33^{aeg} ± 5.81	18.72*

Legend: Data presented is the mean of three readings at each growth phase; different letters within the variety indicate significant differences at P< 0.05; ± indicates Standard Error.

Table 38: Pearson Correlation coefficient (r value) between Root colonization (RC) and Spore density (SD) and between root colonization and pH, EC, N, P, K, Fe, Mn, Cu, and Zn at different ecologies.

Parameter	Lowland		Midland		Upland	
	2015	2016	2015	2016	2015	2016
RC v/s SD	0.21	0.10	- 0.18	0.11	0.31	0.04
RC v/s pH	- 0.11	0.21	- 0.14	- 0.41	- 0.72*	- 0.84**
RC v/s EC	0.40	0.31	- 0.27	0.52	- 0.31	0.59
RC v/s OC	- 0.44	- 0.41	0.58	0.78*	0.61	0.69*
RC v/s N	- 0.46	0.18	- 0.42	0.79*	- 0.01	- 0.49
RC v/s P	- 0.07	0.16	- 0.02	0.05	- 0.31	0.21
RC v/s K	- 0.39	- 0.28	0.59	- 0.49	- 0.59	- 0.53
RC v/s Fe	- 0.23	0.18	0.45	- 0.57	- 0.36	- 0.54
RC v/s Mn	- 0.42	0.14	0.51	0.63	- 0.46	- 0.55
RC v/s Cu	- 0.46	- 0.02	0.47	- 0.74*	- 0.28	0.21
RC v/s Zn	0.09	- 0.07	0.55	- 0.58	- 0.68*	0.45

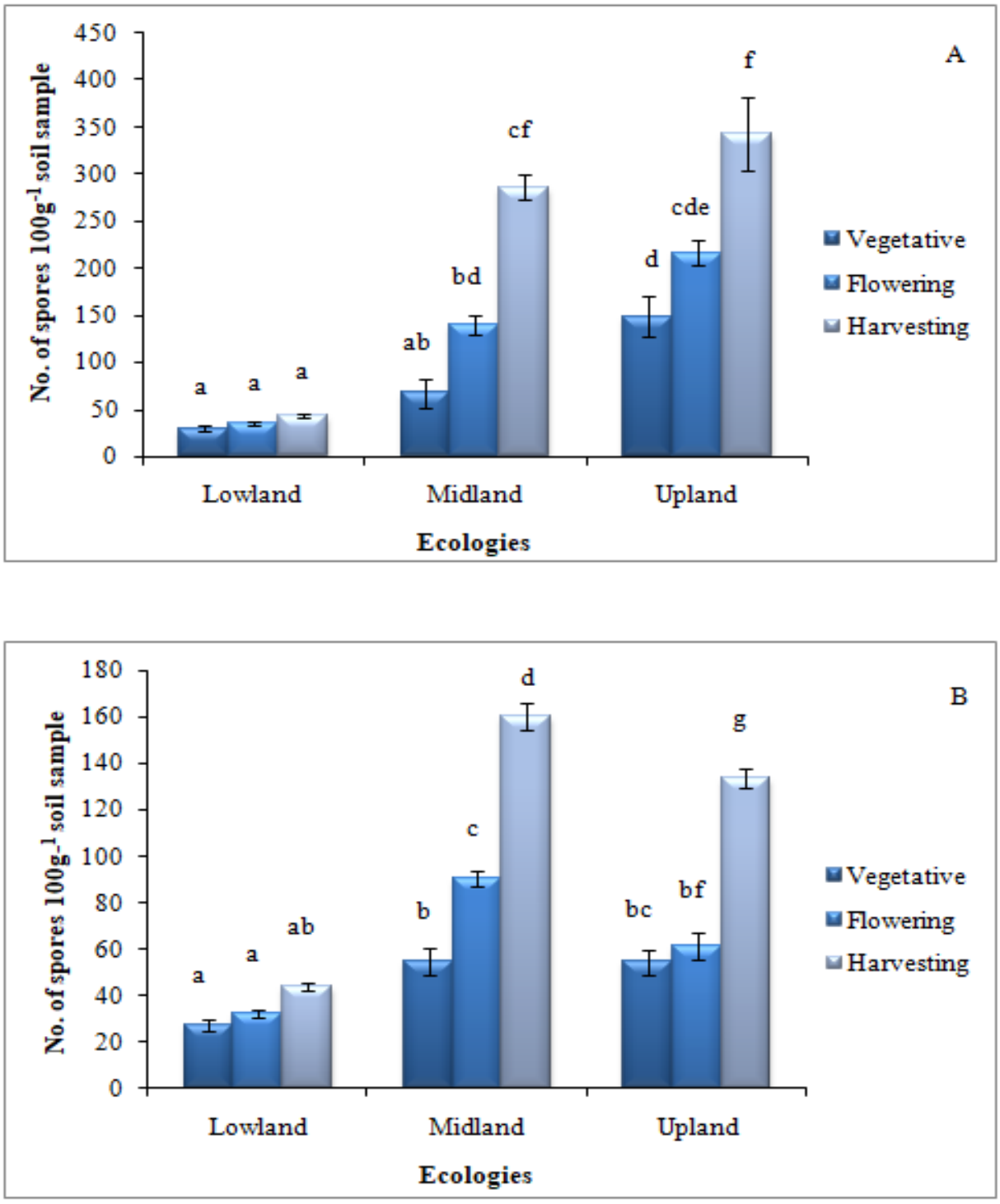
Legend: * significant at $P < 0.05$; **significant at $P < 0.01$. OC = Organic Carbon; N = Nitrogen; P = Phosphorus; K = Potassium; Fe = Iron; Mn = Manganese; Cu = Copper; Zn = Zinc.

6.3.4. AM spore density

The present study revealed that phenology of different rice varieties growing in different ecosystems had significant effect on spore density **(Table 39 and 40)**. AM fungal spore density increased at the harvesting stage compared to the vegetative and flowering stage irrespective of rice variety and ecology **(Fig. 21)**.

Significant difference in mean spore density was observed for the two years of study. The mean maximum spore density was observed in the year 2015 (145.52 spores 100g soil^{-1}) as compared to 2016 (73.06 spores 100g soil^{-1}) at $p < 0.05$ when, $F_{(1,160)} = 25.87$. Mean spore density was maximum in upland (159.72 spores 100g soil^{-1}) followed by midland (133.17 spores 100g soil^{-1}) and minimum in the lowlands (34.98 spores 100g soil^{-1}) at $p < 0.05$, $F_{(2,159)} = 34.93$. In relation to phenology, a significant difference existed in mean spore density at the different growth stages *viz*., vegetative (63.70 spores 100g soil^{-1}), flowering (95.91 spores 100g soil^{-1}) and harvesting (168.26 spores 100g soil^{-1}) stage at $p < 0.05$, $F_{(2,159)} = 20.21$.

Fig. 21: Influence of varied ecology on mean spore density.



Legend: Year of study: A - 2015, B –2016; Error bars represents Standard Error; Values on the column affected by the same letter are not significantly different between the growth stage of each years of study.

Table 39: Influence of plant phenology on spore density at different ecological sites in 2015.

Rice variety	Lowland (*Khazan*)			Midland (*Ker)*			Upland (*Morod*)			$F_{(8,18)}$, p < 0.05
	Vegetative	Flowering	Harvesting	Vegetative	Flowering	Harvesting	Vegetative	Flowering	Harvesting	
Jyoti	18.00^{a} ±1.52	27.00^{ab} ±1.54	46.33^{b} ±1.33	34.33^{ab} ±1.20	175.67^{c} ±1.76	291.33^{d} ±4.67	225.00^{e} ±2.89	243.33^{e} ±10.37	465.67^{f} ±2.33	1381.07*
Khonchri	35.00^{a} ±1.15	39.33^{a} ±1.20	45.00^{a} ±1.52	41.00^{a} ±1.53	106.67^{b} ±5.55	238.00^{c} ±1.15	146.67^{d} ±2.03	162.33^{e} ±3.38	201.67^{df} ±3.48	810.21*
Jaya	36.00^{a} ±0.57	37.00^{a} ±1.15	38.67^{a} ±0.88	127.00^{b} ±1.15	137.67^{b} ±2.90	328.33^{c} ±6.00	74.33^{d} ±1.85	245.00^{e} ±0.88	362.67^{f} ±2.40	2492.51*

Legend: Data presented is the mean of three readings at each growth phase; different letters within the variety indicate significant differences at P< 0.05; ± indicates Standard Error.

Table 40: Influence of plant phenology on spore density at different ecological sites in 2016.

Rice variety	Lowland (*Khazan*)			Midland (*Ker)*			Upland (*Morod*)			$F_{(8,18)}$, p < 0.05
	Vegetative	Flowering	Harvesting	Vegetative	Flowering	Harvesting	Vegetative	Flowering	Harvesting	
Jyoti	19.67^{a} ± 3.53	26.67^{ab} ±1.45	37.33^{bf} ±2.40	35.67^{bef} ± 1.86	93.33^{d} ± 1.45	167.33^{e} ± 2.91	41.67^{f} ± 2.60	47.00^{fg} ± 2.89	142.67^{h} ± 4.06	396.96*
Khonchri	32.33^{a} ± 2.33	38.33^{ab} ± 2.33	45.67^{bc} ± 1.86	52.67^{c} ± 2.40	79.33^{d} ± 4.67	175.33^{e} ±1.76	45.00^{bcf} ± 2.08	52.67^{cg} ±02.33	118.33^{h} ± 0.88	397.55*
Jaya	29.67^{a} ± 2.91	32.33^{a} ± 2.02	45.33^{b} ±1.20	76.33^{c} ± 1.76	98.33^{d} ± 2.33	138.67^{e} ± 3.71	76.33^{c} ±2.19	84.33^{c}± 0.88	140.33^{e} ± 1.86	334.29*

Legend: Data presented is the mean of three readings at each growth phase; different letters within the variety of study indicate significant differences at P< 0.05; ± indicates Standard Error.

6.3.5. Correlation between spore density and adaphic factors

In 2015, a non significant correlation was observed between spore density and the adaphic factors studied in the lowlands and midlands. However, spore density showed a positive correlation with OC (r = 0.71, p = 0.05) in uplands **(Table 41)**.

In 2016, a significant positive correlation with spore density was observed in lowlands for pH (r = 0.90, p = 0.01), EC (r = 0.68, p = 0.05), P (r = 0.70, p = 0.05), K (r = 0.76, p = 0.05), Fe (r = 0.88, p = 0.01), Mn (r = 0.86, p = 0.01) and significant negative correlation with Zn (r = -0.68, p = 0.05) N (r = -0.69, p = 0.05). In midlands no significant correlation and in uplands a significant positive correlation was observed with OC (r = 0.69, p = 0.05) and with P (r = 0.77, p = 0.05) **(Table 41).**

Table 41: Pearson Correlation coefficient (r value) between spore density (SD) and pH, EC, N, P, K, Fe, Mn, Cu and Zn.

Parameter	Lowland		Midland		Upland	
	2015	2016	2015	2016	2015	2016
SD v/s pH	- 0.28	0.90**	- 0.19	- 0.54	- 0.36	- 0.03
SD v/s EC	0.06	0.68*	- 0.43	0.62	0.62	- 0.44
SD v/s OC	0.27	0.01	- 0.04	- 0.37	0.71*	0.69*
SD v/s N	0.12	- 0.69*	- 0.23	- 0.02	0.14	- 0.57
SD v/s P	0.41	0.70*	0.32	- 0.28	0.16	0.77*
SD v/s K	0.51	0.76*	- 0.10	- 0.63	- 0.60	- 0.52
SD v/s Fe	0.45	0.88**	0.23	0.29	- 0.37	0.25
SD v/s Mn	- 0.10	0.86**	- 0.34	- 0.28	- 0.14	- 0.03
SD v/s Cu	0.21	0.63	- 0.56	- 0.38	- 0.51	0.35
SD v/s Zn	0.57	- 0.86**	- 0.66	- 0.54	- 0.56	- 0.61

Legend: * = Correlation is significant at the 0.05 level; **Correlation is significant at 0.01 level; OC = Organic Carbon; N = Nitrogen; P = Phosphorus; K = Potassium; Fe = Iron; Mn = Manganese; Cu = Copper; Zn = Zinc.

6.3.6. AM spore diversity

6.3.6.1. AM diversity Index

Simpson's (D) and Shannon diversity (H) index at the species level were calculated (**Table 42; Fig. 22).** There was no significant difference in the Simpson's Index for the two years at the three studied ecologies. However Shannon's index was highest in the midland and least in upland in the year 2016. Mean Simpson's Index was significantly higher at 5% level ($P<0.05$) in the midland (0.63) as compared to lowland (0.50) and upland (0.40). Mean Shannon's Index was significantly higher in midland (0.93) compared to lowland (0.82) and upland (0.60).

Table 42: Diversity Index of AM fungal communities at the different ecologies.

Ecological parameter	2015			2016			$F_{(5,48)}$, $p<0.05$
	Lowland	Midland	Upland	Lowland	Midland	Upland	
Simpson's Index (*D*)	0.47^{a} ±0.07	0.45^{a} ±0.06	0.46^{a} ±0.03	0.52^{a} ±0.07	0.58^{a} ±0.08	0.35^{a} ±0.07	1.30
Shannon's Index (*H*)	0.78^{ab} ±0.13	0.76^{ab} ±0.12	0.67^{ab} ±0.05	0.86^{ab} ±0.13	1.10^{a} ±0.17	0.52^{b} ±0.11	2.22*

Legend: Data presented is the mean of nine readings at each growth stage; Different letters within the row indicate significant differences at $P< 0.05$; ± indicates Standard Error; Mean Simpson's Index was 0.50^{ab}; 0.63^{a} and 0.40^{b} in the lowland, midland and upland respectively at $p<0.05$, $F_{(5,48)}=4.51$*, Mean Shannon's Index was 0.82^{ab}; 0.93^{a} and 0.60^{b} in the lowland, midland and upland respectively $p<0.05$, $F_{(5,48)}=3.39$*.

6.3.6.2. AM species richness

A total of 17 AM fungal species belonging to seven genera *viz*., *Acaulospora* (8) **(Plate 7 a, b, c, d)**, *Rhizoglomus* (1), *Tricispora* (1) **(Plate 8 f)**, *Claroideoglomus* (2) **(Plate 7 e, f),** *Funneliformis* (1) **(Plate 8 e)**, *Archaeospora* (1) and *Gigaspora* (3) **(Plate 8 a, b, c)** with species number given in parenthesis were recorded from the different ecosystems. The study revealed that *Acaulospora* was the most dominant genus in the studied ecosystems. In comparison to the different ecologies and phenology maximum species richness was recorded in lowlands (9 spp.) for both years during the harvesting stage.

In lowlands, *Acaulospora scrobiculata, A. delicata, A. dilatata, A. laevis, A. tuberculata, A. soloidea, Archaeospora myriocarpa, Funneliformis mosseae, Rhizoglomus fasciculatum* and

Tricispora nevadensis were recorded in both years **(Table 43)**. In midlands, *Acaulospora scrobiculata, A. bireticulata, A. rehmii, A. dilatata, Gigaspora ramisporophora, Claroideoglomus claroideum* and *Tricispora nevadensis* were recorded in 2015 **(Table 44)**. In 2016, three additional species *viz., Funneliformis mosseae, Gigaspora albida* and *G. decipiens* were recorded. In the uplands *A. scrobiculata, A. bireticulata, Claroideoglomus claroideum, C. etunicatum* and *Tricispora nevadensis* were recorded in both years **(Table 45**).

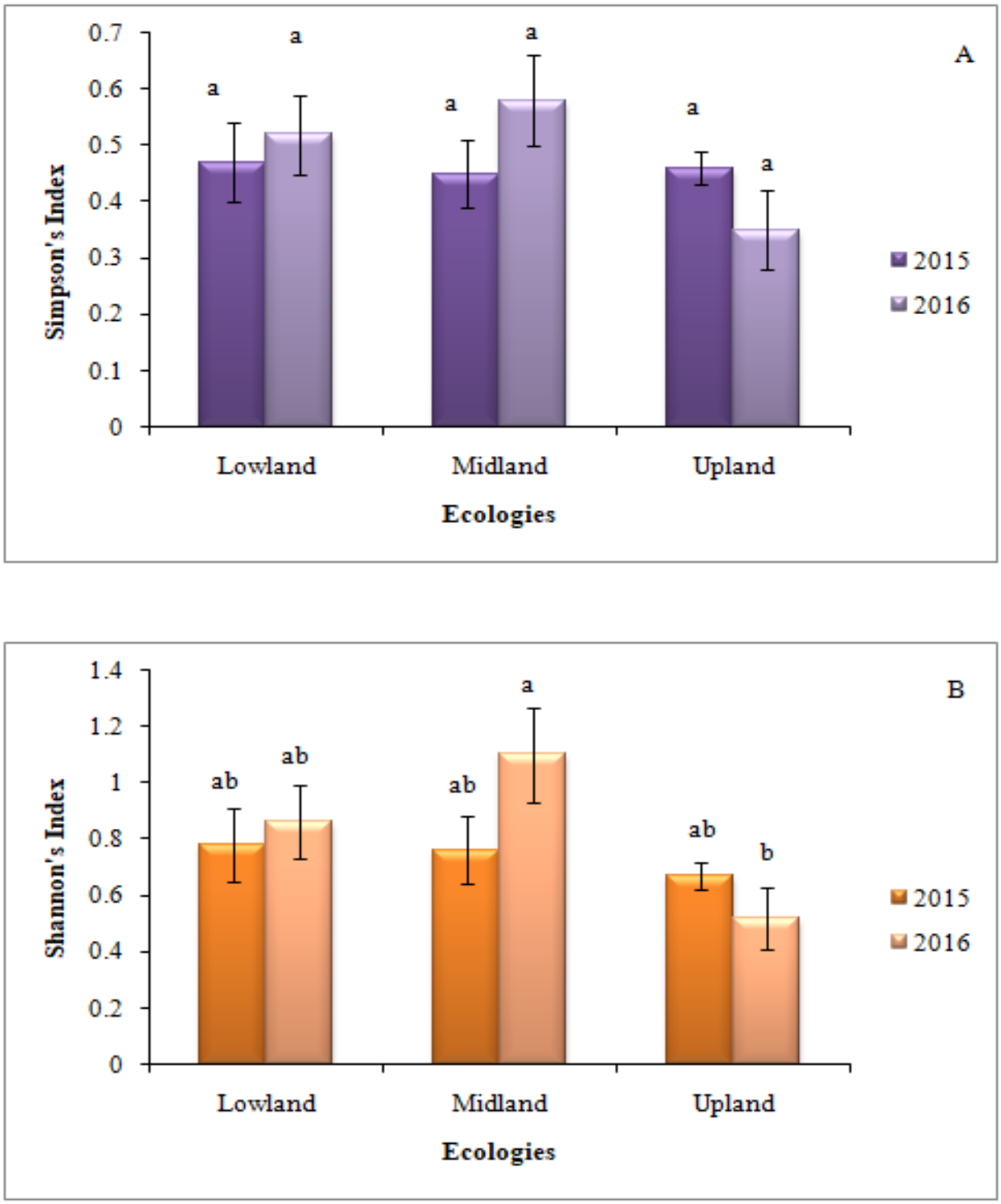
6.3.6.3. Relative Abundance (RA) of AM fungi

The relative abundance of AM species in the different ecologies varied. In lowlands, the genus *Acaulospora* showed maximum RA for both years. In midlands, the genus *Gigaspora* showed maximum RA in both the years and in uplands the genus *Claroideoglomus* showed maximum RA for both years **(Fig. 23)**. The potential correlation between AM fungal abundance and physico-chemical properties of the soil in the different ecosystems was performed using Canonical correspondence analysis (CCA). In the CCA plot **Fig. 24**, the resulting ordination is presented in **Tables 46 and 47**. Eigen values of the first and second axis were 0.59 and 0.48 respectively. Cumulative percentage variance of genera data showed 34.54% and 62.60% of variability on the first and second axis respectively. Genus *Claroideoglomus* shows a high RA in uplands which is closely related to the high OC, N, Mn and Fe content in the soil. Midlands showed high abundance of the genus *Gigaspora*, and this may be related to high content of Zn and low P. In the lowlands, the genus *Acaulospora* showed high RA as it showed tolerance to EC fluctuations, Cu and K content.

6.3.6.4. Frequency of occurrence of AM fungi

In the lowlands, frequency of occurrence was highest for *A. scrobiculata* and *R. fasciculatum* as they were recorded in all the growth stages for both years **(Table 43)**. In midlands, frequency of occurrence was maximum in *A. scrobiculata and G. ramisporophora* **(Table 44)**, while in uplands, frequency of occurrence was highest for *C. claroideum* and *T. nevadensis* **(Table 45)**.

Fig.22: AM fungal diversity in different ecologies.



Legend: Diversity Index: A – Simpson's, B –Shannon's; Error bars represents Standard Error; Values on the column affected by the same letter are not significantly different between the ecologies of each year of study.

Table 43: Distribution and RA of AM fungi in lowlands.

Sr. No.	AM species	Relative Abundance (%)						Frequency of occurrence %
		2015			2016			
		Vegetative	Flowering	Harvesting	Vegetative	Flowering	Harvesting	
1.	*Acaulospora scrobiculata* Trappe	73.15	53.33	46.7	37.56	49.89	13.39	100
2.	*Acaulospora delicata* Walker Pfeffer & Bloss	-	10.56	12.61	-	15.12	16.66	66.66
3.	*Acaulospora dilatata* Morton	-	-	10.72	-	-	12.89	33.33
4.	*Acaulospora laevis* Gerd. & Trappe	-	-	11.30	-	-	10.94	33.33
5.	*Acaulospora tuberculata* Janos & Trappe	-	-	11.30	-	-	9.48	33.33
6.	*Archaeospora myriocarpa* Sieverd. & Schenck	2.53	-	4.72	10.88	-	-	50.00
7.	*Acaulospora soloidea* Vaing. & Rodrigues	-	1.23	1.41	-	-	19.94	50.00
8.	*Funneliformis mosseae* Gerdemann & Trappe.	10.06	6.99	-	5.08	27.06	9.22	83.33
9.	*Rhizoglomus fasciculatum* (Thaxter) Walker & Koske	14.24	21.68	0.70	46.46	5.83	3.86	100
10.	*Tricispora nevadensis* Palenzuela, Ferrol, Azcon- Aguilar & Oehl	-	6.17	0.47	-	2.08	3.57	66.66
Species Richness		4	6	9	4	5	9	-

Legend: - = Absent; Data presented is the mean of three readings at each growth stage.

Table 44: Distribution and RA of AM fungi in midlands.

Sr. No.	AM species	Relative Abundance (%)						Frequency of occurrence %
		2015			2016			
		Vegetative	Flowering	Harvesting	Vegetative	Flowering	Harvesting	
1.	*Acaulospora scrobiculata* Trappe	6.71	15.92	16.99	3.20	13.26	7.20	100
2.	*Acaulospora bireticulata* Rothwell & Trappe	-	-	2.56	-	-	3.32	33.33
3.	*Acaulospora dilatata* Morton	5.69	-	-	-	-	-	16.66
4.	*Acaulospora rehmii* Sieverd. & Toro	8.16	-	5.99	6.77	-	2.55	66.66
5.	*Gigaspora ramisporophora* Sieverd. & Schenck	79.42	78.31	41.73	85.37	31.15	26.32	100
6.	*Gigaspora albida* Schenck & Sm.	-	-	-	-	24.43	26.60	33.33
7.	*Gigaspora decipiens* Gerd. & Trappe	-	-	-	-	27.93	24.54	33.33
8.	*Claroideoglomus claroideum* (Schench & Sm) Walker & Schűβler	-	-	22.09	-	-	8.54	33.33
9.	*Funneliformis mosseae* Gerdemann. & Trappe	-	-	-	4.64	-	-	16.66
10.	*Tricispora nevadensis* Palenzuela, Ferrol, Azcon- Aguilar & Oehl	-	5.76	10.61	-	3.20	0.88	66.66
Species Richness of AM Fungi		4	3	6	4	5	8	-

Legend: - = Absent, Data presented is the mean of three readings at each growth phase.

Table 45: Distribution and RA of AM fungi in upland.

Sr. No.	AM species	Relative Abundance (%)						Frequency of Occurrence %
		2015			2016			
		Vegetative	Flowering	Harvesting	Vegetative	Flowering	Harvesting	
1.	*Acaulospora scrobiculata* Trappe	19.06	18.43	-	22.94	27.01	-	66.66
2.	*Acaulospora bireticulata* Rothwell & Trappe	-	-	8.97	-	-	7.58	33.33
3.	*Claroideoglomus claroideum* (Schench & Sm) Walker & Schűβer	68.01	48.23	54.93	75.81	21.27	62.76	100
4.	*Claroideoglomus etunicatum* (Becker & Gerd) Walker & Schűβler	-	19.84	27.54	-	50.86	29.40	66.66
5.	*Tricispora nevadensis* Palenz Ferrol. Azcon- Aguilar & Oehl.	12.91	13.48	8.54	1.23	0.84	0.24	100
Species Richness		3	4	4	3	4	4	-

Legend: - = Absent; Data presented is the mean of three readings at each growth stage.

Table 46: CCA variable scores for gerera at different ecologies.

AM Genera	Axis 1	Axis 2
Acaulospora	-0.24	0.54
Funneliformis	-0.49	0.85
Rhizoglomus	-0.22	1.49
Archaespora	-0.17	1.56
Tricispora	0.62	-0.17
Gigaspora	-0.87	-0.86
Claroideoglomus	1.14	-0.32
Eigen value	0.59	0.48
Variation %	34.54	28.05
Cumulative %	34.54	62.60

Table 47: Biplot scores for soil variables at different ecologies.

Soil variable	Axis 1	Axis 2
pH	0.12	-0.22
EC	-0.22	0.32
OC	0.87	-0.08
N	0.82	-0.04
P	-0.08	-0.23
K	0.14	0.72
Fe	0.39	0.42
Mn	0.57	0.10
Cu	-0.09	0.12
Zn	-0.54	-0.13

Legend: OC = Organic Carbon; N = Nitrogen; P = Phosphorus; K = Potassium; Fe = Iron, Mn = Manganese; Cu = Copper; Zn = Zinc.

Fig.23: Genera wise spore abundance in rice cultivated at three different ecologies.

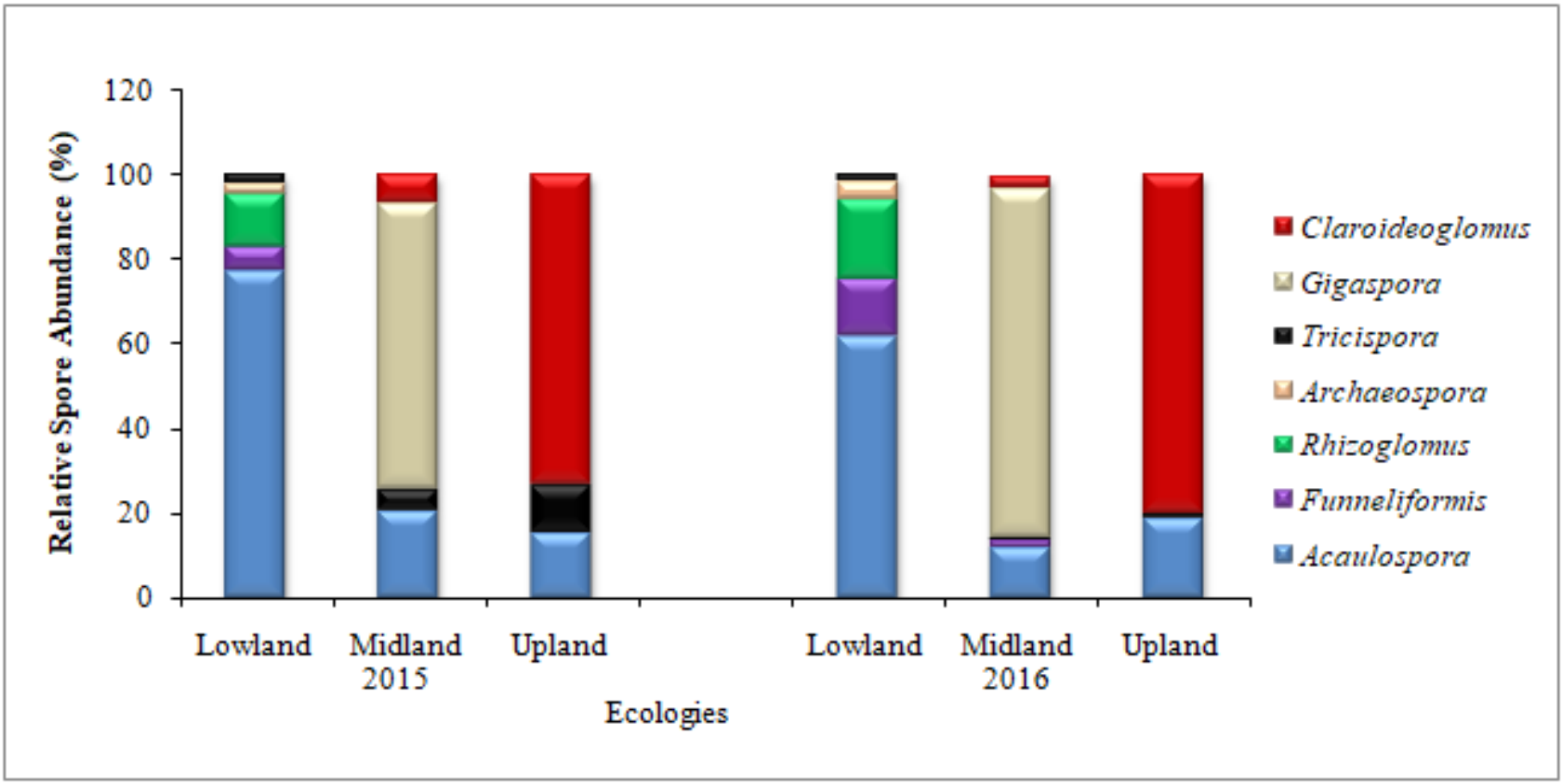
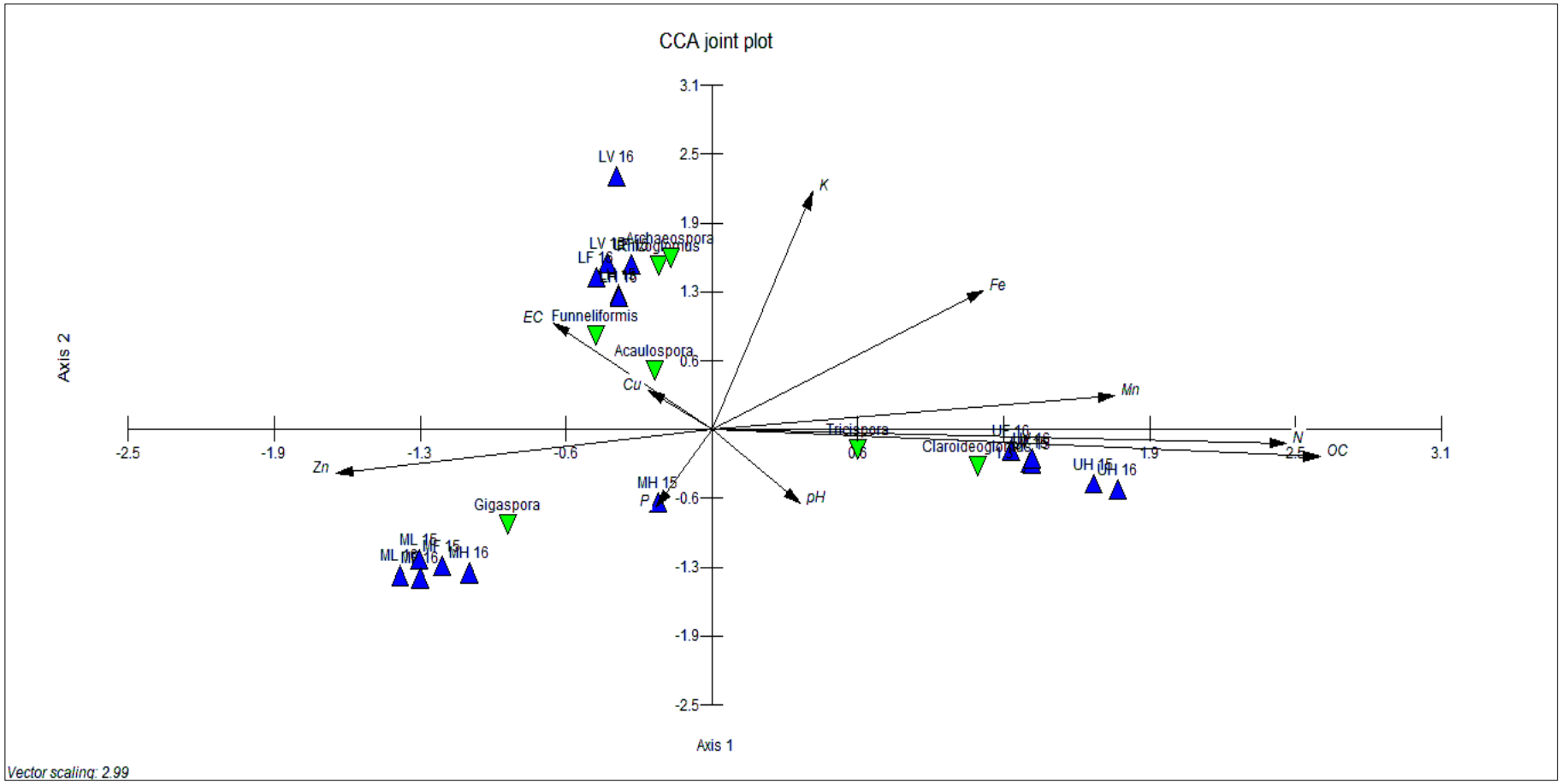
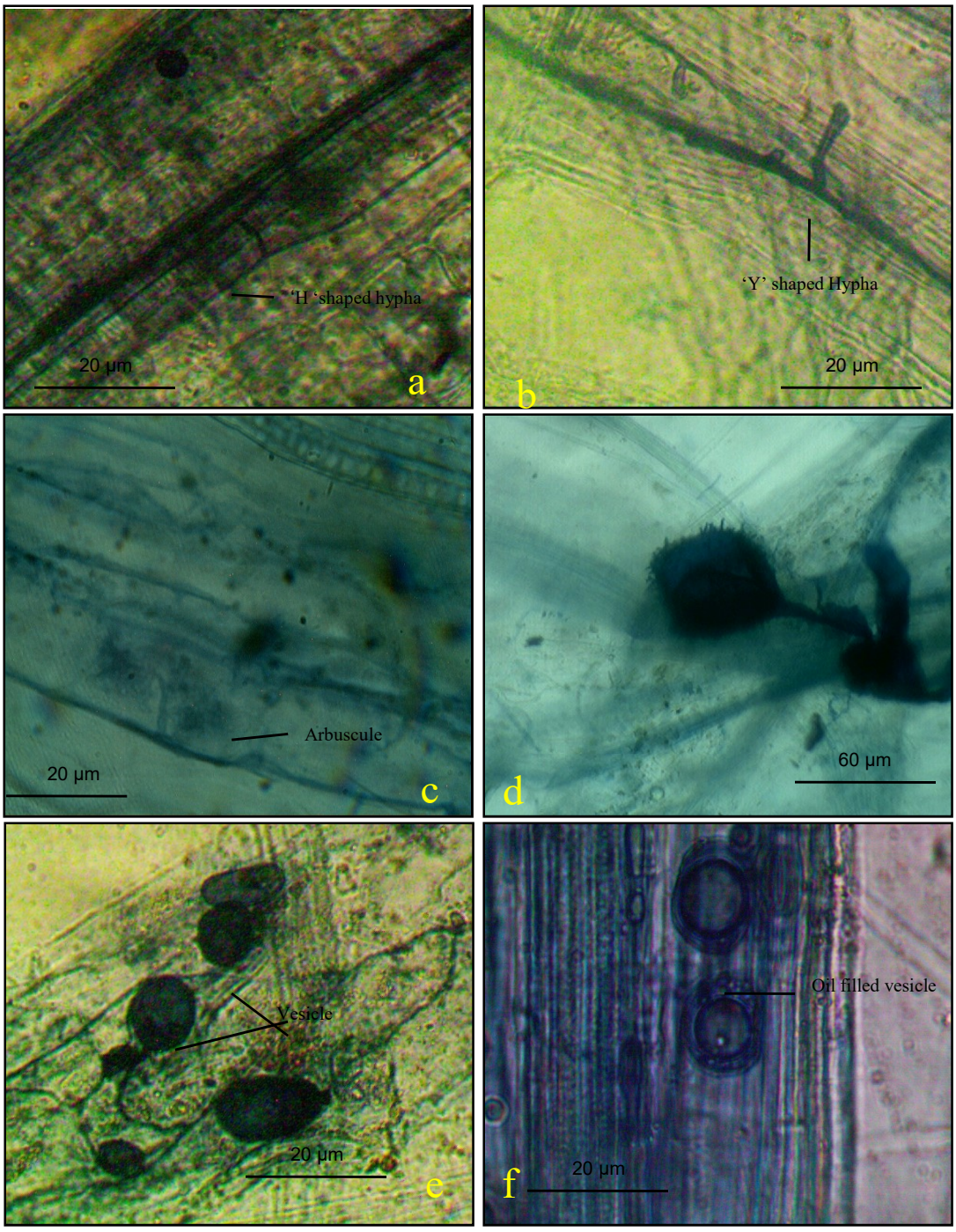


Fig. 24: CCA of genus wise relative abundance distribution of AM genera cultivated in different ecological sites for the years 2015 and 2016.



Legend: Diagram of CCA of soil properties pH, EC, OC, N, P, K, S, Fe, Mn, Cu, Zn and Genus wise relative abundance distribution of AM genera at the different land types: LV-Lowland vegetative stage: LF- Lowland flowering stage: LH- Lowland harvesting; MV- Midland vegetative stage: MF- Midland flowering stage: MH-Midland harvesting; Upland vegetative stage: UF- Upland flowering stage: UH-Upland harvesting; 15 = year 2015; 16 = year 2016

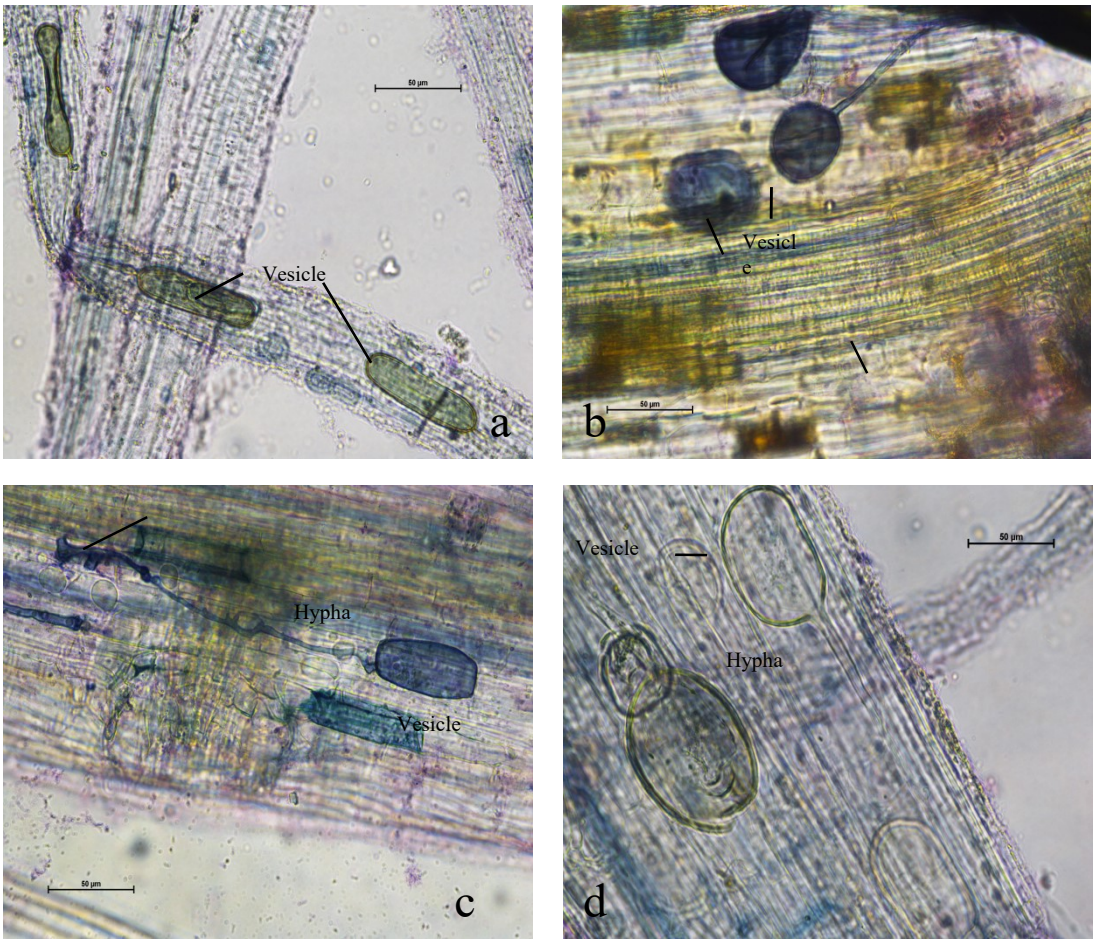
Plate IV



AM fungal colonization in lowlands.

a.'H' shaped hyphae in *O. sativa* var. Assgo; **b**. 'Y' shaped hypha in *O. sativa* var. Bello; **c.** Arum- type arbuscules in *O. sativa* var. Jyoti; **d**. Auxillary cell; **e.** Hyphae with vesicles in *O. sativa* var. Sheidi; **f**. Oil filled vesicles in *O. sativa* var. Korgut.

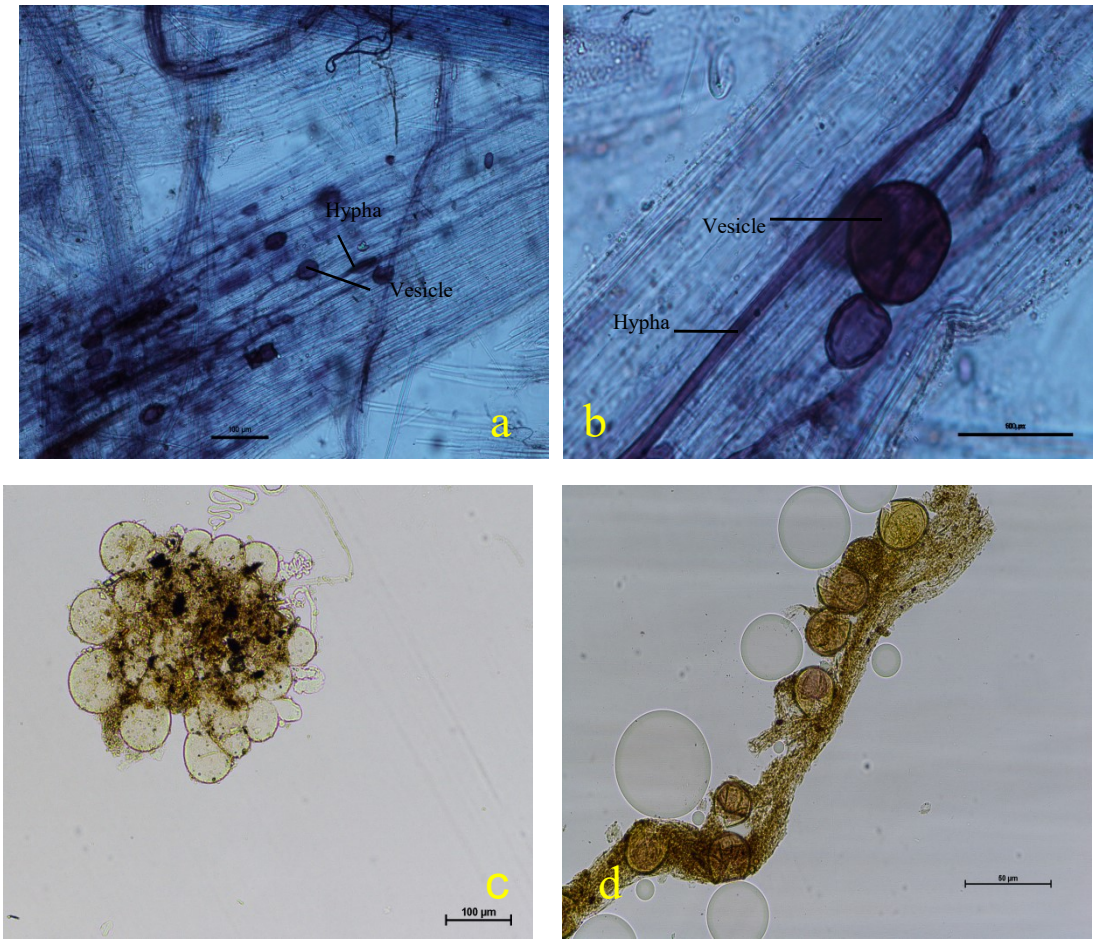
Plate V



AM Fungal colonization in midlands.

a. Elongated vesicles in *O. sativa* var. Jyoti; **b.** Globose vesicle in *O. sativa* var. Jaya; **c**. Irregular shaped vesicles in *O. sativa* var. Khonchri; **d**. Subglobose vesicle in in *O. sativa* var. Khonchri.

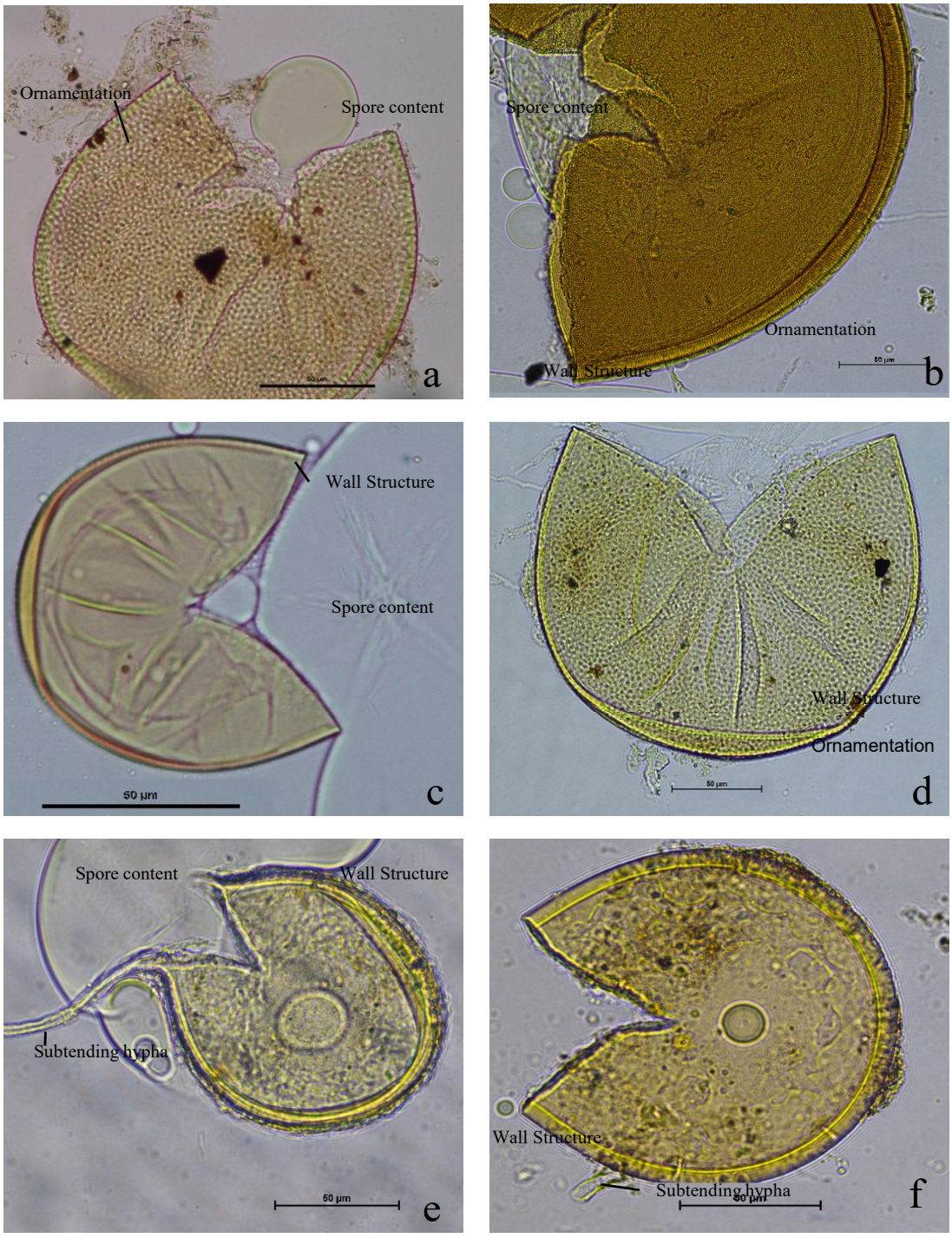
Plate VI



AM Fungal colonization in uplands.

a. Hyphae with vesicles in *O. sativa* var. Jyoti; **b.** Vesicles; **c**. Sporocarp of *Glomus* species; **d**. Extra radical spores of *Glomus* species.

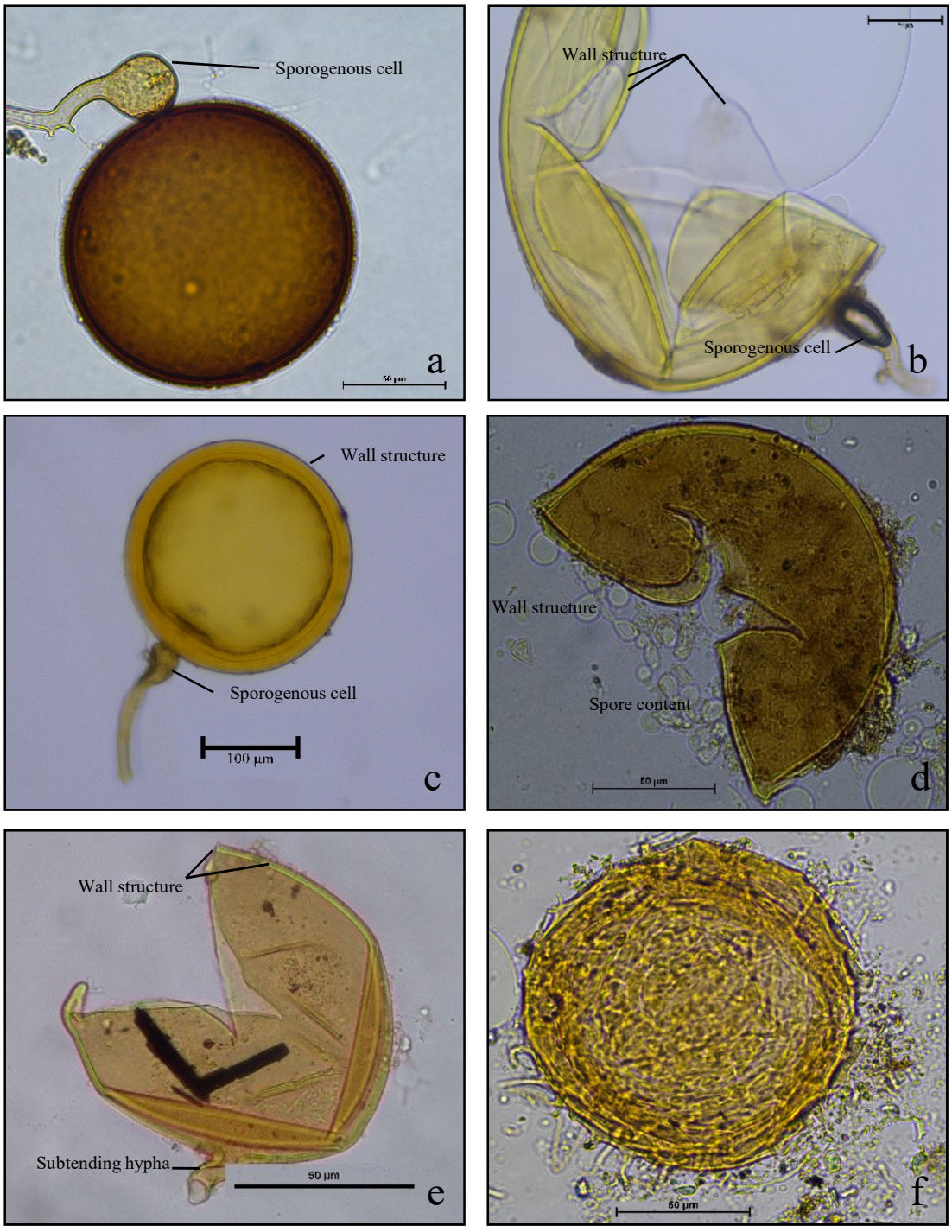
Plate VII



AM fungal species extracted from the study sites.

a. *Acaulospora bireticulata* Rothwell & Trappe; **b.** *A. rehmii* Sieverd. & Toro; ***c.*** *A. dilatata* Morton; **d.** *A. scrobiculata* Trappe; **e.** *Claroideoglomus claroideum* (Schench & Sm) Walker & Schűßler; **f.** *Claroideoglomus etunicatum* (Becker & Gerd) Walker & Schűßler.

Plate VIII



AM fungal species extracted from study sites.

a. *Gigaspora ramisporophora* Sieverd. & Schenck; **b.** *Gi. albida* Schenck & Sm; **c**. *Gi. decipiens* Gerd. & Trappe; **d.** *Glomus microcarpum* Tulasne & Tulasne; **e.** *Funneliformis mosseae* (Nicolson & Gerd.) Walker & Schüssler; **f.** *Tricispora nevadensis* (Palenz., Ferrol, Azćon- Aguilar & Oehl) Oehl. Palenz.

6.4. Discussion

In relation to different ecological sites studied, it was evident that roots of rice cultivated in the three ecologies showed root colonization. However there was significant difference in the rate of AM colonization at the three ecologies, which may be due to the varying soil and climatic situations (Rajan *et al.* 2000). According to Lumini *et al.* (2010) and Miller (2000), there is reduction in the rate of AM colonization in rice cultivated in lowlands and in high input cropping methods. Minimum AM fungal root colonization was recorded in the lowland irrespective of rice variety. According to Vallino *et al.* (2009), AM colonization is considerably low in flooded conditions. Here the roots are submerged under water for a longer period compared to other ecologies and show the presence of aerenchyma as in wetland plants (Justin and Armstrong 1987). The reduction in AM colonization under flooded condition may not only be due to root architecture but may also be due to anatomical changes like increase in aerenchyma and decrease in cortical cells which are required for AM colonization. According to Vallino *et al.* (2014) negative correlation between aerenchyma and AM colonization is known to exist. In the midland and upland, rice is cultivated under shallow flooded conditions, where the level of water changes with the progression of the life cycle of rice. According to Vallino *et al.* (2014), with the shift in soil condition from submerged to exposed there exists a rapid adaptation by the roots to the new situation which coincides with the changes in the rate of AM colonization.

Irrespective of the ecologies studied, AM fungal colonization gradually increased from vegetative to flowering stage and then gradually decreased at harvesting stage. Increased AM colonization reflects the high P requirements of plants in the early growing stages till the flowering stage which then decreases indicating reduction of photosynthate allocation to mycorrhizal roots (Saito *et al*. 2004).

Average spore density in rhizosphere soil of rice cultivated in the three ecologies showed significant variation. From the three studied ecologies minimum spore density was recorded in the lowlands. According to Nopamornbodi *et al*. (1987), low spore density is due to flooding which results in anaerobic soil conditions and nutrients accessibility (Augé 2001). However

some AM species survive in flooded condition. According to Miller and Beaver (1999), some AM species may require less oxygen and they could be concentrated near the plant roots, obtaining oxygen directly from the root or as oxygen diffuses from the root into the rhizosphere .

According to Bentivenga and Hetrick (1992), sporulation of AM fungi is stimulated due to a decrease in plant nutrient requirement. Irrespective of rice variety or ecology, AM spore density intensified at harvesting stage. Similar results were observed by Janos (1980) and Redhead (1977). Production of spores is a means of production induced by root senescence during root turnover at plant maturity or triggered by stress as a survival strategy (Smith and Read 1997). The rise in AM spore population at harvest stage is an indication of the end of optimum plant growth and coincides with a period of slow growth or senescence of host roots (Muthukumar *et al*. 2003).

The function of soil nutrient concentration on rate of colonization by AM fungi and spore density was investigated during the study. There was no significant correlation between root colonization and spore density which is in agreement with Miller (2000). However a significant correlation was observed between root colonization and N content. Hepper (1983) demonstrated that application of nitrates increased AM fungal colonization in lettuce, while Azizah and Hable (1989) demonstrated that application by ammonium nitrate increased colonization in *Leucaena.* AM fungal colonization depends on soil moisture and P availability (Wang *et al.* 2010, Selvaraj *et al.* 2004) besides physiological growth rate and turnover of plant root (Lugo *et al*. 2003). A significant correlation was observed between spore density and soil pH. These results are in agreement with the findings of Kruckelmann (1975) and Nemec *et al.* (1981) in arable soils and rhizosphere soils of *Citrus*, respectively. Spore density and AM colonization showed correlation with increase in OC in the soil. According to Douds *et al.* (1997), OC could enhance spore production and improve colonization, AM fungal hyphae grew best in soils with high amount of OC content. According to Zhao (1999), variation in spore density is a combined effect of seasonality, edaphic factors, age of host plant and spore dormancy.

The composition, richness, distribution, RA of AM fungal species and diversity index are related to regional differences and soil factors. The present study recorded variation in AM species richness at different phenological stages for the three different ecologies. Maximum species richness was recorded at the harvesting stage in the lowland. This could be attributed to high tolerance of AM fungi to soil hypoxia or even anoxia (Wang *et al.* 2011). High species richness in the lowlands may be important for buffering an ecosystem against disturbances (Vogt *et al.* 1997). The study revealed that *Acaulospora* was the most dominant and frequent genera in the studied ecologies. *Acaulospora* species are mostly detected in soils with pH of 6.5 or lower (Siqueira *et al*. 1989).

In lowland, frequency of occurrence was high for *A. scrobiculata* and *R. fasciculatum.* Genus *Acaulospora* showed maximum RA for both years of study. In midlands, the genus *Gigaspora* showed maximum RA in both the years. Similar observation was reported by Toppo *et al.* (2012) suggesting genus *Gigaspora* may be better adapted to semi-aerobic to anaerobic soils. Distribution of *Gigaspora* in soils with pH above 5.5 was three times higher than in soils having less than 5.5 pH (Trindade *et al.* 2006) and may reflect low soil organic matter content as reported by Porter *et al.* (1987). In uplands, frequency of occurrence was higher for *Claroideoglomus claroideum* and *Tricispora nevadensis*. Genus *Claroideoglomus* showed maximum RA for both the years. Predominance of *Glomus* in aerobic soil condition of uplands has been reported in earlier studies (Maiti *et al.* 1995, Toppo *et al.* 2012). This may be related to their competitive interaction and adaptability to aerobic conditions allowing them to establish better than others (Songachan and Kayang 2011). Maximum diversity index observed in the midland indicated shared dominance of many AM fungal genera. The lowest diversity observed in uplands indicates dominance of a few AM genera.

6.5. Conclusion

All the rice fields in the different ecologies were conventionally managed. Each ecological site had a different cultivation practice. The difference in ecology and cultural practices can cause changes in growth suitability of AM fungi. Hence AM fungi tolerant to conditions in a

particular ecology would flourish. In the present study, rice varieties cultivated in all ecologies recorded AM colonization. The highest colonization was observed in the midlands and least in the lowlands. There was no significant difference in spore density in midlands and uplands but was significantly less in lowlands. Highest species richness was recorded at the harvesting stage in the lowland. However, the mean AM diversity index was highest in the midlands. In the present study dominance of different genera in different ecologies *viz.,* genus *Acaulospora* in lowlands, *Gigaspora* in midlands and *Claroideoglomus* in uplands were recorded. As AM fungal colonization in the native soil is better in efficacy (Oliveira *et al*. 2005), cost effectiveness and adaptation with lesser negative ecological consequences in terms of invasive species (Schenck and Perez, 1990), they appear more suitable to be employed as inocula for different ecosystems. Thus the present study suggests the suitability of *Acaulospora* inocula in lowland (*Khazans*), *Gigaspora* in midland (*Ker*) and *Claroideoglomus* in Upland (*Morod*) fields.

CHAPTER 7

Preparation of pure cultures of dominant AM fungal species and their mass multiplication.

7.1. Introduction

Arbuscular mycorrhizal fungi are world widely distributed soil fungi forming symbiosis with most plants. Their association in natural and semi-natural ecosystem has resulted in diversity, increased plant productivity and increased plant resistance against biotic and abiotic stress (Smith and Read 2008). Due to their functional attributes which includes increase in plant growth and yield (Sharma and Srivastava 1991), improved crop uniformity (Aguirre-Medina and Velazco-Zebadua 1994), reduced losses due to environmental stresses and root diseases (Sharma *et al.* 1992, Loth and Hofner 1995), improved transplant establishment (Menge *et al.* 1978) and their advantage of having an extremely broad host range, they can be considered to be used as bioinoculants to improve plant production in agriculture, horticulture and forestry (Souza *et al*. 2010, Ijdo *et al.* 2011).

Despite the functional attributes of AM fungi, inoculum production has its limitations. Spores collected from the field may be difficult to be accurately identified at the species level as they may be parasitized, not viable, less in number, changed appearance as spore wall structure is prone to deterioration caused by various factors that include root pigments, soil chemistry, temperature, moisture and microbial activity (https:invam.wvu.edu/methods/cultures/trap-culture). Preparation and establishment of trap cultures of the field collected rhizosphere soil samples is a widely used alternative to increase unparasitized, viable spore number, which can be used for authentic identification and for initiation of monospecific culture.

From the previous chapter it has been observed that Genus *Acaulospora* is dominant and abundant in the *Khazan* ecosystem and hence the present study is aimed to prepare pure culture isolated from the rhizosphere soil of rice cultivated in the *Khazan* ecology.

7.2. Materials and Methods

7.2.1. Soil Trap Culture preparation

Multiplication of AM fungi using soil trap cultures was carried out as described under **3.2.4.** The mixture of soil and cut roots was transferred in zip-loc polyethelene bags, labeled and stored at 4ºC, and used for AM spore identification and preparation of monospecific culture **(Plate IX a)**.

7.2.2. Sterilization of soil

Soil was sterilized by oven heating the soil at 85ºC twice for three hours daily for two days (Sylvia and Jarstfer 1992).

7.2.3. Root processing

Root processing was carried out as described under **3.2.2.**

7.2.4. Isolation of AM spores

Isolation of AM spores was carried out as described under **3.2.5.**

7.2.5. Estimation of AM spore density

Estimation of AM spore density was carried out as described under **3.2.6.**

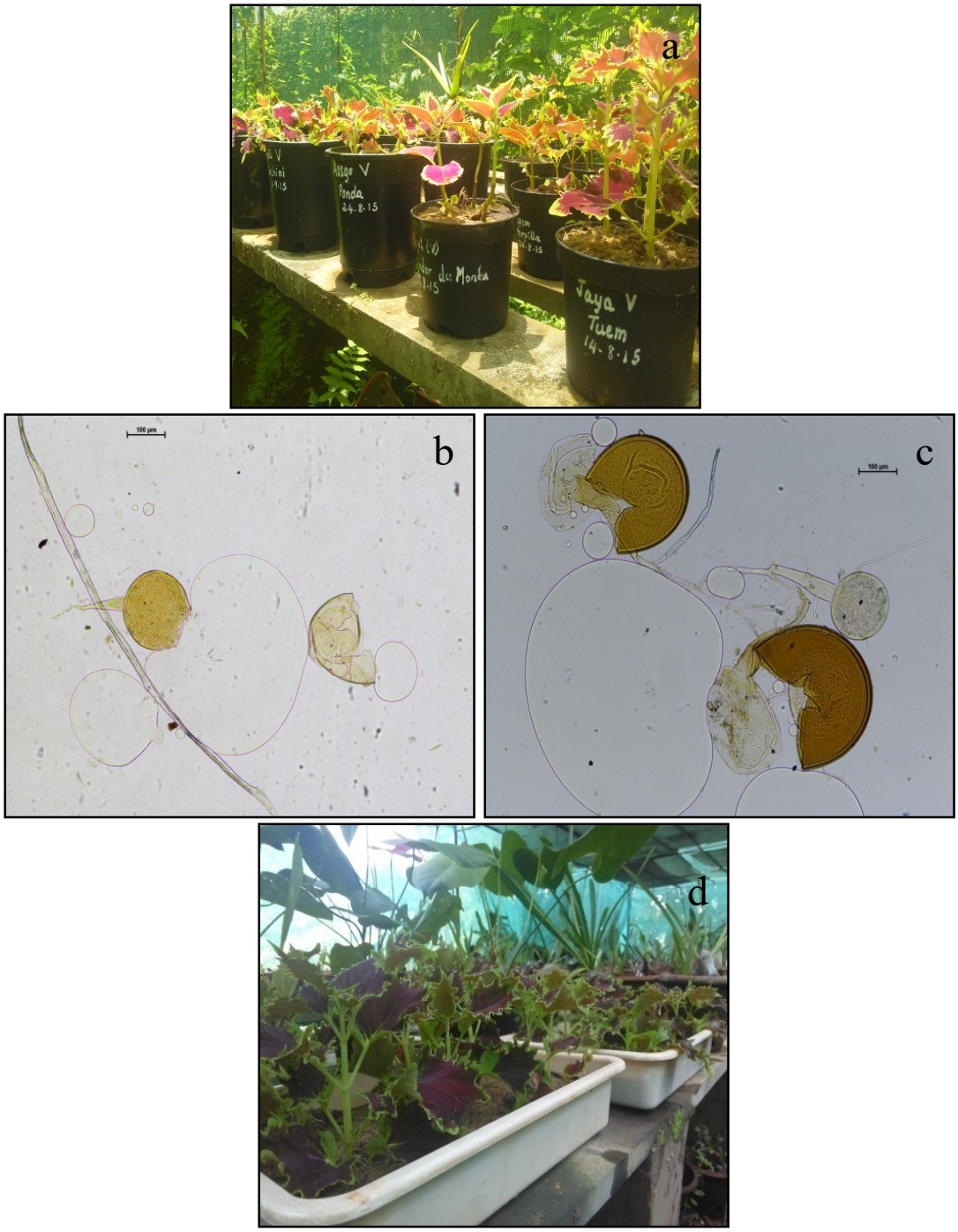
7.2.6. Taxonomic identification of AM fungi

Taxonomic identification of AM fungi was carried out as described under **3.2.7.**

7.2.7. Single species (Pure/ starter) culture

Single species (Pure) culture was prepared using similar looking spores isolated from trap cultures (Gilmore 1968). After repeated washing, the spores (100) belonging to a single morphotype are separated and stored in a Petri-dish at 4ºC. The isolated pores were examined by viewing under Olympus stereo microscope SZ2-ILST (10 x 4.5 zoom) for any changes in morphology. Before inoculation, any soil particles or hyphal fragments adhered to the spores were removed and spores were then washed with distilled water. Plastic pots (15cm dia.) were filled with sterilized sand in which isolated spores (single species) were placed along with

Plate IX



Preparation of pure cultures.

a. Trap cultures; **b.** Spores of *Acaulospora scrobiculata* extracted from monospecific culture; **c.** Spores of *A. rehmii*; **d.** Mass multiplication of AM fungi inocula using cuttings of *Plectranthus scutellarioides* (L.) R. Br. as host plant

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Whatman filter paper at a depth of 2-3cm and then covered with sterilized sand. *Plectranthus scutellarioides* (L) R. Br. was used as host plant (5 cuttings per pot). Pots were then maintained in the green house, provided with Hoagland's solution (Hoagland and Arnon 1950) minus P once in 15 days. After 3 Months, watering was stopped and the plants were cut at the level of the soil. The contents of the pots were allowed to dry and then harvested and used as inoculum for mass production.

7.2.8. Mass production of Single species (Pure/ starter) culture

Mass production of single species was carried out by substrate based production system. *Plectranthus scutellarioides* (L) R. Br. was used as the host plant and the desired single AM species (starter culture) were cultivated in sterilized sand and this was scaled up using medium sized trays (Gaur and Adholeya 2002). The whole system setup was cultivated in the green house. Trays showing successful AM colonization were maintained for a period of 3 months in the green house for the completion of life cycle. After 3 months the plants are allowed to dry, after which the shoot portion was cut off at the soil surface. The mixture of sand and cut roots is then transferred in zip-loc polythelene bags, labeled and stored at 4ºC and used as inocula for further study **(Plate IX d)**.

7.3. Results

7.3.1. Trap culture

From the six study sites, 14 AM fungal species were recovered using trap cultures *Acaulospora* (7), *Glomus* (2), *Entrophospora* (1), *Funneliformis* (1), *Rhizoglomus* (1), *Archaeospora* (1) and *Tricispora* (1) with species number given in parenthesis.

7.3.2. Single species (Pure/ starter) culture

From the results observed in the previous chapters, genus *Acaulospora* was found to be dominant and abundant in the *Khazan*s, hence was used to prepare pure culture. Of the seven species identified, pure cultures of two species *viz.*, *A. scrobiculata* and *A. rehmii* were prepared successfully **(Plate IX b and c)**.

7.3.3. Mass production of single species

From the pure culture of two AM fungal species, *A. scrobiculata* could be successfully upscaled **(Plate IX d)** and maintained as a live culture in the glasshouse of Goa University Arbuscular Mycorhizal Culture Collection (GUAMCC).

7.4. Discussion

Natural soil is an important source of consortium of indigenous AM fungi and often used as a source of inocula. Stutz *et al.* (2000) have reported that frequent collection of rhizosphere soil samples of successive trap cultures, greatly assist in assessment of AM species composition in natural habitats. Freshly collected rhizosphere soil samples can be maintained by using trap cultures and this minimizes the loss or viability of spores. It is necessary to sterilize all the components *viz.*, sand, containers, host plant and spores of the culture system prior to initiation of AM fungal culture. Sand being an effective and cheapest substrate was sterilized and used for mass production. Containers used for culture preparation were shielded from contaminated soil, splashing water and crawling insects. In addition, specific isolates of AM fungi were kept well separated to avoid cross-contamination. Large containers have been shown to result in higher spore population (Ferguson and Menge 1982) and hence during upscaling of AM inocula medium sized trays were used. *Plectranthus scutellarioides* (L) R. Br. cuttings were washed in distilled water to prevent contamination and were used as host plant due to its suitability to multiply AM fungal inocula (Sadhana 2014). In the present study, Hoagland's solution minus P was used as nutrient medium supplied to the growing cultures. According to Abbott and Robson (1991), addition of P to soil reduces AM colonization and sporulation. Before harvesting of spores, the aerial portion of the plant was cut close to the surface of the soil as suggested by Vilarino *et al.* (1992), wherein they demonstrated better sporulation and higher amount of extrametrical hyphae in red clover when the aerial part of the host was removed.

7.5. Conclusion

Trap and monospecific cultures are the most widely adopted methods used to maintain AM spores or inocula as it requires relatively low technical support and expenses. These methods are

least artificial as they require a live host and help in understanding the biology of AM fungal life cycle.

CHAPTER 8

Effect of dominant AM fungal species on growth, yield and grain quality characteristics of selected rice variety grown in *Khazan* lands of Goa.

8.1. Introduction

The beneficial effect of inoculation of crop plants with AM fungi is widely known. Its association is able to increase growth in a number of agricultural crops (Tinker 1975). Sanni (1976) demonstrated increased growth in rice plants after inoculation with *Gigaspora gigantia*. Sivaprasad *et al*. (1990) revealed that AM fungi increased grain and straw yield in wetland rice. Secilia and Bagyaraj (1994a, 1994b) reported increased grain yield, P and Zn content of wetland rice in pot culture. Numerous studies have shown that AM symbiosis induces a significant change in plant host architecture (Gutjahr *et al*. 2009) and harvest index in rice under laboratory condition (Li *et al.* 2012). Gupta and Ali (1993) reported significant increase in grain yield by AM fungi in pot and field conditions. Diedhiou *et al*. (2016) demonstrated the need to analyse the impact of AM inoculants in field conditions, as they obtained different results in pot and field experiments. This, discrepancy was due to the impact of anoxic condition caused by flooding in the field and functioning of AM symbiosis. Although the presence of AM fungi is widespread, a bioinoculant product is best used when the native AM fungal population is low or ineffective, as mere presence of AM fungal isolates does not mean benefits are provided to the host plant (Adholeya *et al.* 2005). Field experiments have demonstrated that inoculation by AM fungi increased root colonization and plant productivity of host plant (Lekberg and Koide 2005, Lehmann *et al.* 2012).

In Goa reclaimed wetlands and mangrove areas where tidal influence is regulated by construction of embankments and sluice gates are called as *Khazan* ecosystems. These ecosystems have always been nature's defense to its own excesses such as high tides, storms or floods. They are predominantly rice and fish fields. However due to labour costs, scarcity of labour, increasing lack of awareness through generation and the need to earn a better livelihood,

large tracts of these lands are used for different purposes like construction and pisiculture throughout the year instead of paddy farming which provides lesser economic returns, as compared to fishing and construction activities (https://www.terii.org/article/goa-village-strives-protect-fast-vanishingkhazans). Often embankments and sluice gates are not maintained regularly and as *Khazans* are regions adjacent to the estuaries get inundated with sea water during high tide or in monsoon season, hence reducing its fertility. Due to these constrains, to increase rice production in a sustainable and affordable way, a cheaper alternative source of nutrient input is needed for cultivation of rice in the *Khazan* lands.

Grain quality is one of the most important factors in rice production. Parboiling is precooking of rice within the husk. It is a hydrothermal treatment given to paddy to improve its quality and quality. It increases the total head yield of paddy, reduces nutrient loss and incidence of breakage during milling (Chukwu 1999). In other words, it is directly related to market value and nutrition as it is the staple food in many countries. Ayamdoo *et al*. (2013) stated that parboiling condition of 18-20 hours soak and 60 minutes steaming yield optimum physical attributes such as milling quality, head rice yield, less breakage, colour, gelatinization and high water absorption when cooked. However little is known about the use of AM fungal inocula in influencing the grain quality.

From the previous chapters it is clear that *Acaulospora* was dominant in the studied *Khazan* sites. Hence, an attempt has been made to mass multiply and use as an inoculum. Hence, an attempt has been made to mass multiply *A. scrobiculata* so that it could be attempted as a inoculum. Hence the present objective was to evaluate the effectiveness of *A. scrobiculata* as a bioinoculant on growth, yield and grain quality in *Khazan* grown rice. Besides, a study on its effect on cultivation practices was also attempted.

8.2. Materials and Methods

8.2.1. Plant and AM fungal culture

Oryza sativa var. Korgut a salt tolerant, tall variety of rice which can withstand submergence in water was acquired from the Zonal Agricultural Office Government of Goa, Duler **(Plate X a)**,

Mapusa. Pure pot cultures of the dominant AM species *viz*., *Acaulospora scrobiculata* Trappe were employed as a bio-inoculum for the field study **(Plate X b)**.

8.2.2. Bioassay

8.2.2.1. Preparation of planting material

Seeds of *Oryza sativa* var. Korgut were surface sterilized with 0.2% sodium hypochloride for 3 minutes, washed in distilled water 3-4 times to remove any traces of sodium hypochloride, later primed (soaked in water overnight) and allowed to germinate. The germinated seeds were then sown in nursery trays.

8.2.2.2. Preparation of nursery trays

Soil used in the nursery trays was sterilized and consisted of a mixture of field soil from Sikeri and sand in the ratio 1:3 (w/w). Pure cultures of *A. scrobiculata* were raised using *Plectranthus scutellarioides* (L) R. Br. (coleus) as host grown in sterilized sand in the green house. The colonized roots and soil from pure culture were used as inoculum that contained approximately 90 - 95 spores per gram. Inoculum (500g) was placed 3-5 cm below the soil surface in the nursery trays prior to transferring the germinated seeds. Eight trays were maintained as control *i.e.* without AM and the other eight trays as treated *i.e*. with AM inoculum **(Plate X c)**. Approximately 100 germinated seedlings were transferred to each tray. The trays were watered daily to field capacity. The seedlings were then allowed to grow for 20 days to initiate AM colonization under natural light, temperature and humidity **(Plate X c, d and e)**. Before transplantation, the seedlings were randomly checked to ensure AM colonization.

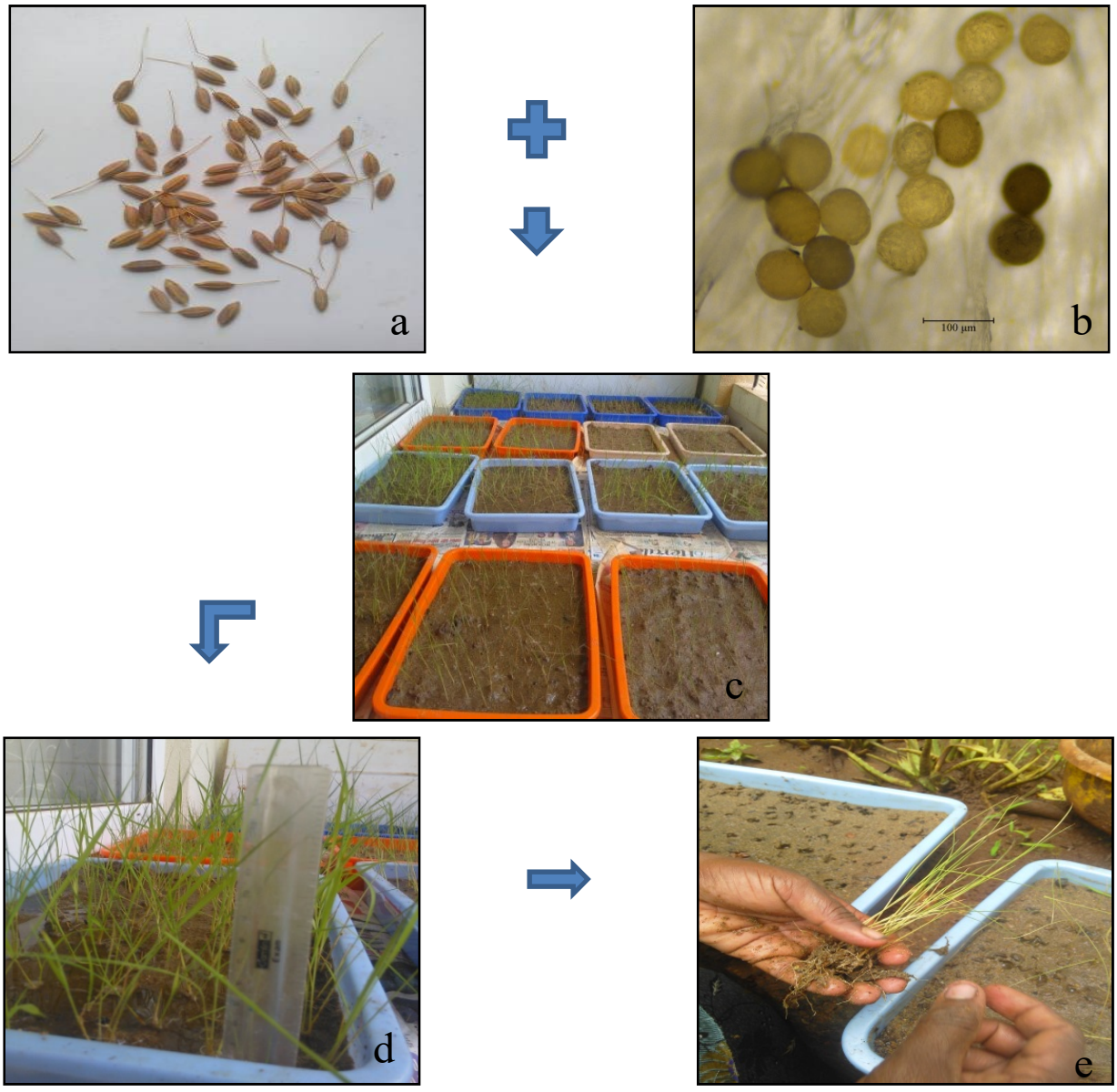
8.2.2.3. Isolation of AM spores

Isolation of AM spores was carried out as described under **3.2.5** before the preparation of the field at Sikeri **(Plate XI a)** for the experiment.

8.2.2.4. Estimation of AM spore density

Estimation of AM spore density in the experimental field at Sikeri was carried out before the beginning of the experiment as described under **3.2.6.**

Plate X



Inoculation with AM fungi in Nursery.
a. Seeds of *O. sativa* var. Korgut; **b.** Spores of *A. scrobiculata*;
c & d. Raising of nursery; **e.** Seedlings for transplantation.

Plate XI

Field Experiment.

a. Experimental site; **b.** Preparation of field for transplantation; **c.** Transplantation in progress; **d.** After 20 days from transplantation; **e.** At harvest; **f.** Panicles of treated plants.

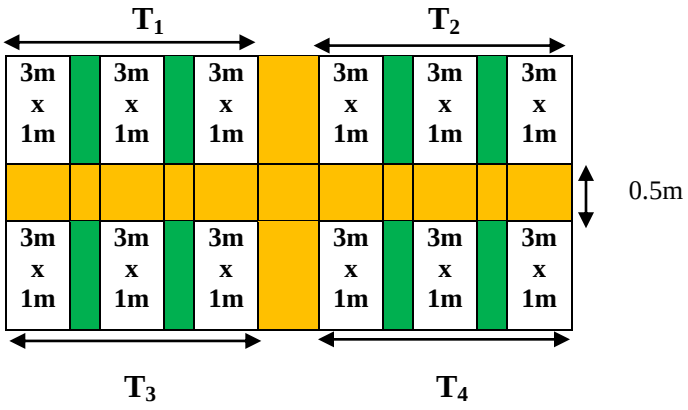
8.2.2.5. Preparation of Field

Ploughing, leveling, building of field *bunds* was carried out prior to transplantation **(Plate XI b)**. The AM colonized (inoculated) and uninoculated seedlings were then transplanted **(Plate XI c)** in July 2017 with a ball of soil containing the inoculums.

8.2.2.6. Experimental Design

The experiment consisted of a completely randomized block design with two treatments *i.e*. with and without AM inoculum, with 1 and 3 rice plantlets per hill and as described below ts (**Fig. 25**).

Fig. 25: Layout of experimental field.



1. T_1 (AM + one plant hill^{-1})
2. T_2 (uninoculated + one plant hill^{-1})
3. T_3 (AM + three plants hill^{-1})
4. T_4 (uninoculated + three plants hill^{-1})

Three replicates of each treatment were maintained. There were four main plots, each plot consisted of 3 subplots with a size of 3m x 1m and inter subplot spacing of 25cm and inter plot spacing of 50cm giving a total of 12 subplots with 65 plant hills each. Spacing between rice hills was 20 x 20cm. The field was submerged under water throughout the experimental period **(Plate XI d and e)**.

8.2.2.7. Fertilization and irrigation

No fertilizers were applied to the experimental plot. During September, with the decrease in rain and opening of sluice gates the water level in the fields decreased (5-7 cm) while at harvesting the water level was further reduced (1-3 cm).

8.2.3. Climate Data

The climate data *viz*., maximum and minimum temperature, relative humidity and rainfal during the months of study was obtained from Meteorological Department of ICAR-CCARI, Goa.

8.2.4. Soil Analyses

Analyses of the soil sample were carried out at the Soil Science Laboratory, ICAR-CCARI, Goa as described under **3.2.1.2**. Composite soil sample collected from the experimental site at Sikeri (15° 35' 18'' N, 73° 53' 20''E) was analysed before commencing the experiment and after harvest for different treatments.

8.2.5. Root processing

Root processing was carried before transplantation of rice seedlings. Besides, roots of five plants for each treatment were processed at the vegetative, flowering, and harvesting stage as described under **3.2.2.**

8.2.6. Estimation of AM fungal root colonization

Root samples before transplantation from five plants for each treatmentt at the vegetative, flowering, and harvesting stage were analysed for AM colonization as described under **3.2.3.**

8.2.7. Agronomic variables

8.2.7.1. Growth parameters

Five hills per treatment at the vegetative, flowering, and harvesting stage were randomly selected to analyse various parameters *viz.*, plant height, number of tillers and leaf number. A meter scale was used to measure the plant height from the base to the shoot tip. Fresh and dry shoot, root and total plant biomass of five randomly selected hills from each treatment at the vegetative,

flowering and harvesting stage were recorded. For dry biomass, plants were oven dried at 70°C for three days till a constant weight was attained.

8.2.7.2. Yield characteristics

Yield characteristics were recorded at the end of the cropping season (late October 2017) which was marked by uniform yellowing of the grain **(Plate XI. e, f)**. During harvesting, five hills per treatment were randomly selected to analyse the various parameters *viz.*, number of panicles per hill, number of fertilized seeds per hill, number of unfertilized seeds per hill, number of grains per hill and panicle length. The panicle length was measured using a meter scale from the base to the tip of the panicle. In each subplot, an area of 1m x 1m (*i.e.* 25 hills) was employed to quantify panicle number and yield. Later the panicles were cut and put into labeled bags. The rice seeds were then separated from the straw for each treatment and weighed. The crop yield (kg per hectare) was calculated. Weight of 1000 randomly selected seeds was recorded using an analytical weighing balance. Harvest Index (HI) was estimated as follows:

HI (%) = grain yield / above ground biomass (Amanula and Inamullah 2016).

Sterility percentage is ratio of unfertilized seeds to total number of seeds was also calculated.

8.2.8. Mycorrhizal Dependency

8.2.8.1. Relative Field Mycorrhizal Dependency (RFMD) Index

To determine the extent of increase in growth of rice due to AM colonization, RFMD Index was calculated at vegetative, flowering and harvesting stage (Plenchette *et al*. 1983).

RFMD Index = Dry weight of mycorrhizal plant/ Dry weight of non mycorrhizal plant x 100

8.2.8.2. Mycorrhizal efficiency index (MEI)

According to Norris *et al*. (1992) evaluation of growth development brought by AM inoculation in unsterile soil consisting of indigenous AM fungi is called as mycorrhizal efficiency index. It is effective in assessing the level to which the introduced AM species competes with native AM species to bring about plant growth response (Mehrotra 2005). MEI percent was calculated at vegetative, flowering and harvesting stage using the following formula

MEI% = weight of inoculated plant – weight of uninoculated plant /weight of inoculated plant x 100 (Bagyaraj 1994).

8.2.9. Winnowing

The seeds of different treatments were cleaned separately to remove unwanted seeds and other unwanted materials by winnowing, air dried and stored separately for two months before parboiling

8.2.10. Parboiling

Rice seeds from each replicate of a treatment were separated into two portions. One portion was analyzed as raw rice and the other as parboiled. The traditional parboiling process comprising of three important steps *viz.,* soaking (steeping), steaming and drying was employed. Seeds soaked overnight in water at room temperature were open steamed for 45 minutes and later sun dried. The raw and parboiled seeds for each treatmentwere further dehusked separately by hand pounding and later the husk was separated from the grain by winnowing (Otegbayo 2004).

8.2.11. Assessment of nutrient components

The nutritional components *viz.,* carbohydrates, proteins and mineral content were analysed in triplicate per treatment for raw and parboiled rice. After winnowing the rice grains were rinsed with distilled water, air dried and powdered using a mixer grinder. The powder was stored in zip-loc bags until further analyses.

8.2.11.1. Estimation of Total Carbohydrates

Total carbohydrates in raw and parboiled rice grain were estimated using the Phenol sulphuric acid method (Nielsen 2010). Powdered rice sample (100mg) for each treatment was taken in a test tube with 5mL of 2.5N HCl and kept in a boiling water bath for hydrolysis for three hours. After cooling at room temperature the samples were neutralized by adding sodium carbonate until effervescence ceased. The solution was then filtered and volume made to 100mL with distilled water (DW) and centrifuged. The resultant supernatant was used to estimate the carbohydrate content.

Standard solution of glucose was prepared by dissolving 100mg of glucose in 100mL of DW. Working standard was prepared with 10mL of glucose standard solution diluted to 100mL of DW. A series of test tubes was prepared by taking 0.2, 0.4, 0.6, 0.8 and 1mL of prepared working standard and 0.1 and 0.2mL of the sample solution in separate test tubes. The volume in each of the test tubes was then made to 1mL with DW. A blank was set with DW. In each of the test tubes 1mL of 5% phenol and 5mL of 96% sulphuric acid was added and shaken well. After 10 minutes the test tubes were placed in a water bath at 30°C for 20 minutes. Optical density was read at 490nm using a visible spectrophotometer (Bio Era BE/CI/SP/VS-01). The amount of total carbohydrate present in the rice was calculated using a standard calibration graph.

8.2.11.2. Estimation of Protein

Protein content from raw and parboiled rice grains was estimated in triplicates per treatment, by using Lowry's method (Lowry *et al.* 1951). Powdered rice sample (500mg) was taken in a test tube with 10mL of extraction buffer, centrifuged and the supernatant was used to estimate the protein content. The extraction buffer was prepared as follows: (Reagent A) 2% sodium carbonate in 0.1N sodium hydroxide; (Reagent B) 0.5% copper sulphate in 1% potassium sodium tartrate; (Reagent C) alkaline copper solution was prepared by mixing 50mL of Reagent A with and 1mL of B prior to use.

Standard protein solution was prepared by dissolving 50mg of bovine serum albumin (BSA) (fraction V) in a standard flask and the volume was made to 50mL with DW. Working standard was prepared by taking 10mL of standard protein and making it to 50mL with DW. A series of test tubes were prepared with 0.2, 0.4, 0.6, 0.8 and 1mL of working standard and 0.1 and 0.2mL of the sample solution was taken in separate test tubes. A tube with 1mL of DW was used as a blank. In each tube, 5mL of Reagent C was added, mixed well, allowed to stand for 10 minutes, and was followed by addition of 0.5mL of Folin-ciocalteau reagent. The tubes were mixed well and allowed to stand at room temperature in the dark for 30 minutes. The absorbance was read at 660nm using a visible spectrophotometer (Bio Era BE/CI/SP/VS-01). A standard graph was drawn and the protein content was estimated.

8.2.11.3. Estimation of Mineral Content

Mineral content in raw and parboiled rice was analysed in triplicates per treatment at the Soil Science Laboratory, Natural Resource Management Section ICAR-CCARI, Old Goa. The following procedure described by Singh *et al.* (2005) was employed for nutrient analyses.

Wet oxidation method was adopted for the release of mineral elements. The procedure for wet oxidation is as follows: One gram of dried rice powder sample was taken in a 150mL conical flask, to which 10ml of concentrated HNO_3 was added and a funnel was placed on the flask. The flask was kept overnight for pre-digestion in a chamber. When the solid sample was not visible at the end of pre-digestion, 10mL of concentrated HNO_3 and 4mL of $HClO_4$ was added to the flask. The flask was then kept on a hot plate in acid-proof digestion chamber at 100°C for one hour. After one hour the temperature was raised to 200°C till the contents of the flask become colourless and only white dense fumes appeared. The flask was then removed from the hot plate, cooled and 30mL of DW was added. The contents of the flask were then filtered using a Whatman No. 42 filter paper into a volumetric flask making its volume to 100mL with DW, which was used as an aliquot for the estimation of Fe, Mn, Cu and Zn concentration using Atomic Absorption Spectrophotometer (Analytikjena novAA400P).

8.2.12. Statistical Analysis

8.2.12.1. Analysis of soil data of each treatment after the experiment was carried out as described as under **3.2.4.1.**

8.2.12.2. Analysis of data of AM fungi colonization, growth parameters, yield and nutrient characteristics for each treatment were carried out as described under **3.2.9.1.**

8.2.12.3. To understand the effect of AM colonization, planting density individually and their interaction if any on growth parameters, yield characteristics and nutrients, univariate analysis of variance (ANOVA) was carried out as described under **5.2.9.4.**

8.2.12.4. To understand the relationship between yield characteristics and physico-chemical properties of soil at various treatments, CCA was carried out as described under **5.2.9.3.**

8.3. Results

8.3.1. Climate Data

The climatic data recorded during the study period is presented in **Fig. 26.** The average maximum and minimum temperatures during the growth period of rice were 31.25°C and 23.86°C, respectively. The average relative humidity was 93.73% and rainfall was 476.95mm.

Fig. 26: Climatic data recorded during the study period.

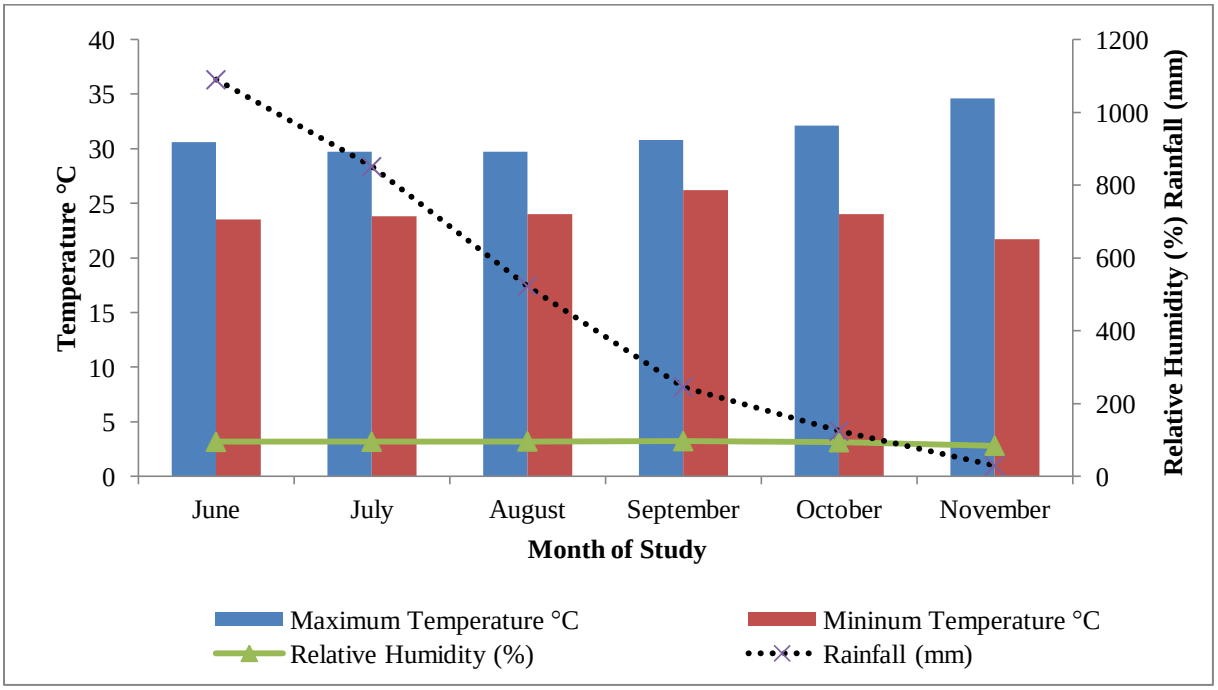


Table 48: Physico-chemical properties of soil before and after rice cultivation.

Parameter	pH	EC (dS/m)	OC (%)	N ($kgha^{-1}$)	P ($kg\ ha^{-1}$)	K ($kg\ ha^{-1}$)	S (ppm)	Fe (ppm)	Mn (ppm)	Cu (ppm)	Zn (ppm)
Before Cultivation											
Treatment	4.26 ±0.33	0.004 ±0.003	2.82 ± 0.05	1384.02 ± 48.22	13.47 ± 01.44	440.53 ± 7.57	2099.22 ± 54.07	34.49 ± 0.23	44.47 ± 0.67	0.01 ± .00	07.49 ±0.04
After Cultivation											
T_1	4.89^{a} ± 0.01	0.011^{a} ± 0.00	1.96^{a} ± 0.06	150.52^{a} ± 26.11	06.85^{a} ± 0.33	430.19^{a} ± 9.41	369.01^{a} ± 11.17	18.53^{a} ± 0.45	71.48^{a} ± 1.16	0.83^{a} ± 0.11	3.54^{a} ± 0.67
T_2	4.83^{a} ± 0.01	0.009^{a} ± 0.003	2.09^{a} ± 0.01	175.61^{a} ± 50.69	12.66^{a} ± 2.04	417.87^{a} ± 0.97	248.43^{a} ± 7.97	20.69^{b} ± 0.27	67.21^{b} ± 0.15	0.58^{a} ± 0.03	2.30^{ab} ± 0.13
T_3	4.17^{b} ± .008	0.007^{b} ± 0.005	1.92^{a} ± 0.02	175.61^{a} ± 21.72	08.61^{a} ± 01.01	404.36^{ab} ± 7.46	312.76^{a} ± 15.47	21.29^{b} ± 0.10	49.37^{c} ± 0.05	0.20^{b} ± .008	1.14^{b} ± 0.02
T_4	4.26^{c} ± 0.01	0.007^{b} ± 0.005	2.36^{b} ± 0.03	217.43^{a} ± 68.45	12.00^{a} ± 0.81	384.64^{b} ± 1.42	226.30^{a} ± 20.33	21.26^{b} ± 0.40	51.70^{c} ± 0.44	0.18^{b} ± 0.01	0.97^{bc} ± 0.04
$F_{(3,18)}$	981.66*	19.50*	28.25*	0.36	5.10	10.33*	1.27	14.73*	307.43*	28.61*	12.00*

Legend: T_1: AM +1 plant $hill^{-1}$; T_2: Uninoculated +1 plant $hill^{-1}$; T_3: AM +3 plants $hill^{-1}$; T_4: Uninoculated +3 plants $hill.^{-1}$;
Data presented is mean of three readings for each treatment; different letters within the same column stage indicate significant differences at $P< 0.05$; ± indicates Standard Error; OC = Organic Carbon; N = Nitrogen; P = Phosphorus; K = Potassium; Fe = Iron; Mn = Manganese; Cu = Copper; Zn = Zinc.

8.3.2. Soil analyses

Results of physico-chemical properties of field soil before and after rice cultivation are depicted in **Table 48.**

8.3.3. AM fungal root colonization

Results of root colonization in *O. sativa* L. var. Korgut are depicted in **Table 49.** Root colonization was recorded in all the treatments at the vegetative, flowering and harvesting stage. However, the extent of colonization varied significantly at different growth stages for each treatment except T_2 treatment, as well as between different treatments at each growth stage at $p < 0.05$. Root colonization was significantly higher in AM inoculated plants compared to uninnoculated plants. Irrespective of growth stage, maximum percent colonization was recorded in T_1 treatment. Mean percent colonization showed significant difference in the treatments and was 25.33%, 5.60%, 18.00% and 5.20% in T_1, T_2, T_3 and T_4 treatments, respectively.

Table 49: AM root colonization in plants subjected to different treatments and at different growth stages.

Treatment	Root colonization (%)			
	Vegetative	Flowering	Harvesting	$F_{(2,12)}$
T_1	$24.00_a^a \pm 2.19$	$37.20_a^b \pm 1.49$	$14.80_a^c \pm 1.35$	42.82*
T_2	$3.60_b^a \pm 0.40$	$6.40_b^a \pm 0.97$	$6.80_b^a \pm 1.74$	02.19
T_3	$20.80_a^a \pm 1.01$	$22.40_c^a \pm 1.72$	$10.80_{ab}^b \pm 0.48$	27.96*
T_4	$2.40_b^a \pm 0.40$	$4.80_b^b \pm 0.48$	$8.40_b^c \pm 0.74$	28.50*
$F_{(3,19)}$	82.72*	145.09*	8.52*	

Legend: T_1: AM +1 plant hill^{-1}; T_2: Uninoculated +1 plant hill^{-1}; T_3: AM +3 plants hill^{-1}; T_4: Uninoculated +3 plants hill.$^{-1}$; Data presented is mean of five reading;± indicates Standard Error. For each treatment and percent root colonization values in a column affected by the same letter as a subscript are not significantly different at $P < 0.05$. For percent colonization at different growth stage for each treatment, values in a row affected by the same letter as a superscript are not significantly different at $P < 0.05$. Mean percent colonization for different treatments was $25.33^a \pm 2.62$, $5.60^b \pm 0.73$, $18.00^c \pm 1.51$ and $5.20^b \pm 0.72$ at T_1, T_2, T_3 and T_4 respectively at $p<0.05$, $F_{(3,56)}=37.89$*.

8.3.4. Agronomic variables

8.3.4.1. Plant growth parameters

Data on the effect of AM colonization and planting density on various growth parameters are given in **Table 50.**

In the flowering and harvesting stage there was no significant difference in plant height for the different treatments except at the vegetative stage in T_2 treatment. Maximum plant height was recorded in T_3 treatment and least was observed for T_2 treatment across all treatments and growth stages **(Fig. 27)**.

There was significant difference in tiller number in different treatments and at different growth stages. Maximum number of tillers in the vegetative stage was recorded in T_3 treatment, at the flowering and harvesting stage in T_1 treatment and the least was observed in T_2 treatment **(Fig. 28)**.

In the vegetative and flowering stage, maximum leaf number was recorded in T_3 treatment. However, in the harvesting stage maximum leaf number was recorded in T_1 treatment and least was recorded in T_2 treatment **(Fig. 29)**.

No significant difference in root biomass was observed between the treatments in vegetative and flowering stage. However, maximum root biomass was recorded for T_3 treatment. At the harvesting stage, there was significant difference between T_2 and T_1 as well as T_3 and T_4 treatments. However, maximum root biomass was recorded in T_3 treatment **(Fig. 30)**.

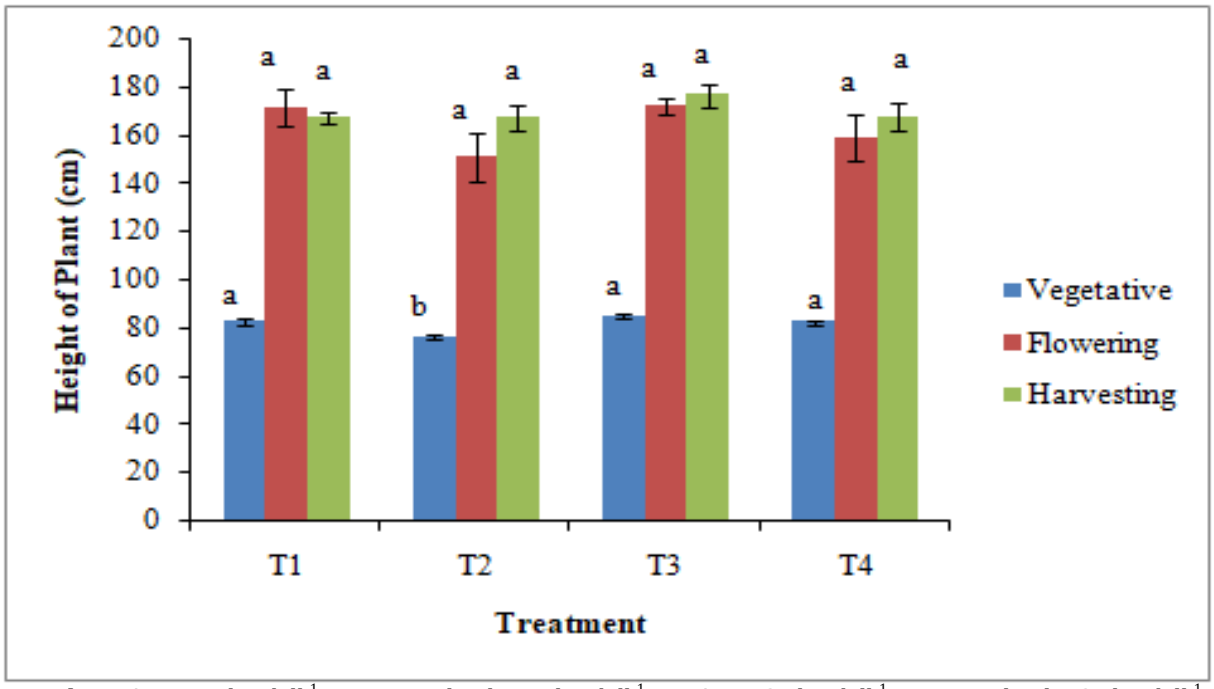
For shoot biomass, at the vegetative stage, there was no significant difference between the treatments. However maximum shoot biomass was recorded in T_3 treatment. At the flowering and harvesting stage there was a significant difference in shoot biomass. Maximum shoot biomass in the flowering and harvesting stage was observed in T_3 and T_1 treatments, respectively **(Fig. 31)**.

Table 50: Effect of AM colonization and planting density on growth parameters in rice.

Growth stage	Treatment	Plant height (cm)	No. of tillers hill^{-1}	No. of leaves hill^{-1}	Root biomass hill^{-1} (g)	Shoot biomass hill^{-1} (g)	Total Plant biomass hill^{-1} (g)	Shoot Root Ratio
Vegetative	T_1	82.50^{a}± 1.37	1.80^{a} ± 0.20	5.80^{a}± 0.37	0.55^{a}± 0.08	3.50^{a} ± 0.38	4.04^{a}± 0.44	6.69^{a} ± 0.68
	T_2	75.80^{b}± 1.00	1.40^{ab} ± 0.24	5.20^{a}± 0.37	0.39^{a} ± 0.02	2.69^{a}± 0.27	3.08^{a}± 0.29	6.82^{a}± 0.40
	T_3	85.00^{a} ± 0.70	3.60^{c} ± 0.60	15.00^{b}± 2.68	0.60^{a} ± 0.03	3.74^{a}± 0.40	4.34^{a} ± 0.42	6.18^{a}± 0.46
	T_4	82.30^{a}± 0.96	3.00^{bc} ± 0.00	13.80^{b}± 1.20	0.40^{a} ± 0.05	2.84^{a}± 0.24	3.25^{a} ± 0.24	7.85^{a}± 1.71
$F_{(3,16)}$		14.18*	9.13*	11.97*	3.62	2.27	2.80	0.51
Flowering	T_1	171.60^{a}± 7.40	8.80^{a}± 0.37	10.40^{a}± 0.67	0.92^{a} ±0.05	4.81^{a}± 0.16	5.73^{a} ± 0.18	5.32^{a}± 0.38
	T_2	150.60^{a}± 10.10	2.00^{b} ± 0.00	6.40^{b}± 0.24	0.97^{a} ± 0.30	3.43^{b}± 0.12	3.80^{b}± 0.49	6.17^{a} ± 2.54
	T_3	171.90^{a}± 3.19	7.80^{a}± 0.73	22.20^{c}± 0.73	1.42^{a} ± 0.07	6.99^{c} ± 0.12	8.41^{c}± 0.15	4.95^{a}± 0.25
	T_4	158.70^{a}± 9.64	7.20^{a}± 0.73	22.20^{c}± 0.01	0.73^{a} ± 0.11	4.50^{a}± 0.23	5.23^{a} ± 0.31	6.80^{a}± 1.03
$F_{(3,16)}$		1.66	30.28*	165.36*	2.86	77.41*	36.70*	0.35
Harvesting	T_1	166.90^{a}± 2.59	12.40^{a}± 0.50	33.00^{a}± 2.30	7.88^{a} ± 0.45	39.07^{a} ± 3.39	46.95^{a} ± 3.60	4.97^{a} ± 0.40
	T_2	166.90^{a}± 5.33	2.40^{b}± 0.24	11.40^{b} ± 1.12	5.00^{b} ± 0.53	21.27^{b}± 1.58	26.27^{b}± 1.95	4.35^{a}± 0.34
	T_3	176.60^{a}± 4.65	10.80^{ac}±1.50	20.60^{c}± 1.50	7.82^{a} ± 0.67	34.93^{a}±1.99	42.75^{a}± 2.48	4.54^{a} ±0.31
	T_4	167.40^{a}± 5.59	7.20^{c}± 1.52	19.80^{c}± 2.20	7.91^{a}± 0.67	18.09^{b} ±0.95	26.01^{b}± 0.99	2.37^{b}± 0.27
$F_{(3,16)}$		1.03	15.74*	23.19*	5.95*	22.09*	19.98*	11.73*

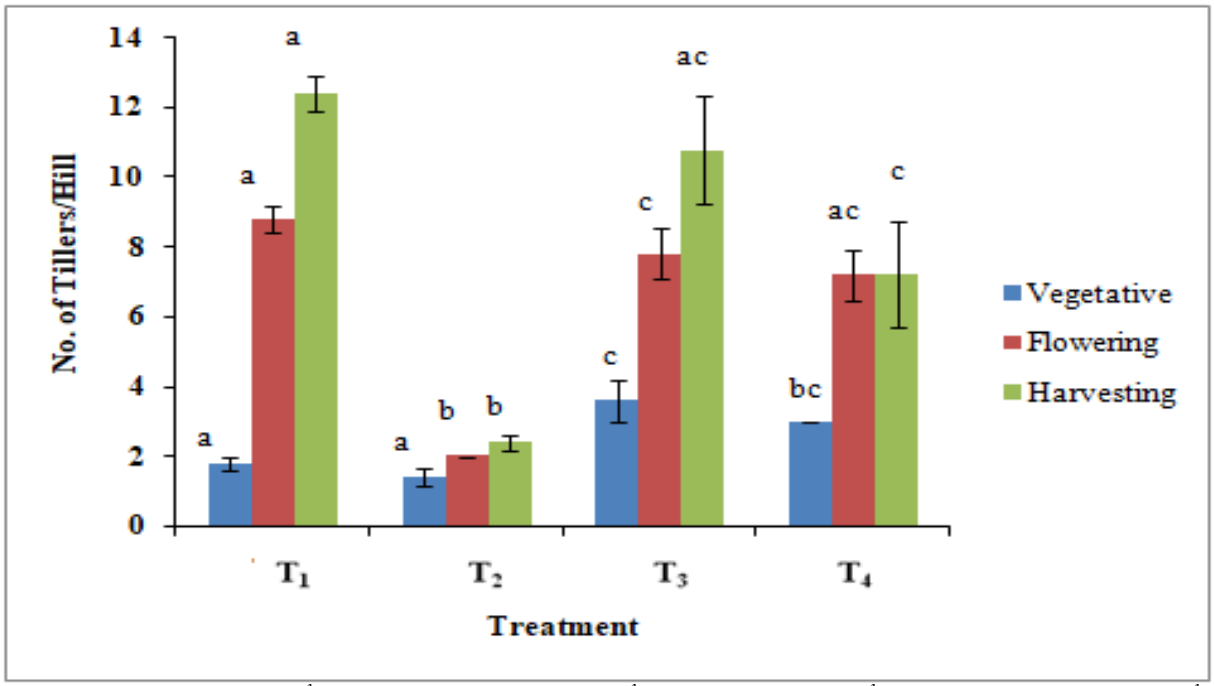
Legend: T_1: AM +1 plant hill^{-1}; T_2: Uninoculated +1 plant hill^{-1}; T_3: AM +3 plants hill^{-1}; T_4: Uninoculated +3 plants hill.$^{-1}$ Data presented is the mean of five readings at each growth phase; different letters between the different treatment of study indicate significant differences at $P< 0.05$; ± indicates Standard Error.

Fig. 27: Effect of AM colonization and planting density on plant height.



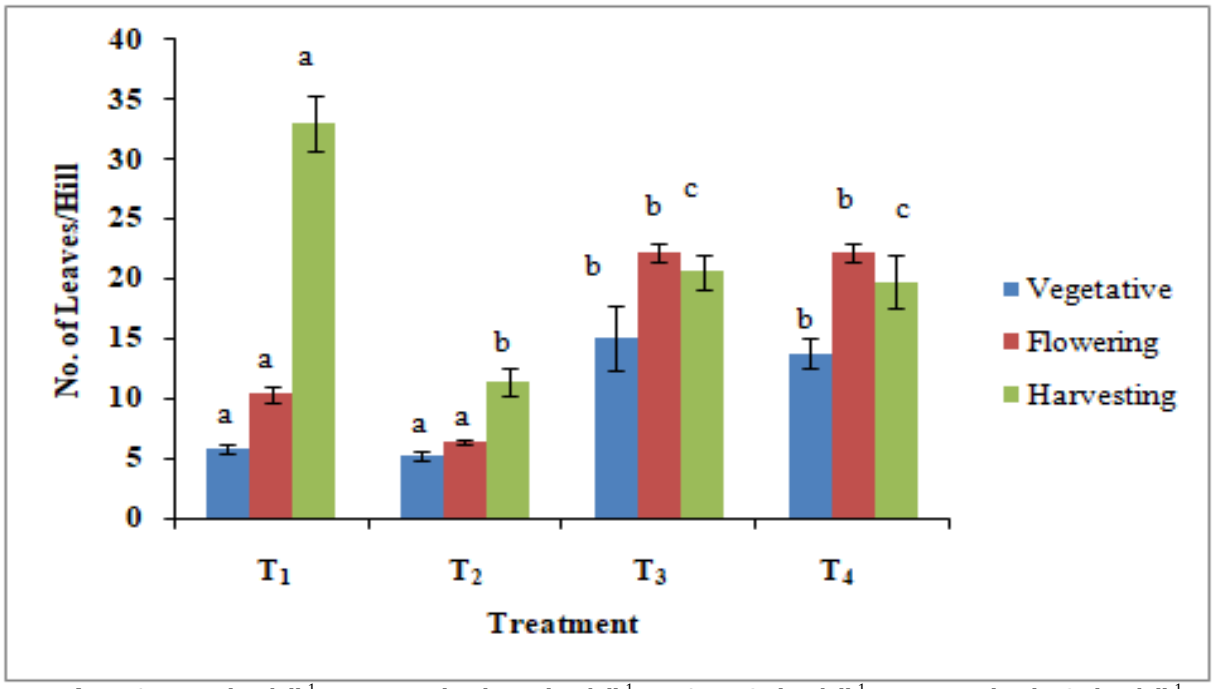
Legend: T_1: AM + 1 plant hill^{-1}; T_2: uninoculated + 1 plant hill^{-1}; T_3: AM + 3 plant hill^{-1}, T_4: ninoculated + 3 plant hill^{-1}; Error bars represents Error bars; Values on the column affected by the same letter are not significantly different between the growth stages.

Fig. 28: Effect of AM colonization and planting density on tiller number.



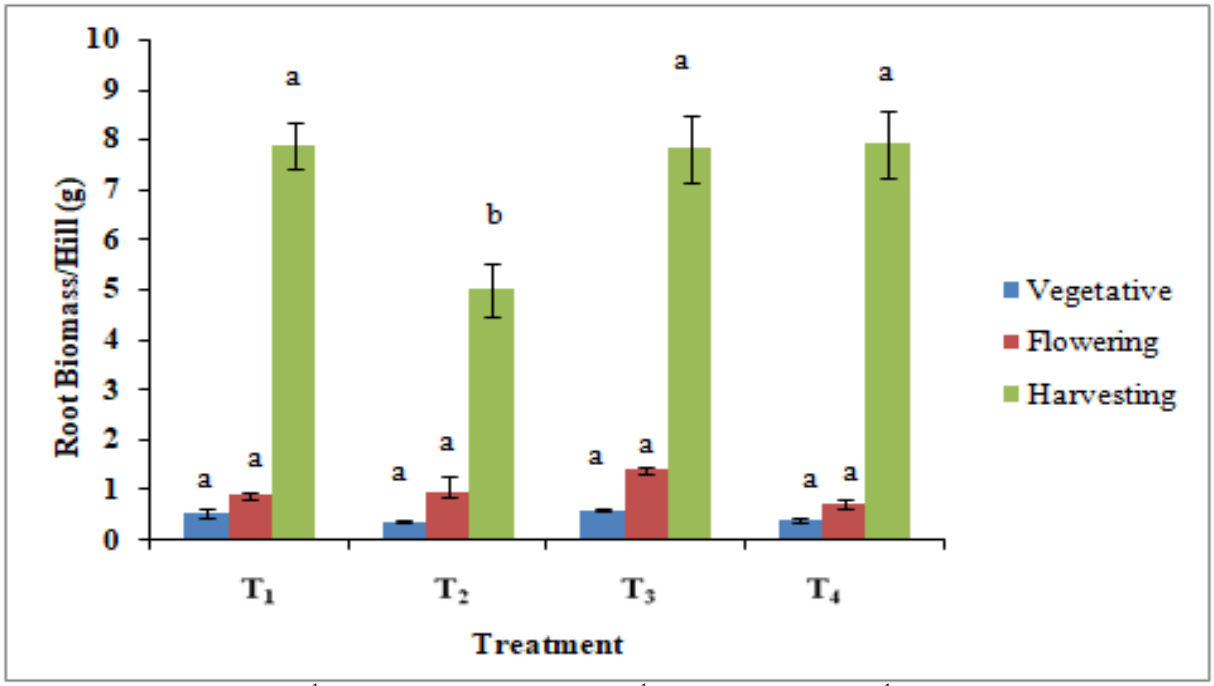
Legend: T_1: AM + 1 plant hill^{-1}; T_2: uninoculated + 1 plant hill^{-1}; T_3: AM + 3 plant hill^{-1}, T_4: uninoculated + 3 plant hill^{-1}; Error bars represents Error bars; Values on the column affected by the same letter are not significantly different between the growth stages.

Fig. 29: Effect of AM colonization and planting density on leaf number.



Legend: T_1: AM + 1 plant hill^{-1}; T_2: uninoculated + 1 plant hill^{-1}; T_3: AM + 3 plant hill^{-1}, T_4: uninoculated + 3 plant hill^{-1}; Error bars represents Error bars; Values on the column affected by the same letter are not significantly different between the growth stages

Fig. 30: Effect of AM colonization and planting density on root biomass.

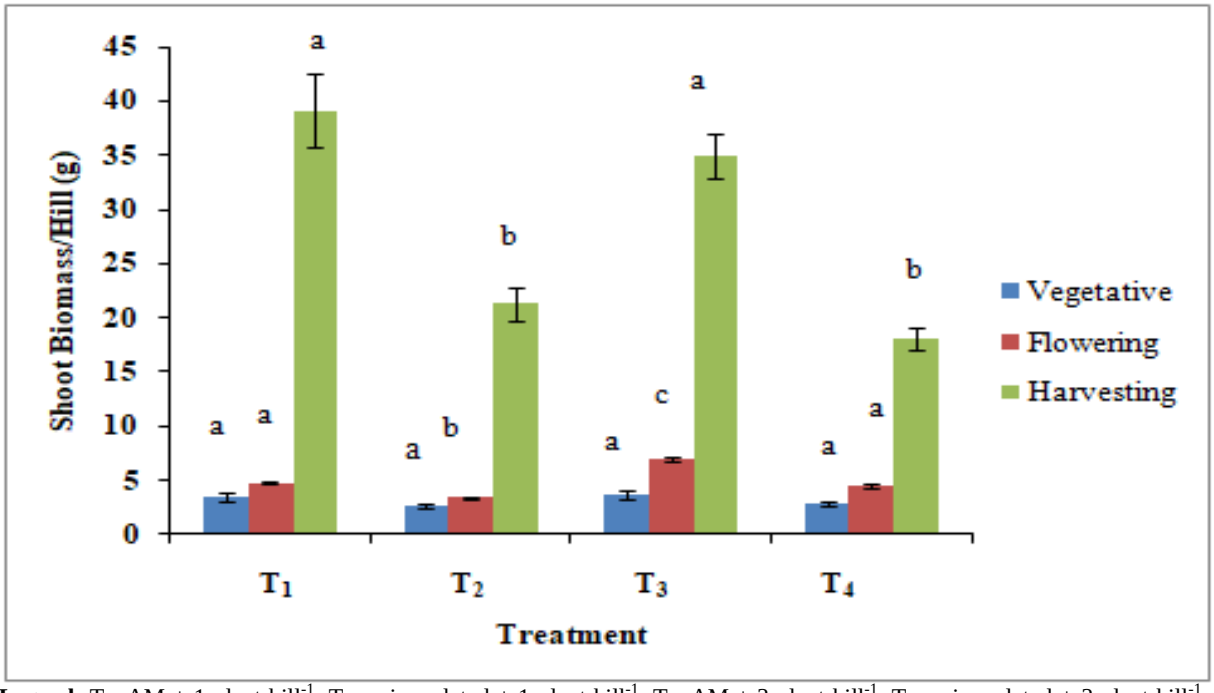


Legend: T_1: AM + 1 plant hill^{-1}; T_2: uninoculated + 1 plant hill^{-1}; T_3: AM + 3 plant hill^{-1}, T_4: uninoculated + 3 plant hill^{-1}; Error bars represents Error bars; Values on the column affected by the same letter are not significantly different between the growth stages.

No significant difference was observed in plant biomass at the vegetative stage. However, maximum plant biomass was recorded in T_3 treatment. During the flowering and harvesting stage, a significant difference was observed among the different treatments. Maximum plant biomass at the flowering and harvesting stage was recporded in T_3 and T_1 treatments, respectively **(Fig. 32)**.

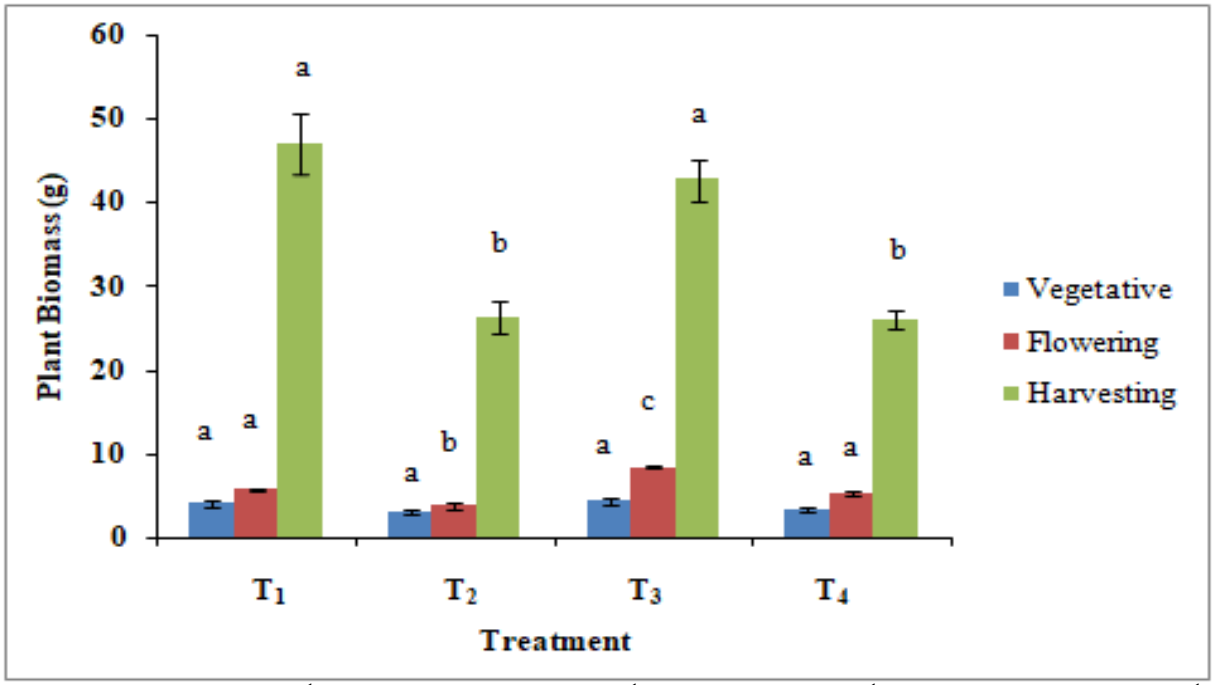
There was no significant difference in shoot root ratio at the vegetative and flowering stage among the different treatments. Maximum shoot root ratio being obtained in T_4 treatment, while at harvesting stage, the maximum shoot root ratio was observed in T_1 treatment **(Fig. 33)**.

Fig. 31: Effect of AM colonization and planting density on shoot biomass.



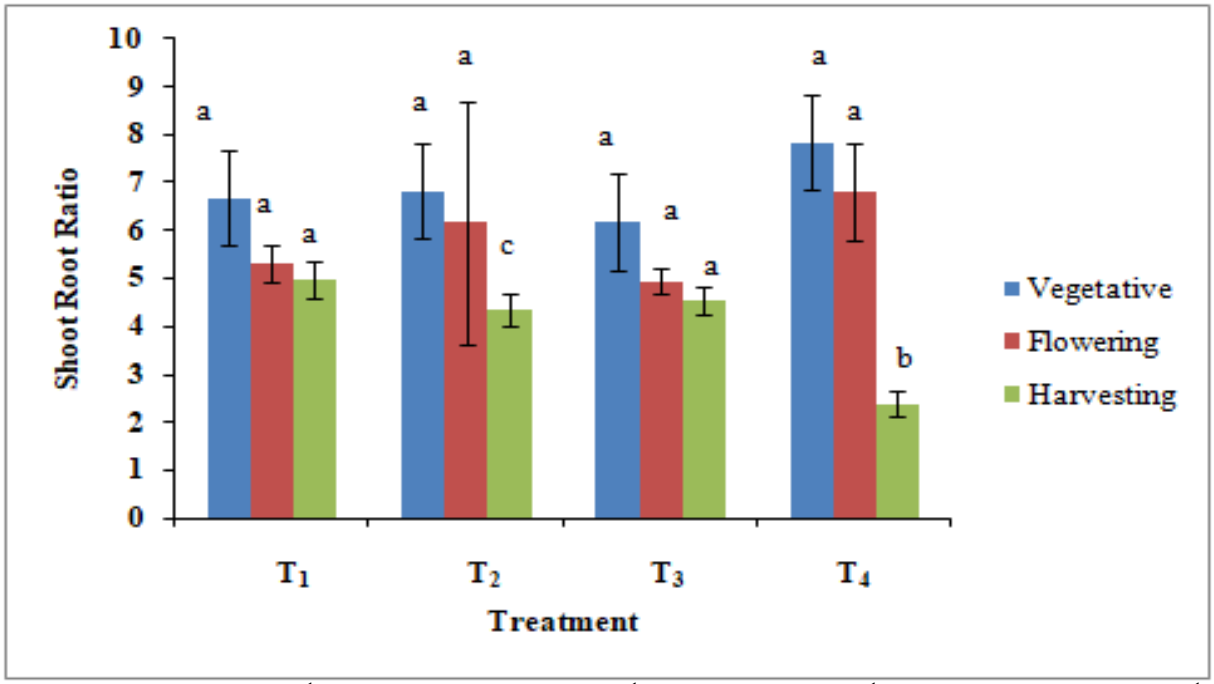
Legend: T_1: AM + 1 plant hill^{-1}; T_2: uninoculated + 1 plant hill^{-1}; T_3: AM + 3 plant hill^{-1}, T_4: uninoculated + 3 plant hill^{-1}; Error bars represents Error bars; Values on the column affected by the same letter are not significantly different between the growth stages.

Fig. 32: Effect of AM colonization and planting density on plant biomass.



Legend: T_1: AM + 1 plant hill^{-1}; T_2: uninoculated + 1 plant hill^{-1}; T_3: AM + 3 plant hill^{-1}, T_4: uninoculated + 3 plant hill^{-1}; Error bars represents Error bars; Values on the column affected by the same letter are not significantly different between the growth stages.

Fig. 33: Effect of AM colonization and planting density on shoot root ratio.



Legend: T_1: AM + 1 plant hill^{-1}; T_2: uninoculated + 1 plant hill^{-1}; T_3: AM + 3 plant hill^{-1}, T_4: uninoculated + 3 plant hill^{-1}; Error bars represents Error bars; Values on the column affected by the same letter are not significantly different between the growth stages.

8.3.4.2. Effect of AM inoculation and planting density on growth parameters

Results of AM inoculation, planting density and its interaction on various growth parameters are depicted **Table 51**.

At the vegetative stage, AM inoculation had significant effect on plant height, leaf number, root, shoot and total plant biomass. Results revealed that the planting density had significant effect on plants height and tiller number. However, the interaction of AM inoculation and planting density did not have any significant effect on growth parameters.

In the flowering stage, AM inoculation had significant effect on tiller number, leaf number, shoot and total plant biomass. Planting density had significant effect on tiller number, leaf number and shoot biomass. However, the interaction between AM inoculation and planting density had effect on tiller number, leaf number and shoot biomass. At the harvesting stage, AM colonization had significant effect on tiller number, leaf number, root, shoot and total plant biomass, and shoot root ratio. Planting density had an effect on root biomass and shoot root ratio. The interaction between AM inoculation and planting density had significant effect on tiller, leaf number, root biomass and shoot root ratio.

Table 51: Univarate two-way ANOVA assessing the effect of AM colonization and planting density and its interaction on growth parameters during vegetative, flowering and harvesting stage.

Growth Parameter	Source	Vegetative					Flowering					Harvesting				
		df	***SS***	***MS***	***F***	***sig.***	***df***	***SS***	***MS***	***F***	***sig.***	***df***	***SS***	***MS***	***F***	***sig.***
Plant height	**AM colonization**	1	110.45	110.45	20.28*	0.00	1	1462.05	1462.05	4.49	0.05	1	105.80	105.80	0.96	0.34
	Planting density	1	101.25	101.25	18.59*	0.00	1	88.20	88.20	0.27	0.61	1	130.05	130.05	1.18	0.29
	AM Colonization x Planting density	1	20.00	20.00	3.67	0.07	1	76.05	76.05	0.23	0.63	1	105.80	105.80	0.96	0.34
	Error	16	87.10	5.44			16	5206.40	325.40			16	1760.80	110.05		
	Total	20	13222838.00				20	539517.50				20	576368.50			
No. of tillers hill^{-1}	**AM colonization**	1	1.25	1.25	2.17	0.16	1	68.45	68.45	44.88*	0.00	1	231.20	231.20	36.99*	0.00
	Planting density	1	14.45	14.45	25.13*	0.00	1	22.05	22.05	14.45*	0.00	1	12.80	12.80	2.04	0.17
	AM Colonization x Planting density	1	0.05	0.05	0.08	0.77	1	48.05	48.05	31.50*	0.00	1	51.2	51.2	8.19*	0.01
	Error	16	9.20	0.57			16	24.40	1.52			16	100.00	6.25		
	Total	20	145.00				20	995.00				20	1740			
No. of leaves hill^{-1}	**AM colonization**	1	4.05	4.05	0.36	0.55	1	20.00	20.00	10.00*	0.00	1	627.20	627.20	36.73*	0.00
	Planting density	1	396.05	396.05	35.52*	0.00	1	952.20	952.20	476.10*	0.00	1	20.00	20.00	1.17	0.29
	AM Colonization x Planting density	1	0.45	0.45	0.04	0.84	1	20.00	20.00	10.00*	0.00	1	540.80	540.80	31.67*	0.00
	Error	16	178.40	11.15			16	32.00	2.00			16	273.2	17.07		
	Total	20	2559.00				20	5706.00				20	10450.00			
Root biomass	**AM colonization**	1	0.15	0.15	10.32*	0.00	1	0.49	0.49	3.38	0.08	1	9.70	9.70	5.59*	0.03
	Planting density	1	0.00	0.00	0.36	0.55	1	0.08	0.08	0.56	0.46	1	10.18	10.18	5.87*	0.02
	AM Colonization x Planting density	1	0.00	0.00	0.17	0.67	1	0.68	0.68	4.64*	0.04	1	11.05	11.05	6.37*	0.02
	Error	16	0.23	0.01			16	2.34	0.14			16	27.73	1.73		
	Total	20	5.13				20	23.98				20	1082.12	1082.12		
Shoot biomass	**AM colonization**	1	3.61	3.61	6.45*	0.02	1	18.72	18.72	129.87*	0.00	1	1499.91	1499.91	63.41*	0.00
	Planting density	1	0.19	0.19	0.34	0.56	1	13.20	13.20	91.59*	0.00	1	66.68	66.68	2.81	0.11
	AM Colonization	1	0.00	0.00	0.01	0.90	1	1.55	1.55	10.76*	0.00	1	1.15	1.15	0.04	0.82

	x Planting density															
	Error	16	8.96	0.56			16	2.30	0.14			16	378.43	23.65		
	Total	20	216.55				20	522.57				20	18009.29			
Plant biomass	**AM colonization**	1	5.25	5.25	7.97*	0.01	1	32.51	32.51	64.42*	0.00	1	1750.88	1750.88	58.48*	0.00
	Planting density	1	0.26	0.26	0.39	0.53	1	21.09	21.09	41.80*	0.00	1	24.75	24.75	0.82	0.37
	AM Colonization x Planting density	1	0.02	0.02	0.03	0.86	1	1.95	1.95	3.88	0.06	1	19.34	19.34	0.64	0.43
	Error	16	10.54	0.65			16	8.07	0.50			16	479.02	29.93		
	Total	20	286.70				20	734.81				20	27469.78			
Shoot root ratio	**AM colonization**	1	4.03	4.03	0.85	0.37	1	9.15	9.15	0.94	0.34	1	9.77	9.77	17.16*	0.00
	Planting density	1	0.33	0.33	0.07	0.79	1	0.08	0.08	0.00	0.92	1	7.27	7.27	12.77*	0.00
	AM Colonization x Planting density	1	2.91	2.91	0.61	0.44	1	1.24	1.24	0.12	0.72	1	2.99	2.99	5.26*	0.03
	Error	16	75.85	4.74			16	155.54	9.72			16	9.10	0.58		
	Total	20	1031.47				20	840.80				20	358.65			

Legend: *df* – Degree of freedom; *SS*- Sum of Squares; *MS*- Mean Square; *F*- Fisher's value; * significant at $P < 0.05$

8.3.3.3. Yield characteristics

Results on the effect of AM colonization and planting density on yield characteristics are depicted in **Table 52**, **Fig. 34**.

There was significant difference between the number of panicles per hill. Maximum panicle number per hill was recorded in T_3 treatment. Unfertilized seeds per hill did not show significant difference between the various treatments. However, maximum number of unfertilized seeds was observed in T_4 treatment. Number of fertilized seeds per hill, number of grains per hill, panicle length, yield, weight of 1000 seeds and Harvest index showed a significant difference between the different treatments, and maximum values for each of thse parameters were recorded in T_1 treatment. Number of panicles per m^2 was significantly varied for the different treatments with maximum number observed in T_3 treatment. Significant difference was also observed for sterility percentage with maximum sterility level observed in T_4 treatment.

8.3.3.4. Effect of AM inoculation and planting density on growth parameters

Results on the effect of AM inoculation, planting density and its interaction on various growth parameters are depicted in **Table 53**.

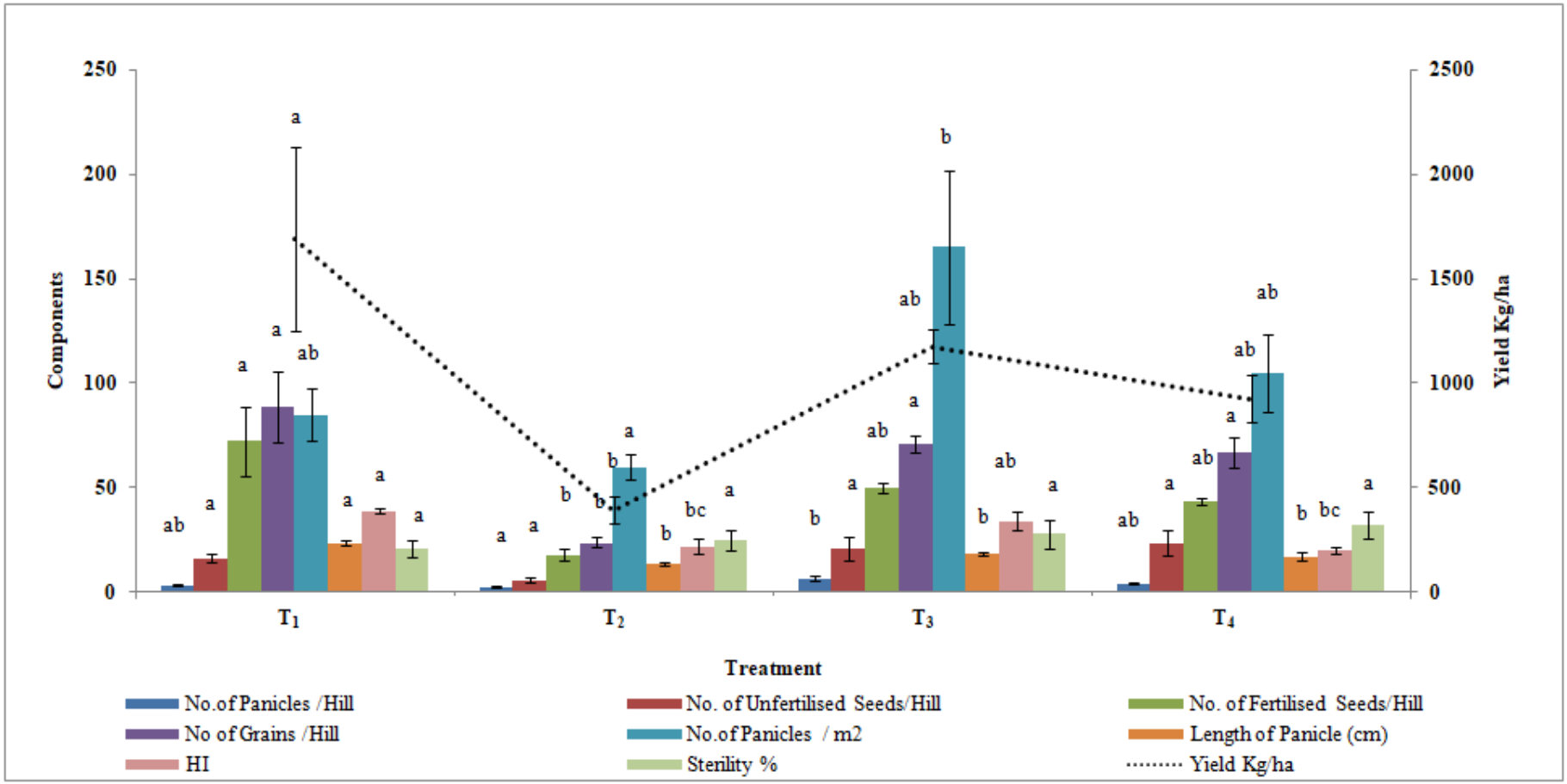
Mycorrhizal inoculation had a significant effect on number grains per hill, panicle length, yield, weight of 1000 seeds and harvest index. Planting density had a significant effect on number of panicles per hill, number of unfertilized and fertilized seeds, number of panicles per m^2 and weight of 1000 seeds. The interaction of AM inoculation and planting density had a significant effect on number of fertilized seeds, number of grains per hill and panicle length. However, AM inoculation, planting density and its interaction did not have any effect on sterility percentage.

Table 52: Effect of AM colonization and planting density on yield and characteristics.

Treatment	No. of panicle hill^{-1}	No. of unfertilised seeds hill^{-1}	No. of fertilised seeds hill^{-1}	No. of grain hill^{-1}	No. of panicles m$^{2-1}$	Length of panicle (cm)	Yield kg ha^{-1}	Weight of 1000 seeds (g)	HI	Sterility %
T_1	3.40^{ab} ± 0.50	16.35^{a}± 1.93	72.20^{a}± 16.64	88.57^{a}± 16.71	85.00^{ab}± 12.74	23.56^{a}± 1.02	1687.50^{a} ± 439.63	67.32^{a} ± 0.31	0.39^{a}± 0.01	20.60^{a} ± 4.13
T_2	2.40^{a}± 0.24	5.80^{a}± 1.11	17.93^{b}± 2.56	23.73^{b} ± 2.41	60.00^{a}± 6.12	13.43^{b}± 0.52	391.30^{b}± 64.39	65.30^{b} ± 0.73	0.22^{bc} ±0.03	24.80^{a} ± 4.70
T_3	6.60^{b}± 1.46	21.00^{a} ± 5.53	49.99^{ab} ± 2.60	70.99^{a} ± 3.86	165.00^{b}± 36.74	18.28^{b}± 0.79	1175.43^{ab} ± 81.37	65.61^{ab}± 0.19	0.34^{ab} ± 0.04	28.00^{a}± 6.83
T_4	4.20^{ab}± 0.73	23.40^{a} ± 6.17	43.50^{ab} ± 1.89	66.90^{a} ± 7.02	105.00^{ab}± 18.37	17.30^{b} ± 1.89	925.10^{ab}± 117.72	64.08^{b}± 0.26	0.20^{bc} ± 0.01	32.30^{a} ± 6.35
$F_{(3,16)}$	4.25*	3.29	6.78*	8.67*	4.25*	12.58*	5.33*	9.59*	9.18*	0.77

Legend: T_1: AM +1 plant hill^{-1}; T_2: Uninoculated +1 plant hill^{-1}; T_3: AM +3 plants hill^{-1}; T_4: Uninoculated +3 plants hill.$^{-1}$; Data presented is the mean of five reading; ± indicates Standard Error; Different letters between the different treatments of study indicate significant differences at $P< 0.05$.

Fig. 34: Effect of AM colonization and planting density on yield characteristics.



Legend: T_1: AM +1 plant hill^{-1}; T_2: Uninoculated +1 plant hill^{-1}; T_3: AM +3 plants hill^{-1}; T_4: Uninoculated +3 plants hill.$^{-1}$; Error bars represents Standard Error; Values on the column affected by the same letter are not significantly different between the treatments at $p < 0.05$.

Table 53: Univarate two-way ANOVA assessing the effect of AM colonization and planting density and its interaction on yield characteristics.

Parameters	Source	*df*	*SS*	*MS*	*F*	*sig.*
No. of panicles hill^{-1}	**AM colonization**	1	14.45	14.45	3.82	0.06
	Planting density	1	31.25	31.25	8.27*	001
	AM colnization x Planting density	1	2.45	2.45	0.64	0.43
	Error	16	60.40	3.77		
	Total	20	453.00			
No. of unfertilized seeds hill^{-1}	**AM colonization**	1	83.15	83.15	0.90	0.35
	Planting density	1	618.71	618.71	6.71*	0.02
	AM colnization x Planting density	1	209.82	209.82	2.27	0.15
	Error	16	1474.83	92.17		
	Total	20	7922.98			
No. of fertilized seeds hill^{-1}	**AM colonization**	1	4615.02	4615.02	12.58*	0.00
	Planting density	1	14.09	14.09	0.03	0.84
	AM colnization x Planting density	1	2853.42	2853.42	7.76*	0.01
	Error	16	5878.24	367.39		
	Total	20	55508.92			
No. of grains hill^{-1}	**AM colonization**	1	5938.49	5938.49	13.59*	0.00
	Planting density	1	819.81	819.81	1.87	0.19
	AM colnization x Planting density	1	4611.98	4611.98	10.55*	0.00

	Error	16	6991.45	436.96		
	Total	20	96604.54			
No. of panicles/m^2	**AM colonization**	1	9031.25	9031.25	3.82	0.06
	Planting density	1	19531.25	19531.25	8.27*	0.01
	AM colnization x Planting density	1	1531.25	1531.25	6.49	0.43
	Error	16	37750.00			
	Total	20	283125.00			
Length of panicle	**AM colonization**	1	154.23	154.23	22.27*	0.00
	Planting density	1	2.49	2.49	0.36	0.55
	AM colnization x Planting density	1	104.60	104.60	15.10*	0.00
	Error	16	110.79	6.92		
	Total	20	6955.49			
Yield Kg ha^{-1}	**AM colonization**	1	1793824.01	1793824.01	10.97*	0.01
	Planting density	1	354.25	354.25	0.00	0.96
	AM colnization x Planting density	1	820377.81	820377.81	5.02	0.05
	Error	16	1307432.34	163429.04		
	Total	20	17022			
Weight of 1000 seeds	**AM colonization**	1	15.73	15.73	16.94*	0.00
	Planting density	1	10.68	10.68	11.50*	0.00

	AM colnization x Planting density	1	0.30	0.30	0.32	0.57
	Error	16	14.86	0.92		
	Total	20	86043.19			
Harvest Index	**AM colonization**	1	0.11	0.11	25.67*	0.00
	Planting density	1	0.00	0.00	1.60	0.22
	AM colnization x Planting density	1	0.00	0.00	0.26	0.60
	Error	16	0.72	0.00		
	Total	20	1.84			
Sterility percentage	**AM colonization**	1	90.31	90.31	0.57	0.46
	Planting density	1	277.51	277.51	1.75	0.20
	AM colnization x Planting density	1	0.01	0.01	0.00	0.99
	Error	16	2525.8	157.86		
	Total	20	16859.25			

Legend: *df* – Degree of freedom; *SS*- Sum of Squares; *MS*- Mean Square; *F*- Fisher's value; * significant at $P < 0.05$.

8.3.3.5. Effect of soil parameters on yield characteristics

The potential correlation of yield characteristics and physico-chemical properties of soil for different treatments was performed by Canonical correspondence analysis (CAA). In the CCA plot, the resulting ordination is presented in **Fig. 35**. The eigen values of the first and second axis were 0.024 and 0.007, respectively. The cumulative percentage variance data showed 72.14% and 19.55% of variability on the first and second axis, respectively. From the biplot, it was observed that the number of grains per hill was the most important variable influencing the difference in the effect of AM colonization and planting density. In addition, variables like number of fertilized seeds per hill, yield, percent colonization, length of panicle and HI, and soil parameters like pH, EC, S, Mn, Cu and Zn influenced the results of the treatments.

8.2.4: Relative field mycorrhizal dependency index (RFMD) and Mycorrhizal efficiency index (MEI)

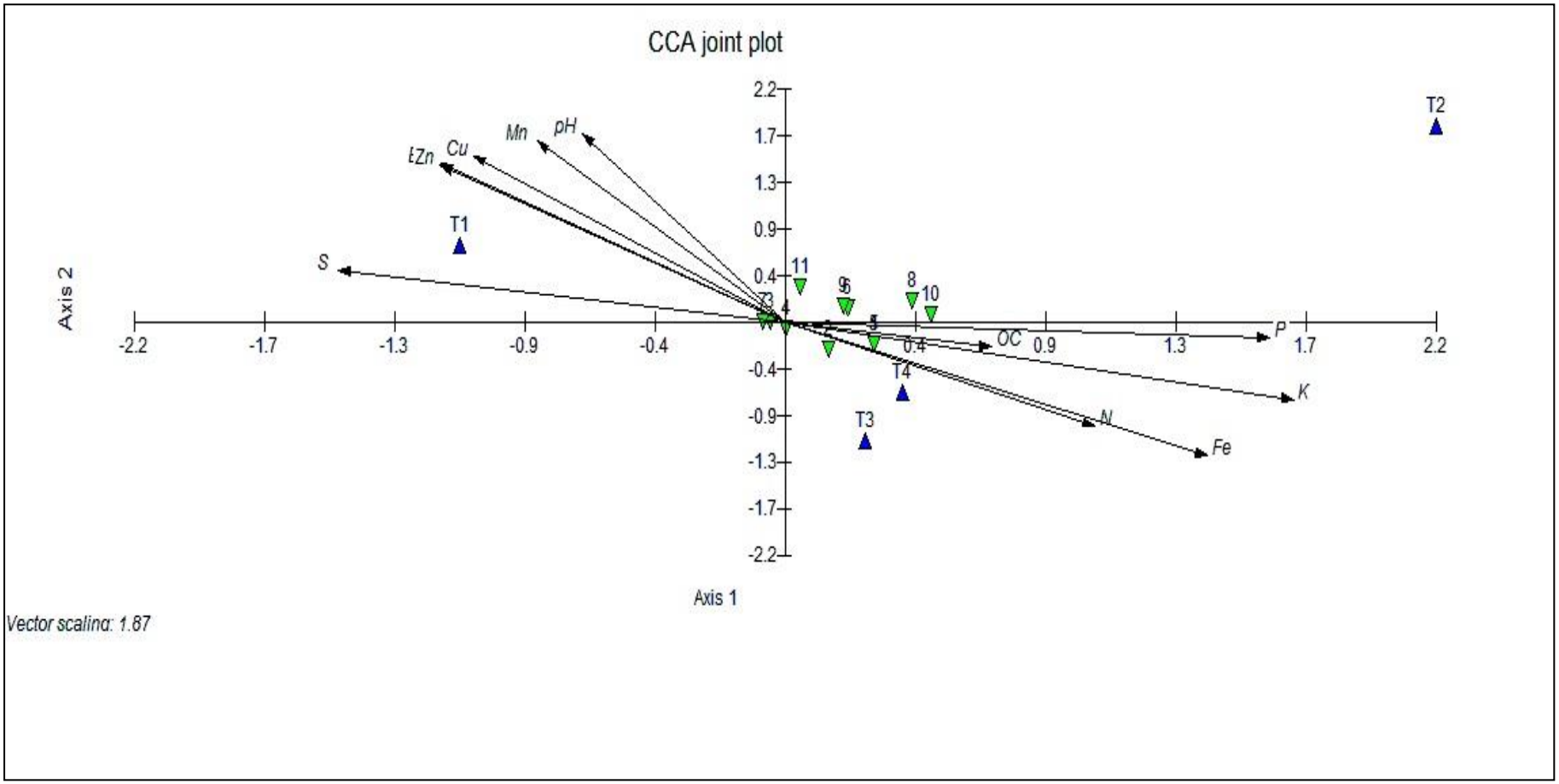
There was no significant variation in RFMD index and MEI between different planting density nor between the different growth stages (**Table 54**). The RFMD index and MEI however showed an increase from the vegetative to harvesting stage when rice was cultivated one plant per hill. However planting at three plants per hill recorded an increase from vegetative to flowering stage while from flowering to harvesting stage there was a decrease.

Table 54: Relative Field Mycorrhizal Dependency (RFMD) and Mycorrhizal efficiency Index (MEI) of rice at different planting density.

No. of plants hill^{-1}	Growth stage	RFMD Index	MEI %
One plant hill^{-1}	Vegetative	139.33 ± 20.13	19.89 ± 10.66
	Flowering	159.98 ± 18.75	34.25 ± 7.11
	Harvesting	182.68 ± 18.24	42,52 ± 6.99
Three plants hill^{-1}	Vegetative	138.33 ± 20.10	21.52 ± 10.76
	Flowering	184.67 ± 9.32	45.31 ± 2.67
	Harvesting	166.27 ± 14.93	38.11 ±4.92

Legend: Data presented is the mean of five readings at each growth stage; ± indicates Standard Error.

Fig. 35: CCA of soil properties and yield characteristics to different treatments.



Legend: T_1: AM +1 plant hill^{-1}; T_2: Uninoculated +1 plant hill^{-1}; T_3: AM +3 plants hill^{-1}; T_4: Uninoculated +3 plants hill.$^{-1}$; 1- No. of panicle hill^{-1}; 2-Unfertilised seeds hill^{-1}; 3-Fertilised seeds hill^{-1}; 4- grains/hill; 5- Panicles m$^{2-1}$; 6- Panicle length; 7- yield Kg ha^{-1}; 8- Weight of 1000 seeds (g); 9- HI; 10- Sterility percentage; 11- Mycorrhizal colonization percentage.

8.3.2.5: Assessment of nutrients

Results of nurrient content *viz*., carbohydrate, protein and mineral content in parboiled and raw rice are depicted in **Table 55**.

Table 55: Variation in nutritional components in *O. sativa* var. Korgut as affected by treatments.

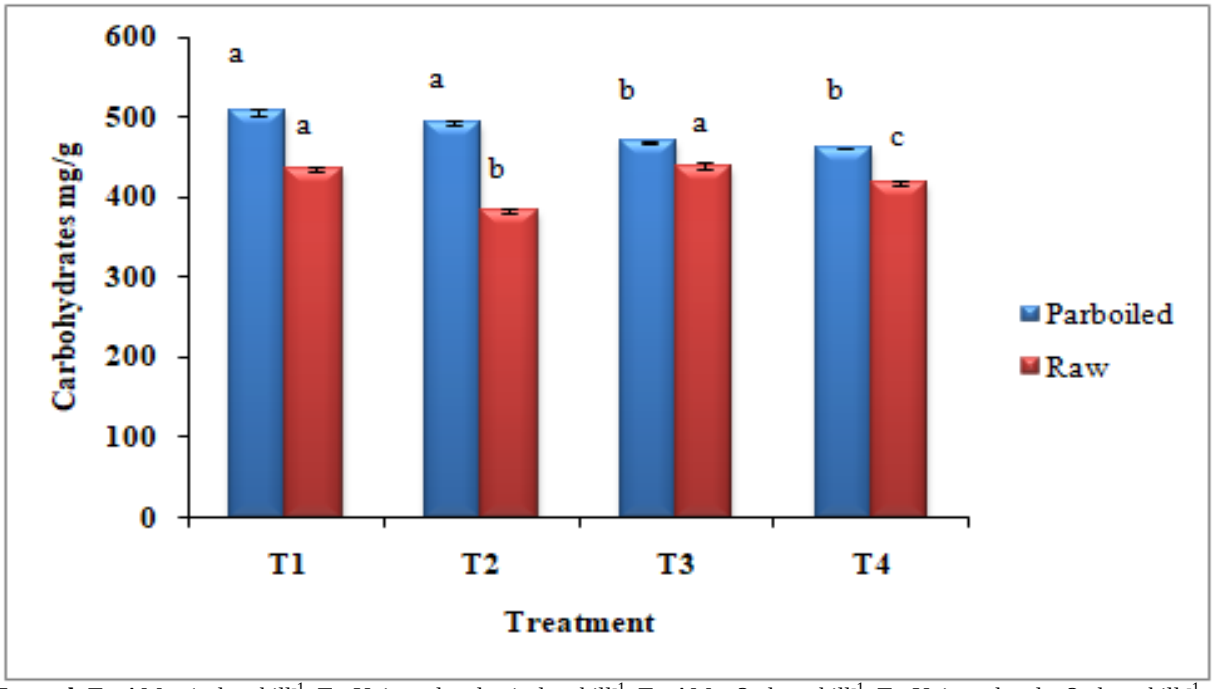
Nutritional Component	Treatment	Parboiled Rice	Raw Rice	$F_{(1,5)}$
Carbohydrate (g/ kg)	T_1	$507.50^{a}_{a}\pm4.33$	$435.83^{b}_{a}\pm3.00$	184.90*
	T_2	$494.33^{a}_{a}\pm3.48$	$384.33^{b}_{b}\pm2.60$	640.58*
	T_3	$469.37^{a}_{b}\pm2.23$	$439.67^{b}_{a}\pm3.75$	44.82*
	T_4	$461.73^{a}_{b}\pm0.93$	$418.70^{b}_{c}\pm2.32$	294.20*
	$F_{(3,11)}$	48.85*	72.09*	
Protein (g/ kg)	T_1	$31.25^{a}_{a}\pm10.82$	$58.75^{a}_{a}\pm5.05$	5.29
	T_2	$30.00^{a}_{a}\pm5.77$	$42.50^{a}_{a}\pm1.44$	4.41
	T_3	$26.25^{b}_{a}\pm3.60$	$56.25^{a}_{a}\pm3.60$	34.56*
	T_4	$20.00^{b}_{a}\pm5.77$	$42.99^{a}_{a}\pm1.75$	14.51*
	$F_{(3,11)}$	0.519	6.73	
Fe (ppm)	T_1	$69.24^{a}_{a}\pm0.10$	$36.50^{b}_{a}\pm0.23$	16459.50*
	T_2	$66.75^{b}_{b}\pm0.10$	$86.82^{a}_{b}\pm0.07$	23479.56*
	T_3	$34.42^{b}_{c}\pm0.19$	$44.31^{a}_{c}\pm0.08$	2147.49*
	T_4	$51.42^{b}_{d}\pm0.11$	$95.08^{a}_{b}\pm0.01$	154024.95*
	$F_{(3,11)}$	776.52*	52353.27*	
Mn (ppm)	T_1	$2.48^{a}_{a}\pm0.13$	$0.02^{b}_{a}0.00$	338.15*
	T_2	$2.01^{a}_{b}\pm0.00$	$0.11^{b}_{b}\pm0.00$	162450.00*
	T_3	$2.41^{a}_{ab}0.10$	$0.16^{b}_{b}\pm0.01$	415.62*
	T_4	$2.35^{a}_{ab}\pm0.01$	$1.16^{b}_{c}\pm0.01$	3204.10*
	$F_{(3,11)}$	5.73*	19271.01*	
Cu (ppm)	T_1	$0.46^{a}_{a}\pm0.14$	$0.32^{a}_{a}\pm0.03$	0.37
	T_2	$0.35^{a}_{a}0.02$	$0.26^{b}_{a}\pm0.02$	0.03*
	T_3	$0.58^{a}_{a}\pm0.13$	$0.52^{a}_{b}\pm0.00$	0.65
	T_4	$0.53^{a}_{a}\pm0.01$	$0.55^{a}_{b}\pm0.01$	0.51
	$F_{(3,11)}$	1.03	39.07*	

Zn (ppm)	T_1	$0.58^{a}{}_{a}0.12$	$0.32^{a}{}_{a}\pm0.04$	3.91
	T_2	$0.37^{a}{}_{a}\pm0.01$	$0.26^{b}{}_{a}\pm0.01$	27.92*
	T_3	$0.48^{a}{}_{a}\pm0.02$	$0.53^{a}{}_{b}\pm0.02$	3.04
	T_4	$0.48^{a}{}_{a}\pm0.02$	$0.55^{b}{}_{b}\pm0.01$	12.16*
	$F_{(3,11)}$	1.79	32.81*	

Legend: T_1: AM Inoculated + 1 plant hill^{-1}; T_2: Uninoculated + 1 plant hill^{-1}; T_3: AM Inoculated + 3 plants hill^{-1}; T_4: Uninoculated+ 3 plants hill^{-1}.
Data presented is mean of three reading;± indicates Standard Error.
For each treatment and nutritional parameter, values in a column affected by the same letter as a lower case are not significantly different at the level of probability indicated. For parboiled and raw rice and particular nutritional parameter, values in a row affected by the same letter as an upper case are not significantly different at the level of probability indicated.

There was a significant difference in carbohydrate content between the different treatments in parboiled rice. Maximum carbohydrate content was recorded in T_1 treatment. However in raw rice there was no significant difference in AM inoculated plants with different planting density. Maximum carbohydrate content was recorded in T_3 treatment. In general, carbohydrate content was significantly higher in parboiled rice compared to raw rice **(Fig. 36)**.

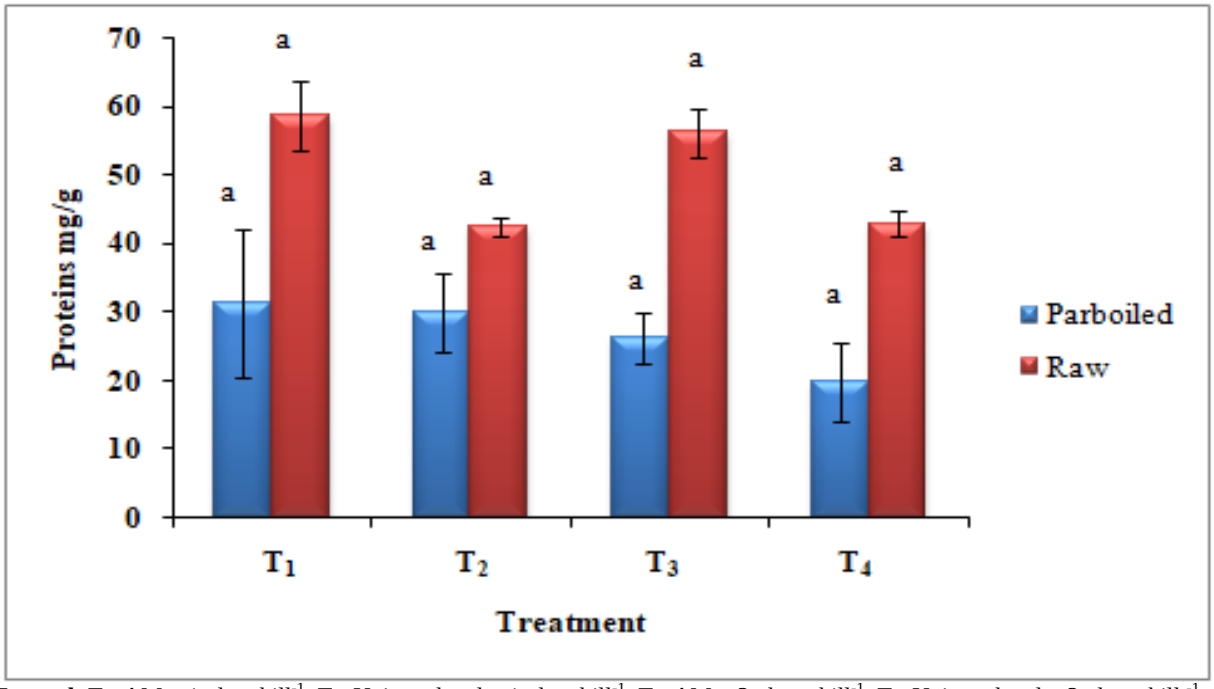
Fig. 36: Effect of AM and planting density on carbohydrate content.



Legend: T_1: AM + 1 plant hill^{-1}; T_2: Uninoculated + 1 plant hill^{-1}; T_3: AM + 3 plants hill^{-1}; T_4: Uninoculated + 3 plants hill.$^{-1}$; Error bars represents standard error. Values on the column affected by the same letter are not significantly different between the growth stage.

No significant difference in protein content was observed between different treatments in parboiled and raw rice. However maximum protein content was recorded in T_1 treatment for both parboiled and raw rice. Protein content was higher in raw rice compared to parboiled rice **(Fig. 37)**.

Fig. 37: Effect of AM and planting density on protein content.

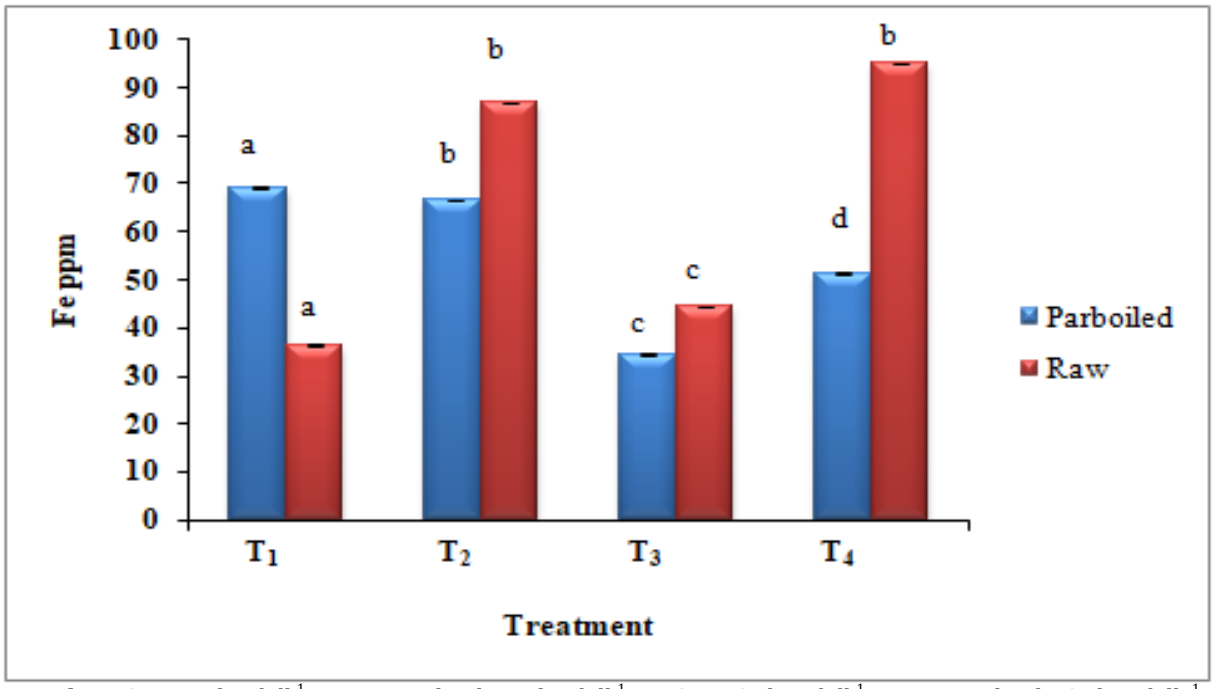


Legend: T_1: AM + 1 plant hill^{-1}; T_2: Uninoculated + 1 plant hill^{-1}; T_3: AM + 3 plants hill^{-1}; T_4: Uninoculated + 3 plants hill.$^{-1}$; Error bars represents standard error; Values on the column affected by the same letter are not significantly different between the growth stage.

Significant difference was observed in Fe content between the different treatments in parboiled and raw rice. Maximum Fe content was observed in T_1 treatment in parboiled and T_4 treatment in raw rice. Fe content was significantly higher in raw rice than parboiled for all treatments except in T_1 **(Fig. 38)**.

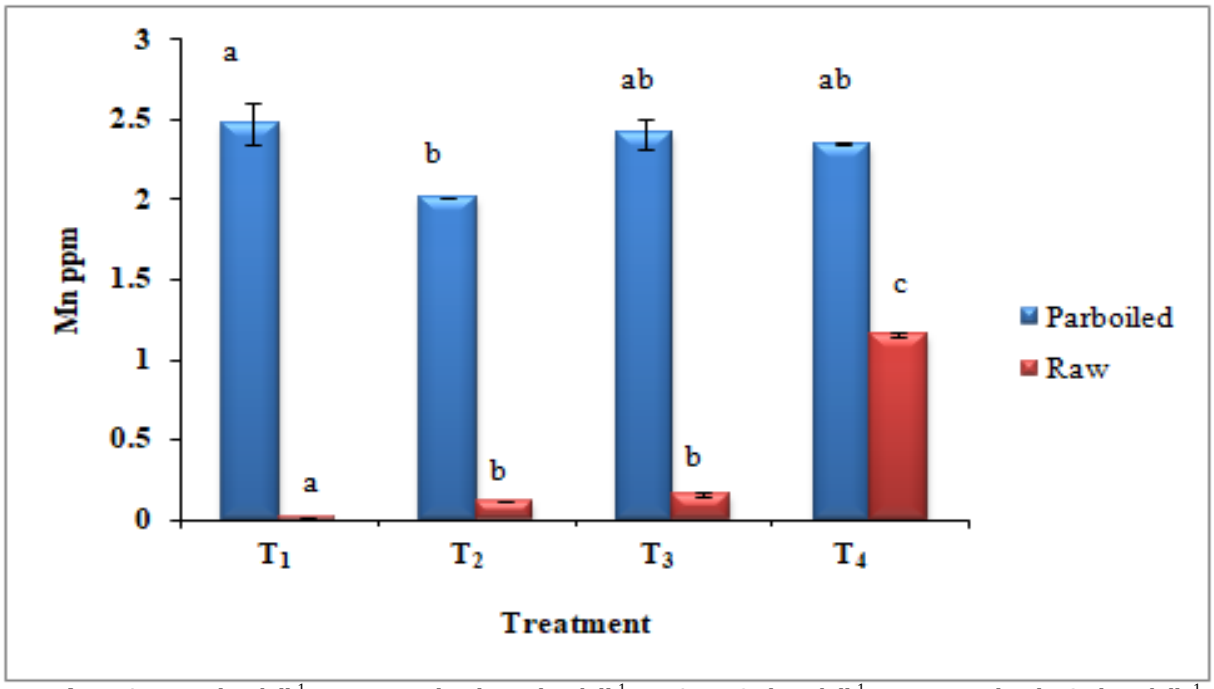
Significance difference was observed in Mn content between the different treatments in parboiled and raw rice. Maximum Mn content was observed in T_1 treatment in parboiled and T_4 treatment in raw rice. Mn content was significantly higher in parboiled rice compared to raw rice **(Fig. 39)**.

Fig. 38: Effect of AM and planting density on Fe content.



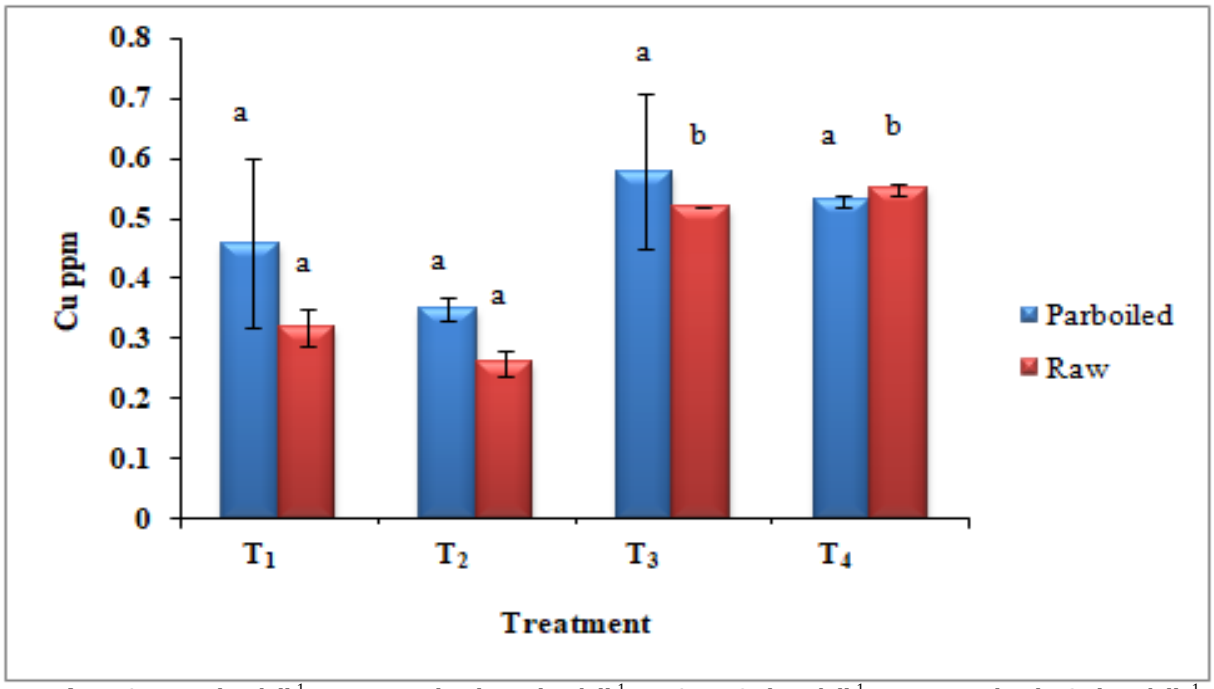
Legend: T_1: AM + 1 plant hill^{-1}; T_2: Uninoculated + 1 plant hill^{-1}; T_3: AM + 3 plants hill^{-1}; T_4: Uninoculated + 3 plants hill.$^{-1}$; Error bars represents standard error; Values on the column affected by the same letter are not significantly different between the growth stage.

Fig. 39: Effect of AM and planting density on Mn content.



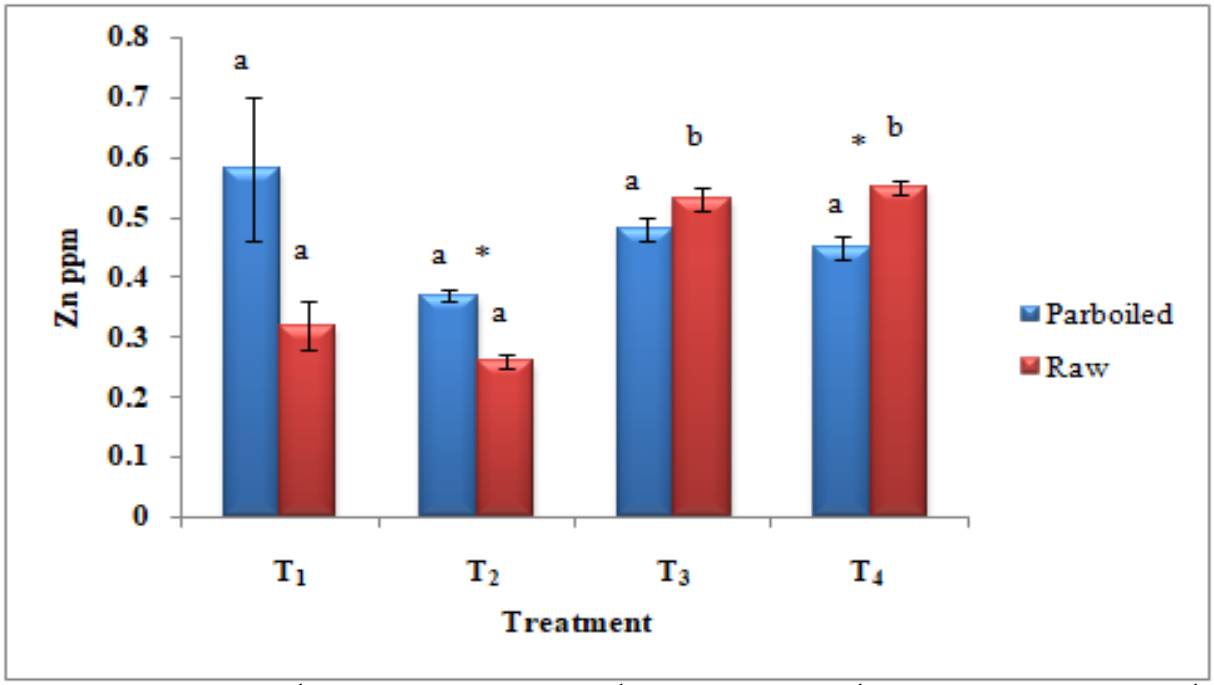
Legend: T_1: AM + 1 plant hill^{-1}; T_2: Uninoculated + 1 plant hill^{-1}; T_3: AM + 3 plants hill^{-1}; T_4: Uninoculated + 3 plants hill.$^{-1}$; Error bars represents standard error; Values on the column affected by the same letter are not significantly different between the growth stage.

Fig. 40: Effect of AM and planting density on Cu content.



Legend: T_1: AM + 1 plant hill^{-1}; T_2: Uninoculated + 1 plant hill^{-1}; T_3: AM + 3 plants hill^{-1}; T_4: Uninoculated + 3 plants hill.$^{-1}$; Error bars represents standard error; Values on the column affected by the same letter are not significantly different between the growth stage.

Fig. 41: Effect of AM and planting density on Zn content.



Legend: T_1: AM + 1 plant hill^{-1}; T_2: Uninoculated + 1 plant hill^{-1}; T_3: AM + 3 plants hill^{-1}; T_4: Uninoculated + 3 plants hill.$^{-1}$; Error bars represents standard error; Values on the column affected by the same letter are not significantly different between the growth stage.

No significant difference in parboiled rice was observed in Cu content between the different treatments. Maximum Cu content was observed in T_3 treatment. Significant difference in Cu content was observed in raw rice with maximum content observed in T_4 treatment. However mean Cu content was higher in parboiled rice **(Fig. 40).**

There was no significant differencein Zn content in the various treatments for parboiled rice. Maximum Zn content was recorded in T_1 treatment. However in raw rice there was a significant difference in Zn cntent in relation to planting density **(Fig. 41).**

8.2.5.1: Effect of AM colonization and planting density on nutrient content

Results on the effects of AM inoculation, planting density and its interaction on the nutrient content in parboiled and raw rice are depicted in **Table 56**.

In parboiled rice, AM inoculation and planting density had a significant effect on carbohydrate, Fe and Mn content in various treatments. An interaction of both these had an effect on proteins, Fe and Mn content. In raw rice, AM inoculation had a significant effect on carbohydrate, protein, Fe and Mn content. Planting density had significant effect on carbohydrate, Fe, Mn, Cu and Zn content. The interaction of AM inoculation and planting density had a significant effect on carbohydrate and Mn content in different treatments.

Table 56: Univarate two-way ANOVA assessing the effect of AM colonization and planting density and its interaction on nutrients.

		Parboiled rice					Raw rice				
Nutrients	**Source**	***df***	***SS***	***MS***	***F***	***sig.***	***df***	***SS***	***MS***	***F***	***sig.***
Carbohydrate	**AM colonization**	1	1378.16	1378.16	36.35*	0.00	1	3938.56	3938.56	148.59*	0.00
	Planting density	1	6458.88	6458.88	170.39*	0.00	1	1094.43	1094.43	41.29*	0.00
	AM colonization x Planting density	1	205.01	205.01	5.40	0.48	1	699.21	699.21	26.38*	0.00
	Error	8	303.24	37.90			8	2119049.88	26.50		
	Total	12	2746903.86				12	2119049.86			
Protein	**AM colonization**	1	619.92	619.92	0.06	0.06	1	653.13	653.13	19.92*	0.00
	Planting density	1	338.67	338.67	0.14	0.14	1	3.03	3.03	0.09	0.76
	AM colonization x Planting density	1	732.42	732.42	0.04*	0.04	1	6.70	6.70	0.20	0.66
	Error	8	1059.37	132.42			8	262.29	32.78		
	Total	12	18248.43				12	31072.33			
Fe	**AM colonization**	1	41.10	41.10	804.04*	0.00	1	7664.39	7664.39	153185.69*	0.00
	Planting density	1	2459.31	2459.31	48104.00*	0.00	1	193.68	193.68	3871.09*	0.00
	AM colonization x Planting density	1	115.01	115.01	2249.58*	0.00	1	0.15	0.15	3.03	0.12
	Error	8	0.40	0.05			8	0.40	0.05		
	Total	12	37197.57				12	59626.28			

Mn	**AM colonization**	1	5.53	5.53	245.46*	0.00	1	0.89	0.89	2017.52*	0.00
	Planting density	1	2.79	2.79	1238.88*	0.00	1	1.05	1.05	2377.83*	0.00
	AM colonization x Planting density	1	2.36	2.36	104.98*	0.00	1	0.61	0.61	1385.67*	0.00
	Error	8	0.18	0.23			8	0.00	0.00		
	Total	12	49.29				12	4.13			
Cu	**AM colonization**	1	0.02	0.02	0.77	0.40	1	0.00	0.00	0.25	0.62
	Planting density	1	0.06	0.06	2.13	0.18	1	0.18	0.18	113.13*	0.00
	AM colonization x Planting density	1	0.00	0.00	0.05	0.82	1	0.00	0.00	3.81	0.08
	Error	8	0.22	0.28			8	0.01	0.00		
	Total	12	3.04				12	2.22			
Zn	**AM colonization**	1	0.01	0.01	1.51	0.25	1	0.00	0.00	0.59	0.46
	Planting density	1	0.00	0.00	0.30	0.59	1	0.19	0.19	95.47*	0.00
	AM colonization x Planting density	1	0.04	0.04	4.07	0.07	1	0.00	0.00	2.38	0.16
	Error	8	0.09	0.01			8	0.01	0.00		
	Total	12	3.06				12	2.29			

Legend: *df* – Degree of freedom; *SS*- Sum of Squares; *MS*- Mean Square; *F*- Fisher's value; * significant at $P < 0.05$

8.3: Discussion

Rice cultivation is best suited in regions having high humidity, sunshine and plenty of water supply. According to Bakumovsky (1983) in rice crop the normal temperature ranged from 25 to 30˚C for the vegetative stage. Singh (2004) reported the optimum temperature during flowering and harvesting stage in rice ranged from 26.5 to 29.5˚C and 20 to 25˚C, respectively. Sharma *et al.* (1991) reported that 1250mm rainfall is required for vegetative growth of rice. In the present study the total rainfall received in the year 2017 (from June to November) was 2861.7mm and from seedling stage to the vegetative stage was 1618.6mm which was excellent for rice cultivation.

The aim of the present study was to analyse the effect of AM inoculation and the planting density under conventional paddy cultivation in the *Khazans*. This involves comparing AM inoculated plants with uninoculated (control) plants cultivated in the field. The experimental field was kept fallow in the previous year. The plot showed poor spore density (5 spores$100g^{-1}$ of soil) and low root colonization (2%). According to Harinikumar and Bagyaraj (1988), reduction in spore density under fallow condition is attributed to 'fallow disorder' that results in reduction in infectivity of spores as AM structure become older. Earlier studies have utilised fields of low colonization (2-3%) to study the effect of AM fungal colonization on Zn uptake (Purakayastha and Chhonkar 2001) and, growth and biomass in rice (Zhang *et al.* 2014).

In Goa, rice in the *Khazans* is cultivated after the onset of rains so that the salts deposited in the field soil are washed away and the sluice gates are closed to prevent the flow of saline water into the fields. *Oryza sativa* var. Korgut was choosen in the present study as it shows tolerance to salinity stress during the seedling stage thereby depicting early seedling vigour which aids in establishment under stressful conditions (Manohara *et al.* 2015). Seedlings of (20 days old) were transplanted in the field. This seems to be a practice wherein 15-20 days old seedling transplantation is preferred in Goa. As this stage, the seedling can resist damage from sudden heavy rainfall, flooding, high tide, can undergo underwater leaf elongation and is also easier to transplant.

Native AM fungal inoculum is more effective due to ecological adaptation (Oliveria *et al.* 2005). Pure culture of *A. scrobiculata* was used as an inoculum for the present study, as the genus *Acaulospora* was dominant and abundant in the *Khazans* of Goa. Besides it is known to be extensively distributed in varied vegetation types (Muthukumar and Udaiyan 2000) and in in both disturbed and undisturbed ecosystem (Jaiswal and Rodrigues 2001). In AM fungal community, *A. scrobiculata* spore population does not affect the population of other AM fungal species (Johnson 1993). Intraradical hyphae were thicker, and vesicles ranged from spherical to oblong to irregular in shape (Muthukumar and Udaiyan 2000).

Rice plants when transplanted, at a spacing of 20 x 20 cm in the field. Munda *et al*. (2007) reported that spacing of 20 x 20 cm was better, they produce more of tillers compared to 25 x 25cm spacing.

Physico-chemical analysis of the field soil before the onset of the experiment revealed that the soil was acidic with higher macro- and micro-nutrients with the exception of P. Nutrients are required for plant growth, while the mobility and accessibility of nutrients is influenced by pH (Marschner 1995). Soil pH is also known to influence AM colonization and extra-radical mycelial colonization (Hayman and Tavares 1985). Host plants also contribute to variation in soil pH, which results in variation in colonization rate. In the present study, the soil pH was low (4.3) and the rate of colonization at the different treatments ranged from 3.6 to 37.2%. Medeiros *et al*. (1994) reported low AM colonization at pH 4. Soil nutrients and available P content is known to alter the rate of AM fungal colonization. When available P is limiting, the plant provides C to AM fungi, thus promoting colonization (Johnson 2010).

In the present study, AM inoculated plants recorded significant increase in root colonization compared to uninoculated plants. Similar results were observed by Zhang *et al.* (2014) who attributed it to absence of effective AM propagules in the field. AM inoculated plants are known to establish faster in field conditions (Bagyaraj and Sreeamula 1982) as they are capable of overcoming transplanting shock and exhibit improved growth, yield and biomass production due to enhanced uptake of nutrients and water absorption (Dai *et al.* 2011). In AM inoculated plants, the percent colonization increased from vegetative to flowering stage and

then gradually decreased at harvesting stage. Similar results have been observed in an earlier study (Kumari and Prabina 2017). The life cycle of AM fungi begins with germination of AM propagates, colonization of host root and ends with sporulation. Sporulation also occurs at harvesting stage of the host plant (Hindumati and Reddy 2011) and hence it results in decreased colonization. But in uninoculated plants there was an increase from vegetative to harvesting stage. This may be due to low levels of AM propagules already present in the soil, which have to compete to bring about colonization in the rice plants hence leading to a delay in colonization and further completion of its life cycle. In the present study, there was significant difference in AM colonization in response to planting density. AM colonization was higher in plants cultivated singly than when planted three plants per hill. These results are in accordance with previous studies (Carey *et al*. 1992, Facelli *et al*. 1999) which reported that with the increase in planting density, the degree of AM colonization decreases. With higher planting densities there is an increasing overlap of P depletion zone created by roots and root hairs resulting in decreasing usefulness of hyphae. According to Allsopp and Stock (1992), a quantity of AM inoculum in the soil, can colonize a given length of root in a given period of time. Hence as root density increases a smaller proportion of the total root length may become colonized in a given period of time. According to Abbott and Robson (1984) greater competition for light at higher densities would result in less carbohydrate availability to AM fungi.

According to Janos (2007), mycorrhizal dependency is the degree of which the plant relies upon mycorrhiza for its growth or yield at a known degree of soil fertility. Mycorrhizal dependency is linked to a given level of soil fertility and P is the element which has the closest relationship to AM development and efficiency (Menge *et al.* 1978). Mycorrhizal dependency shows a direct correlation with percent colonization (Renuka *et al.* 2012). In the present study, when rice plants were cultivated at the rate of one plant per hill, RFMD and MEI increased with growth and plant age. Similar results were observed in *Physalis peruviana* L. in saline soils (Miranda *et al.* 2011). However, when plants were cultivated at a planting density of three plants per hill there was an increase in RFMD and MEI from vegetative to flowering stage and then a decrease was observed.

All the plant growth parameters showed an increase in inoculated plants at all planting densities. The present results revealed that plants inoculated at the nursery stage before transplantation promotes better AM symbiosis. This resulted in enhanced uptake of nutrients thus stimulating plant growth and yield. AM inoculated plants not differ significantly from uninoculated ones in relation to their height at flowering and harvesting stage. Similar results were observed by Oladele and Awodun (2014). It could be possible due to similarity in their genetic character (Islam and Salam 2017). However AM inoculated plants showed significant effect on tiller number and leaf and shoot and root biomass. Similar results were recorded by Oladele and Awodun (2014) in rice. Dar and Bali (2007) reported that the application of biofertilizers in rice cultivated in low lands significantly improved the leaf number, leaf area index and all yield characteristics.

Muhammad *et al.* (1987) reported that plant height in rice remained unaffected irrespective of planting density. According to Islam and Salam (2017), total number of tillers per hill, length of panicle, grains per panicle, yield and weight of 1000 grains was not significantly affected by the number of seedlings per hill. However in the present study maximum yield was recorded in plants singly per hill. Optimum output of a plant is when cultivated at the least density, as the plant gets maximum utilization of radiation, water and nutrients (Mondal *et al.* 2013). The rate of photosynthesis and other physiological phenomena are known to affect growth and development in rice plant (Khan *et al.* 2015). The present study revealed that AM inoculated plants produced higher yield when cultivated at lower density (one plant per hill). According to Mangunath *et al.* (2009) yield increases with increase in number of tillers, panicles and filled spikelets.

Rice is consumed either as parboiled or raw and is directly related to market value. From the study it is observed that raw rice from AM inoculated plants recorded higher carbohydrate and protein content compared to uninoculated plants. However the mineral content showed variation among the AM inoculated plants. According to Gandebe *et al*. (2017) P, N and Mg content showed increased levels in seeds harvested from AM inoculated plants. In the present study, carbohydrate content was higher in parboiled rice and protein content was high in raw rice, while the mineral content showed variation in parboiled and raw rice. Rice is soaked in

water during parboiling, that results in the diffusing of water into the kernel and some of its components leach out. These leached components include protein, sugars and non starch lipids (Chen *et al.* 1999). During the heating process starch in the endosperm is substantially gelatinized reducing stickiness of cooked rice (FAO 1998). Lipase in the bran layer of rice becomes inactive due to heating and hence reduces the tendency for oxidative rancidity improvment (Chukwu and Oseh 2009).

8.5: Conclusion

From the present study it can be concluded that *A. scrobiculata* is efficient in stimulating growth and yield in *O. sativa* L. var. Korgut under field conditions. The plants were pre inoculated with the prepared AM inoculums and cultivated at different densities. The optimum results were obtained in plants inoculated with AM fungi and cultivated at one plant per hill. Effective utilization of AM fungi is highly relevant to sustainable agriculture as AM symbiosis results in clean, efficient and non-polluting environment. The productivity of many agricultural crop plants in soil is dependent on the formation of AM fungi, making this symbiosis an essential factor in low-input sustainable agriculture (Kapoor *et al.* 2002). Application of efficient AM fungal inoculum in rice cultivation is an effective technique for crop and yield improvement.

CHAPTER 9

Summary

Khazans are low-lying lands in Goa. These lands have been used for agriculture which are subjected to inundation by neighbouring rivers. The *Khazans* consists of four main components *viz. bunds, manos, poiem* and the rice fields. It is the *bunds* that protect the fields from inundation; the *manos* are sluice gates that control the flow of water in and out the fields. These gates keep saline water out during the monsoon and the *poiem* is the internal water body. These regions have been the rice bowls of the coastal community. But due to a sharp rise in the standard of living, scarcity of labour, no proper maintenance of sluice gates these agricultural lands have resulted in poor returns. Studies on the association of AM fungi with rice in lowlands are scarce. Hence the present study was undertaken with the aim to evaluate AM diversity in rice cultivated in *Khazan* areas, to study the response of AM fungi to rice plant phenology, to develop pure cultures and to test the efficiency of AM fungi in promoting growth, yield and characteristics at two planting densities.

The main findings of the present investigation are summarized below:

Mycorrhizal studies on eleven different rice varieties cultivated in *Khazan* fields were carried out from six different sites in Goa. AM fungal colonization was recorded in all the rice varieties studied. The association was characterized by presence of intra- and extra-radical hyphae, hyphal coils, arbuscules, vesicles and intra- and extra-radical spores. In the *Khazans* average root colonization was 45.56% and average spore density was 27 spores$100g^{-1}$ of soil. Taxonomic study revealed a total of 14 AM fungal species. *Acaulospora* was the dominant genus, represented by seven species. The other genera recorded were *Glomus* (2), *Entrophospora* (1), *Funneliformis* (1), *Rhizoglomus* (1), *Archaeospora* (1) and *Tricispora* (1) with species number given in parenthesis. The study reports variation in AM colonization, spore density, AM diversity index, species richness and spore abundance suggesting variable influence of several factors at each site which may have influenced the distribution of AM population.

Studies on the physico-chemical characteristics at the different *Khazan* sites revealed differences in soil properties. Soil pH at all the sites was acidic in nature and macro- and micro-nutrients showed variation at all sites. PCA analysis explained the difference in soil characteristics at the different *Khazan* sites. N, Zn and Fe created significant effect on the soils at Neura, K and OC content influenced the soils at Shiroda, Mn content at Salvador do Mundo and pH strongly influenced the soils at Sikeri. Soil analyses at two different sites *viz.*, Tuem and Sikeri at the different growth stages of rice revealed that the soil properties differed in the same field at different growth stage of rice. There was no specific pattern of nutrient distribution at the two sites. PCA studies revealed that that OC, N, P, Zn, Cu and EC influenced the soil characteristics at the two sites. There was a significant difference in *Khazan* soils in relation to K and Mn content compared to midland and upland soil. PCA studies revealed that the distribution of N, OC, Mn and P content brought about variation in soil properties at the different studied ecologies.

The composition and association between AM fungi and a rice variety varied with space (*viz.*, Tuem and Sikeri) and time (different growth stages). Hyphal coils were observed at vegetative stage, vesicles at flowering stage, while arbuscules were few. AM colonization pattern and spore density varied significantly between the growth stages in the two years of study. Mean colonization was higher during flowering and least at harvesting stage, while mean spore density was higher at the harvesting stage. Mean colonization and spore density was significantly higher at Tuem. Six AM fungal genera *viz.*, *Acaulospora*, *Glomus*, *Funneliformis*, *Rhizoglomus, Tricispora* and *Entrophospora* were recovered from rhizosphere soils of the two study sites. In Tuem, *Acaulospora scrobiculata, A. bireticulata, A. delicata* and *A. dilatata* were recorded. In Sikeri, *Rhizoglomus fasciculatum, Funneliformis mosseae, Tricispora nevadensis Glomus microcarpum, Acaulospora scrobiculata and Entrophospora infrequens* were recorded. *Acaulospora* was the dominant genera. *Acaulospora scrobiculata* was common in both the sites. Present study indicated that AM colonization was influenced by plant phenology. In Tuem maximum AM species diversity index was recorded at flowering stage while at Sikeri it was maximum at harvesting stage. The present study showed variation in relative abundance and frequency of distribution of AM fungi at the different growth stages and between the two studied areas. CCA studies in Tuem revealed that RA of *A. scrobiculata*

and *A. bireticulata* was closely related to Cu content in the soil. High RA of *A. delicata* and *A. dilatata was* closely related to P level. In Sikeri RA of *A. scrobiculata* showed strong correlation to Cu and N, RA of *R. fasciculatum* showed a correlation with EC, K, P and Fe content in the soil. RA of *F .mosseae* showed correlation with Cu and N content.

AM studies at different ecologies reported that maximum root colonization in midland (58.55%) followed by upland (51.09%) and minimum in lowland (38.67%). In relation to phenology, maximum colonization was observed at flowering stage (64.87%) followed by harvesting (47.05%) and minimum in the vegetative stage (36.39%). Mean spore density was maximum in upland (159.72 spores 100g soil^{-1}) followed by midland (133.17 spores 100g soil^{-1}) and minimum in the lowlands (34.98 spores 100g soil^{-1}). In relation to phenology maximum spore density was recorded at harvesting (168.26 spores 100g soil^{-1}) followed by flowering (95.91spores 100g soil^{-1}) and least in the vegetative stage (63.70 spores 100g soil^{-1}). A total of 17 AM fungal species belonging to eight genera *viz*., *Acaulospora* (8), *Rhizoglomus* (1), *Tricispora* (1), *Claroideoglomus* (2) *Funneliformis* (1), *Archaeospora* (1) and *Gigaspora* (3) with species number given in parenthesis was recorded from different ecosystems. In lowland, *Acaulospora scrobiculata, A. delicata, A. dilatata, A. laevis, A. tuberculata, A. soloidea, Archaeospora myriocarpa*, *Funneliformis mosseae*, *Rhizoglomus fasciculatum* and *Tricispora nevadensis* were recorded. In midlands, *Acaulospora scrobiculata*, *A. bireticulata, A. rehmii, A. dilatata, Gigaspora ramisporophora, Claroideoglomus claroideum,* and *Tricispora nevadensis, Funneliformis mosseae*, *Gigaspora albida* and *G. decipiens* were recorded. In the uplands *A. scrobiculata, A. bireticulata, Claroideoglomus claroideum, C. etunicatum* and *Tricispora nevadensis* were recorded. AM diversity index was maximum at the midland followed by lowlands and least in upland. Highest species richness was recorded at the harvesting stage in the lowland. Variation in relative abundance in AM fungal distribution was observed in the different ecologies. CCA of correlation between RA of different AM genera with soil characters revealed that Genus *Claroideoglomus* showed high relative abundance in upland which is closely related to the high content of OC, N, Mn and Fe content in the soil. Midland showed high abundance of the genus *Gigaspora*, and is found related to high Zn and low P content. In the lowland, the genus *Acalospora* showed high abundance and showed tolerance to EC fluctuations, Cu and K content.

As genus *Acaulospora* was dominant in the lowland, using *Plectranthus scutellarioides* (L.) R. Br. as host plant trap cultures were prepared. Pure cultures of two AM species *viz.*, *Acaulospora scrobiculata* and *A. rehmii.* were successfully prepared. Of these two AM species, *A. scrobiculata* was successfully mass multiplied and used to evaluate its effect on growth and yield characteristics in rice at different planting densities.

Inoculation of A. *scrobiculata* at two planting densities in rice revealed significant increase in growth and yield compared to uninoculated plants. The study also revealed that plants cultivated at least density produced optimum growth and yield. Effect of inoculation and planting density revealed varied responses. In relation to carbohydrate and protein, AM inoculated plants showed higher content. Parboiled rice recorded higher carbohydrate while protein content was higher in raw rice. Micronutrients induced a varied response between inoculated and uninoculated, at different planting densities, and between parboiled and raw rice. Relative field mycorrhizal dependency index and mycorrhizal efficiency index showed an increase from vegetative to flowering to harvesting stage when rice was cultivated at a planting density of one plant per hill. However, when three plants per hill there was an initial increase from vegetative to flowering stage while it decreased from flowering to harvesting stage. CCA plot of the potential correlation of yield characteristics and physico-chemical properties of the soil to the different treatments revealed that the number of grains per hill was the most important variable. Increase in number of fertilized seeds per hill, yield, percent colonization, length of panicle and HI was observed in plants inoculated with *A. scrobiculata* and cultivated at least planting density. Therefore, it is concluded that cultivation of rice at least planting density and inoculated with efficient AM species would optimize plant growth and yield. Hence it is concluded that AM fungi can be used as bioinoculant and for rice cultivation in the *Khazan* lands.

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Synopsis

Introduction:

In Goa, the coastal saline soils are called as *Khazans*. These soils are found in the agro-climate zone No.11 and No. 12 on the east and west coast of India (Alvares 2002). *Khazans* are traditionally community managed integrated agro-aqua ecosystems (Sonak *et al.* 2005). These lands have been reclaimed from marshy mangrove swamps with an intricate system of dykes (*bunds*) and sluice gates. The gates protect the fields from inundation and control water in and out of the riverlute (Alvares 2002).

Khazans have been a source of livelihood for many, as these lowlands were originally used for paddy cultivation, traditional farming, pisciculture and salt extraction. Assgo, Bello, Damgo, Kalo damgo, Kalo korgut, Kalo novan, Khonchri, Korgut, Muno and Shiedi are rice varieties cultivated in the *Khazans* (Bhonsle 2011). But a sharp rise in the standard of living, scarcity of labour, non-availability of agricultural and technological inputs has resulted in poor returns from agriculture in *Khazans.*

Plants benefit from AM fungal establishment by increasing resistance to environmental stresses, enhance plant nutrient acquisition, water relation, disease resistance and improve soil quality (Smith and Read 2008). AM fungi are soil microorganism known to establish a mutual symbiosis with most of the higher plants (Ferrol *et al*. 2004). In contrast the role of mycorrhizae in wetland plants was regarded to be controversial as mycorrhizal fungi were believed to be unable to survive anaerobic conditions present in wetland soils (Miller 2000). However it is now established that AM fungal association exist in aquatic and wetlands including lakes (Sondergaad and Laegaard 1977, Wigand *et al*. 1998), estuaries (Wigand and Stevenson 1994), rice paddies (Dhillon and Blasing 1992), prairie potholes (Wetzel and van der Valk 1996) and salt marshes (Sengupta and Chaudhuri 1990, Brown and Bledsoe 1996).

Application of AM fungi play a very important role in enhancing crop growth and yield due to increased supply of P to the host plant, increased disease resistance and water uptake (Douds*et*

al. 2005). Therefore, AM fungi offer a great potential for sustainable agriculture by decreasing the use of excessive P fertilizers. Thereby, increasing the scope for improvement of yield of different rice varieties cultivated in *Khazans* of Goa with mycorrhiza as an inoculant. There is a need to identify AM fungal species, its potential as an inoculant in enhancing productivity of rice in the *Khazans* of Goa.

Methodology

- Rice root samples along with its rhizospheric soils were collected.
- Soil was analysed for pH, EC, Organic Carbon (OC) content by rapid titration method (Walkley and Black 1934), estimation of Nitrogen by micro-Kjeldahl method (Jackson 1971), Bray and Kurtz method (1945) for available Phosphorus (P), ammonium acetate method (Hanway and Heidal 1952) for Potassium (K) and using DTPA-$CaCl_2$ – TEA method (Lindsay and Norvell 1978), quantification of micronutrients like available iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn) were carried out.
- Processing of roots for AM fungal colonization was carried out by using Trypan blue staining technique (Phillips and Hayman 1970.
- Estimation of AM fungal root colonization percentage was carried out by root slide method (Read *et al.* 1976).
- Using cuttings of *Plectranthus scutellaroides* (L.) R.Br., trap (Morton *et al.* 1993), monospecific culture (Gilmore 1968) and mass production of single AM species (Gaur and Adholeya 2002) was carried out.
- AM spores were isolated from the rhizosphere soil using wet sieving and decanting method (Gerdemann and Nicolson 1963).
- AM fungal spore density was estimated using method laid by Gaur and Adholeya (1994).
- Identification of AM fungal spores were by using the morphological and taxonomical criteria, comparing them to the descriptions byAlmeida and Schenck (1990), Schenck and Perez (1990), Rodrigues and Muthukumar (2009), Schüßler and Walker (2010), Redecker *et al.* (2013) and International Collection of Vesicular Arbuscular Mycorrhizal Fungi (http://invam.caf.wuv.edu).

- Using Multi-Variate Statistical Package (MVSP) program version 3.1.Simpson's (D) and Shannon's (H) index for diversity studies were conducted for each site separately.
- AM species richness, Relative abundance (RA%), Frequency of occurrence % (Beena *et al.* 2000) for each area was determined.
- For evaluation of the effects of the dominant AM fungal species, sterilization of *Oryza sativa* var. Korgut seeds by 0.2% Sodium hypochloride was carried out. Nursery trays were prepared using colonized roots and soil from pure culture
- Experimental Design consists of a completely randomized block design with two treatments *i.e*. with and without *A. scrobiculata*, with 1 and 3 plantlets per hill and designated as follows:

  T_1 (AM + one plant hill/hill)

  T_2 (Uninoculated + one plant/hill)

  T_3 (AM + three plant/ hill)

  T_4 (Uninoculated + three plants/hill)
- Each treatment had three replicates. There were four main plots, each plot consisted of 3 subplots with a size of 3m x 1m and inter subplot spacing of 25cm and inter plot spacing of 50cm giving a total of 12 subplots with 65 plant hills each. Spacing between rice hills was 20 x 20cm. The field was submerged under water throughout the duration of the experiment.
- Plant growth parameters at vegetative, flowering and harvesting stage were randomly analysed for various parameters *viz.,* plant height, number of tillers, number of leaves, dry plant, root, shoot biomass was recorded and shoot root ratio calculated.
- Relative Field Mycorrhizal Dependency (RFMD) Index (Plenchette *et al*. 1983) and Mycorrhizal efficiency index (MEI) (Bagyaraj 1994) were calculated.
- Yield and its components were recorded at the end of the cropping season. Five hills/treatment were randomly selected to analyse various parameters *viz.*,number of panicles/hill, number of fertilized seeds/hill, number of unfertilized seeds/hill, number of grains/hill and length of panicle. Each subplot, an area of 1m x 1m (i.e. 25 hills) were used as harvesting sample for analyses of number of panicles/m^2 and yield.
- Harvest Index (HI) was estimated (Amanula and Inamullah 2016).
- Sterility percentage is ratio of unfertilized seeds to total number of seeds.

- The traditional parboiling process was employed
- The nutritional components *viz.* total carbohydrates by Phenol sulphuric acid method (Nielsen 2010), proteins by Lowry *et al.* 1951 and for mineral content the procedure laid down by Singh *et al.* (2005) was carried out for parboiled and raw rice.
- Analysis of variance (ANOVA) was carried out for data of soil, AM fungal colonization, spore density, growth parameters, yield and its components; relationship between AM colonization and spore density was determined by Pearson's correlation using IBM SPSS Statistics 20 <0.05 significance level. PCA analysis of soil at each site was carried out using Statistica version 6.0. To understand if there exists an interaction between two independent variables on the dependent variable Two way Analysis (ANOVA) was carried out. Canonical correspondence analysis (CCA) was carried out using Multi Variate Statistical Package (MVSP) program version 3.1 to understand the relationship between AM fungi, adaphic factors and its occurrence.

Objective 1:

To isolate and identify AM fungal spores from rhizosphere soils of different varieties of *Oryza sativa* from *Khazans* of Goa.

In this chapter, the association and diversity of AM fungi associated with the different varieties of rice cultivated in rice dominated *Khazan* areas are covered. One time sampling during the flowering stage of eleven rice varieties cultivated in six talukas of Goa was carried out to asses AM fungal colonization, spore density, its richness and relative abundance.

Observations

All 11 rice varieties cultivated in the six different *Khazan* sites showed AM colonization. Percent AM root colonization in different rice varieties varied from site to site. Maximum root colonization in all the sites studied was observed in variety Jyoti and minimum in variety Korgut The average AM fungal colonization in the different rice varieties cultivated in the *Khazans* was 45.56%. The maximum average root colonization was observed in rice cultivated in Tuem and minimum in Salvador do Mondo. In the present study, hyphal and vesicular

colonization was dominant. The rhizosphere soils showed variation in AM spore number at the different study site. The highest spore density was observed in the variety Assgo and the least was recorded in variety Jyoti. The average number of spores in all the study sites was 27.48 spores $100g^{-1}$ soil sample. The maximum average number of spores $100g^{-1}$ soil sample was observed in Shiroda and minimum in Salvador do Mondo. Fourteen AM fungal species were recorded from six agricultural study sites. *Acaulospora* was the dominant genus, represented by seven species. The other genera recorded were *Glomus* (2 spp.), *Entrophospora* (1 sp.), *Funneliformis* (1 sp.), *Rhizoglomus* (1 sp.), *Archaeospora* (1 sp.) and *Tricispora* (1sp.). Diversity index was highest at Sikeri and least in Neura. *Gigaspora* and *Scutellospora* species were not recorded in any of the study sites. The most abundant genus in the *Khazan* lands was *Acaulospora*.

Objective 2:

To study the physico-chemical properties of rhizosphere soils of rice grown *khazan* lands of Goa.

This chapter deals with soil sample analysis. Soil was collected from six sites at flowering stage. To understand the effect of adaphic factors on the phenology of rice, soil samples were collected and analysed at three different stages from two different *khazans* and from three different ecologies.

Observation

The effect of different adaphic factors in the acidic soil samples was observed.

Objective 3:

Spatio-temporal variation of AM fungi in *O. sativa* var. Jyoti grown in the *khazan* lands of Goa

The chapter deals with spatio-temporal variation of AM fungi in *O. sativa* var. Jyoti cultivated in two different *Khazans viz*. in Tuem and Sikeri. Tuem is in Pernem and Sikeri in Bicholim. The fields were sown by broadcasting the seeds in Tuem and by transplantation in Sikeri.

Fields in Tuem are on a flat low lying area, three meters above the mean sea level and these fields were cultivated continuously for a number of years. The fields in Sikeri are located on a slope, seven meters above the mean sea level and these fields were cultivated for the first time. At the vegetative (August), flowering (September) and harvesting (October) stage of rice, roots and rhizosphere soil samples were collected and analysed for two years. The present work was initiated to understand the variation of AM fungi in relation to the different growth stages or phenology of rice plant in the two selected *Khazans* of Goa.

Observation

AM fungal colonization was observed in *O. sativa* var. Jyoti in both the study sites in all growth stages. Hyphal coils during the vegetative stage, vesicles predominant in flowering stage and few arbuscules were observed. Mean colonization was significantly higher at 5% level ($P< 0.05$) during flowering phase and the least during the harvesting stage in both the years at both the study sites. Harvest stage recorded maximum spore density. Six AM fungal genera *viz.*, *Acaulospora*, *Glomus*, *Funneliformis*, *Rhizoglomus Tricispora* and *Entrophospora* were retrieved from the study sites. Six AM species were recovered from Sikeri as compared to four species from Tuem. *Acaulospora scrobiculata* was common in both the sites. Maximum relative abundance and frequency of occurrence was shown by *A. scrobiculata* and *F. mosseae* in all the growth stages and maximum in the vegetative stage in Tuem and Sikeri respectively. Variation in AM diversity was observed between the study sites and at different growth stages. Tuem depicted maximum diversity at flowering stage and Sikeri at harvesting stage.

Objective 4:

Assessment of AM fungal root colonization in different varieties of rice (*Oryza sativa* L.) grown in the *Khazan, Ker* and *Morod* lands of Goa.

Assessment of AM fungal colonization in three rice varieties *viz.*, Jyoti, Khonchri and Jaya from three different ecologies *viz.* lowlands (*Khazan),* midlands (*Ker*) and uplands (*Morod*) of Goa during different growth stages are discussed in this chapter. The study was carried out for two consecutive years. The length of the growing period of rice and its cultivation practices is

different in different ecologies. The aim of the present work was to study the occurrence of native AM fungal and to formulate AM inocula types for different ecosystems.

Observation

Maximum colonization was during the flowering stage in the three studied ecologies. Mean colonization % was highest in the midland followed by upland and minimum was observed in lowlands. Maximum spore density was at harvesting stage. Seventeen AM fungal species belonging to seven genera *viz*., *Acaulospora* (8), *Rhizoglomus* (1), *Tricispora* (1), *Claroideoglomus* (2) *Funneliformis* (1), *Archaeospora* (1) and *Gigaspora* (3) with species number given in parenthesis were found from different ecologies and plant phenology. Lowlands recorded maximum species richness. In lowlands *Acaulospora scrobiculata, A. delicata, A. dilatata, A. laevis, A. tuberculata, A. myriocarpa, A. soloidea*, *Funneliformis mosseae*, *Rhizoglomus fasciculatum* and *Tricispora nevadensis* were recorded. Genus *Acaulospora* showed maximum relative abundance. In midlands, *Acaulospora scrobiculata, A. bireticulata, A. rehmii, A. dilatata, Gigaspora raremisporophora, G. albida, G. decipiens, Claroideoglomus claroideum, Tricispora nevadensis* and *Funneliformis mosseae*, were recorded. Genus *Gigaspora* showed maximum relative abundance. In the uplands, *A. scrobiculata, A. bireticulata, Claroideoglomus claroideum, C. etunicatum* and *Entrophospora nevadensis* were recorded. Genus *Claroideoglomus* showed maximum relative abundance. Midlands showed highest AM fungal diversity and least was observed in uplands.

Objective 5:

Preparation of pure culture of dominant AM fungal species and their mass multiplication

Mass multiplication of monoxenic cultures was carried out. As these methods are least artificial which require a live host and help in understanding the biology of AM fungal life cycle were adopted.

Observation

From the six study sites, 14 AM fungal species were recovered using trap cultures. *Acaulospora* (8spp.), *Glomus* (2 spp.), *Entrophospora* (2 spp.), *Funneliformis* (1 sp.) and *Rhizoglomus* (1 sp.) with species number given in parenthesis were recorded. Genus *Acaulospora* was observed to be dominant and abundant in the *Khazans.* Of the eight species identified, pure cultures of *A. scrobiculata* and *A. rehmii* could be obtained. *A. scrobiculata* could be successfully upscaled.

Objective 6:

Evaluation of dominant AM fungal species on growth, yield and grain quality characteristics of selected rice variety grown in *Khazan* lands of Goa.

The aim of this chapter was to evaluate the effectiveness of *A. scrobiculata* Trappa. as a bio-inoculant on *Oryza sativa* var. Korgut cultivated in the *Khazans* on growth, yield, grain quality and its effect on cultivation practices. A completely randomized block design with two treatments *i.e*. with and without *A. scrobiculata*, with 1 and 3 plantlets/hill was conducted in the field in Sikeri and designated as follows: T_1 (AM + one plant/hill); T_2 (Uninoculated + one plant/ hill); T_3 (AM + three plant/hill) and T_4 (Uninoculated + three plants/hill)

Observation

Root colonization was observed in all the treatments (T_1, T_2, T_3 and T_4) at the vegetative, flowering and harvesting stage. The amount of root colonization was significantly higher by mycorrhizal colonization showing considerable reduced colonization in uninnoculated treatments. Maximum colonization % was observed in treatment T_1. In the vegetative stage, flowering and harvesting stage there was no significant difference at $p < 0.05$ in height of the plant except at the vegetative stage for the treatment T_2. There was a significant difference at $p < 0.05$ in the number of tillers in the different treatment at the different growth stages. The maximum number of tillers in the vegetative stage was observed at treatment T_3, in the flowering and harvesting stage maximum was in treatment $T_1$and least was observed in treatment T_2. Significant ($p < 0.05$) effect on the number of tillers depending on its plant density was observed in the vegetative stage, however in the flowering and harvesting stage

significant effect was observed on colonization, on density of plants/hill as well as its interaction. In the vegetative and flowering stage the maximum number of leaves was observed for treatment T_3 however in the harvesting stage maximum number was observed in the T_1 treatment. In the vegetative stage root, shoot, plant biomass and shoot root ratio did not vary significantly at $p < 0.05$ between treatments. Relative field mycorrhizal dependency index and mycorrhizal efficiency index showed an increase from the vegetative to the flowering to the harvesting stage when rice was cultivated one plant per hill however when at three plants per hill there was an increase from vegetative to flowering stage and from flowering to harvesting stage there was a decrease in RFMD and MEI. Significant effect of AM colonization and the density of plants/hill on yield of rice, harvest index and weight of 1000 seeds at $p < 0.05$ were observed for the different treatments, with maximum readings at the T_1 treatment. The number of grains/hill was the most important variable influencing the difference in the effect of mycorrhizal colonization and density of plants/hill to the different treatment. Effect of AM colonization and density of plants/hill on nutritional component of rice depicted that plants inoculated with AM fungi and parboiled rice had higher carbohydrate content than uninoculated and raw rice. Mycorrhizal colonization and the density of plants/hill had a significant effect on carbohydrate content of rice. No significant difference in the protein content between treatments in the parboiled and raw rice, however maximum protein content was observed in treatment T_1. Protein content was higher in raw as compared to parboiled rice.AM fungal colonization and density of plants/hill did not have an effect on protein content in parboiled rice, however mycorrhizal colonization had an effect on protein content in raw rice. AM fungal colonization and density of plants/hill had an effect on Fe and Zn content in parboiled and raw rice. Hence can conclude, use of inoculum and cultivation at the least density/hill certainly has advantages over uninoculated plants and at higher density of plants/hill.

The main observations of the entire work can be summarized as follows:

In the present study AM fungi are able to survive anaerobic condition and colonize rice cultivated in the *Khazans*. Fourteeen AM fungal species were recorded. Genus *Acaulospora* was dominant and abundant in the lowlands, *Gigaspora* in the midlands and *Claroideoglomus* in the uplands hence can be formulated as inocula for different ecosystems.

In relation to evaluation of AM fungi and density of plants/hill on growth, yield and its components, number of grains/hill was the most important variable influencing the difference between treatments. Rice inoculated with *A. scrobiculata* and cultivated one plant/hill was effective in producing the best results with highest yield, weight of seeds, seeds containing highest carbohydrate, Fe and Zn content as well as the highest harvest index.

The study revealed that AM fungi can be used as an inoculant to increase yield, its components and nutritional aspect of *Oryza sativa* var. Korgut.

Research Work Published:

- Xavier Martins WF, Rodrigues BF. 2018. Arbuscular mycorrhizal fungal diversity in *Oryza sativa* (rice) varieties cultivated in *Khazan* lands in Goa. *Kavaka*, 50: 48-52.
- Xavier Martins WF, Rodrigues BF. 2019. Identification of dominant Arbuscular Mycorrhizal fungi in different rice ecosystems. *Agricultural Research*, https://doi.org/10.1007/s-40003-019-00404-y

Papers Presented:

- Xavier Martins WF, Rodrigues BF. 2015. Arbuscular Mycorrhizal fungal diversity in *Oryza sativa* L. (Rice) varieties cultivated in *Khazan* lands In: Asian Mycological Congress Goa.
- Xavier Martins WF, Rodrigues BF.2018. Use of arbuscular mycorrhizal (AM) bio-inoculants for rice cultivation in different land types. National Conference on "Changing Environment: Challenges, Solutions and Strategies" held at Dhempe

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Appendix

Research Papers Published

- Xavier Martins WF, Rodrigues BF. 2018. Arbuscular mycorrhizal fungal diversity in *Oryza sativa* (rice) varieties cultivated in *Khazan* lands in Goa. *Kavaka*, 50: 48-52.

- Xavier Martins WF, Rodrigues BF. 2019. Identification of dominant arbuscular mycorrhizal fungi in different rice ecosystems. *Agricultural Research*, https://doi.org/10.1007/s-40003-019-00404-y

Reaearch Papers Presented

- Presented a poster titled "Arbuscular mycorrhizal fungal diversity in *Oryza sativa* L. (rice) varieties cultivated in *Khazan* lands" October 2015, at the Asian Mycological Congress Goa University.

- Presented a paper titled "Use of arbuscular mycorrhizal (AM) bio-inoculants for rice cultivation in different land types" at the National Conference on "Changing Environment: Challenges, Solutions and Strategies" March 2018, held at Dhempe College of Arts, Science, Miramar, Goa, India.

- Presented a poster titled **"**Impact of arbuscular mycorrhizal fungi and cultivation practise on growth and yield in *Oryza sativa* L. var. Korgut" at the National Conference on "Frontiers in Biopesticides and Biofertilizers," December 2019, at Ravi S. Naik College of Arts and Science, Farmagudi, Ponda-Goa.

KAVAKA 50: 48-52(2018)

Arbuscular mycorrhizal fungal diversity in Oryza sativa (rice) varieties cultivated in Khazan lands in Goa

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ABSTRACT

This study was conducted to assess arbuscular mycorrhizal (AM) fungal diversity associated with rice (Oryza sativa L.) cultivated in the Khazan lands in Goa. AM fungi (Glomeromycota) are vital components of almost all terrestrial ecosystems, forming a mutualistic symbiosis with roots of more than 80% of vascular plants including agronomically important species. Roots of rice varieties from six different agricultural sites were found to be colonized, with AM fungi ranging from 18.0% to 98.0%. Variety Korgu tsho wed the least mycorrhizal colonization while maximum colonization was recorded in variety Jyoti. AM fungal species belonging to four genera viz., Acaulospora, Glomus, Funneliformis and Entrophospora were recorded from the rhizosphere soils and Acaulospora being the dominant genus.

Keywords: Root colonization, spore density, endomycorrhiza

INTRODUCTION

Arbuscular mycorrhiza is a mutualistic association between fungi and plant roots. In this association the fungus receives photosynthetically derived carbon compounds from the green plants and the plants have an increased access to mineral nutrients especially phosphorus (P) (Rivera et al., 2005) and other minerals like K, Fe, Cu, Ca, Mg and Zn (George, 2000; Yaseen et al., 2011). The association also helps to improve the tolerance of the host plant towards biotic (Singh et al., 2000) and abiotic stress (Gaur and Adholeya, 2004).

Goa is the smallest state of the Republic of India, its position is marked by 15° 48' 00" N and 14° 53' 54" N Latitude and 74° 20' 13" E and 73° 40' 33" E Longitude having a total geographical area of 3,61,113 hectares covering both north and south Goa districts (Gune, 1979). Rice (Oryza sativa L.) is the predominant staple food crop of Goa. Rice fields here are called differently, depending on the soil, rainfall conditions and nearness to the riverside. They have been distinguished into Morod (Upland), Ker (Midland) and Khazan (Lowland).

Khazans is a konkani term in Goa for its coastal saline lowland soils. They are integrated agro-aqua ecosystems which are traditionally managed. They have been reclaimed over centuries from marshy mangrove swamps with an intricate system of bunds and sluice gates. The gates protect the fields from inundation and control the water flow in and out of the rivulets. In Goa these lowlands were originally used for paddy cultivation, traditional farming, pisciculture and salt extraction. Paddy fields have been cultivated by using bunds to keep the sea water away and sluice gates to control the inflow of saline water.

Agricultural lands are artificial ecosystems and are subjected to human intervention. Nature's diversity, due to agriculture, is replaced with a small number of cultivated plants. With the change of natural ecosystem to agro-ecosystem and increase in the intensity of agricultural inputs there is a decrease in AM fungal diversity (Oehl et al., 2003; Jefwa et al., 2012). Rice is grown in different ecosystem, when cultivated in the uplands readily forming mycorrhizal association has been reported by Ilag et al. (1987). Barea (1991) has reported that AM fungi can survive in water logged condition. Wetland rice was previously considered to be non mycorrhizal but a positive response to AM fungal inoculation has been observed (Sharma et al., 1988). AM fungi are important in organic and sustainable farming system that relies on biological process rather than agrochemicals (Harrier and Watson, 2004), thus offering a great potential for sustainable agricultural system (Khalil et al., 1992). A better understanding of the field study, based on AM fungal diversity associated with agronomic crops is necessary. Hence, in the present paper, an effort was made to study the AM fungal association in the different varieties of rice cultivated in different Khazan lands of Goa.

MATERIALS AND METHODS

Collection of rhizosphere soil samples: Field visits were conducted during flowering stage in rice dominated Khazan areas of six different talukas of Goa. The mean maximum and minimum temperature recorded during that period were 32.11º C and 23.4º C, respectively with relative humidity ranging from 46 to 95.68%, the seasonal total rainfall was 2595.1 mm as obtained from the Meteorological Department, of ICAR (Central Coastal Agricultural Research Institute, Goa). Three healthy plants of each of the 11 varieties viz., Jyoti, Jaya, Assgo, Bello, Damgo, Kalo korgut, Kalo novan, Khochri, Korgut, Muno and Shiedi (**Table 1**) were collected randomly from different parts of the Khazan lands at each site. While sampling rhizosphere soil was collected along with the roots. Samples were collected within 0-25 cm depth and then mixed thoroughly to obtain a composite sample of approximately 500 g of soil from June 2015 to November 2015 and brought to the laboratory for further analyses.

Soil Analyses: From the composite sample three sub samples were drawn and analyzed separately. Soil pH was measured in 1:1 water solution suspension using a pH meter (LI 120 Elico, India). Electrical conductivity (EC) was measured using conductivity meter (CM 180 Elico, India). Walkley and Black (1934) rapid titration method was used to estimate organic carbon content. Nitrogen was assessed by micro-Kjeldahl method (Jackson, 1971). Available P was estimated using Bray and Kurtz method (1945). Potassium (K) was estimated by ammonium acetate method (Hanway and Heidal, 1952).

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FULL-LENGTH RESEARCH ARTICLE

Identification of Dominant Arbuscular Mycorrhizal Fungi in Different Rice Ecosystems

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Received: 26 October 2018 / Accepted: 27 April 2019

Abstract Rice is a staple food in Goa. It is cultivated in three different ecosystems, viz, lowland (*khazan*), midland (*ker*) and upland (*morod*). The present investigation was carried out for two consecutive years, i.e., 2015 and 2016 to study the diversity of arbuscular mycorrhiza (AM) and identify the dominant species in the three different rice ecosystems of Goa. The native dominant AM species identified from the study can be further employed for developing AM inocula. The study revealed 17 AM fungal species recorded from the three ecosystems and belonged to six genera, viz., *Acaulospora* (9), *Rhizoglomus* (1), *Entrophospora* (1), *Claroideoglomus* (2), *Funneliformis* (1) and *Gigaspora* (3). There was dominance of different genera in different ecosystems. The genus *Acaulospora* was abundant in lowlands, genus *Gigaspora* in midlands and the genus *Claroideoglomus* in upland fields. This study suggests the possibility of using inocula of the dominant AM species in the respective ecosystems for increased plant growth and yield.

Keywords AM fungi · Diversity · Dominance · Inocula

Introduction

One of the important crops grown in many tropical countries of the world is rice (*Oryza sativa* L.). It is grown in different ecosystems defined on the basis of hydrology, roughly classified as irrigated, rainfed lowland, upland and flood prone. Approximately, half of the world rice area is irrigated and of the remainder is distributed among rainfed lowland (25%), uplands (13%) and flood prone (9%) [11, 12, 15]. The degree of flooding is determined by a number of variables such as rainfall pattern and intensity, topography, soil properties and drainage system [16].

Rice is the staple food of Goa. The crop is cultivated in three different topographical situations, i.e., upland (*morod*), midland (*ker*) and lowland (*khazans*) mainly as wet season (*kharif*) crop from June to October. Rice cultivation in uplands is 16.4% of the total rice area in the state. The growing period is 115–120 days. Fields are prepared by plowing early in the season followed by leveling so that the field is ready for sowing before the regular onset of monsoon. Pre-germinated seeds are broadcasted, or plantlets are transplanted by planting uniformly in lines spaced by 20 cm. Broadcasting and transplanting are carried out with a thin film of water. Rice crop cultivated in midland is 32% of the total rice area in the state. The crop grown in this ecosystem has relatively longer growing period (130–135 days). Seedlings are raised in wet or dry nurseries after germination. The seedlings are ready for transplanting after 21–24 days. Three to four seedlings are planted per hill at a distance of 20 × 10 cm. Seedlings are transplanted in fields that are plowed and leveled at the first shower. Rice cultivation in the lowland occupies an area of 32% of the rice area in the state, with varieties having growth duration of 105–115 days. Fields are plowed in the summer. Seeds are either broadcasted or transplanted by raising a nursery. However, in the lowland, it is essential to sow at regular onset of monsoon after ensuring flushing of salts from the fields [24].

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