INFLUENCE OF MYCORRHIZA AND PHOSPHORUS ON PHYSIOLOGICAL PARAMETERS OF LEAVES OF LITCHI (*Litchi chinensis* Sonn.) LAYERS

Abstract

Litchi (*Litchi chinensis* Sonn.), is delicious juicy fruit of India having excellent nutritional quality. It has a great potential to earn foreign exchange in the national and international market through export. Slow plant growth and high rate of mortality in initial stage of plant establishment are the major problem of litchi. Increasing photosynthetic activity through exploiting photosynthetic, components are major target. The carotenoid and chlorophyll content are one of the major components that affect the photosynthetic activity of plant. Arbuscular mycorrhizal (AM) fungi are beneficial symbiotic soil microorganisms and AM technology can find its potential application in the nursery of horticultural industry. When AM fungi have been successfully applied to many wood fruit tree species, little information is available in litchi. Therefore, the pot experiment was undertaken to study the influence of phosphorus (50 mg and 75 mg), mycorrhiza (*G. mosseae* and *G. coronatum*) alone and in combination. The treatment significantly influenced the changes in chlorophyll and carotenoid content in leaves of litchi saplings in nursery stage. After120 days of inoculation both the species of mycorrhiza alone and in combination with phosphorus application were very effective with the highest level of total chlorophyll content of (2.474 mg/g fr.wt) in case T_5 *G. mosseae*10 g + Phosphorus 50 mg. Significantly lowest value of chlorophyll was noted in T_0 Control (2.090 mg/g fr.wt).Carotenoid content was also measured maximum in T_5 *G. mosseae*10 g + Phosphorus 50 mg (0.154 mg/g fr. wt.) as compare to T_0 Control with (0.065 mg/g fr.wt.). Relative water content (RWC) after 60,90 and 120 DAI significantly differentiate. Maximum RWC in case T_5*G. mosseae*10 g + Phosphorus 50 mg (31.43 %) which was statistically equal with *G. coronatum*10 g + P 50 mg (31.14 %). Significantly influencing specific leaf weight of different date of observations. The performance was maximum found in T_5 *G. mosseae* 10 g + Phosphorus 50 mg (7.28 %) as compare to T_0 control (4.44 %). Significant effect of treatments on leaf parameters of litchi layers pertaining number of leaves per flush and length of flush is maximum with T_5*G. mosseae* 10 g + Phosphorus 50 mg (5 - 8) and (10.2 cm).

Key words: *Litchi chinensis*, *mycorrhizae, Chlorophyll, Carotenoid.*

Introduction

Litchi (*Litchi chinensis* Sonn.), is subtropical fruit tree native to the area between southern china, northern Viet Nam and Myanmar belong to the Sapindaceae family, is an important fruit crop that is widely cultivated in the world (Menzal, 2002). The fruit are fleshy drupes with an edible aril surrounded by the pericarp. China is leading producer country with 950 thousand metric tons in term of production in the world (Jiang *et al*., 2012). Litchi has been historically propagated by marcottage, and this is the most common method of propagation employed by commercial nurseries. Other methods of propagation like seeds,

cutting, budding and grafting are not expedient, as they may lead to either long juvenile period or improper establishment of the litchi seedlings (Pandey and Sharma, 1989). Marcottage (air branch-layering, Chinese layering, air-grafting, gootee, guti or marcotting) has been practiced by the Chinese for over 300 (Li and Li, 1949) for propagating litchi. Marcots come into bearing early, although they have a shallow root system and thus lead to obtaining profitable returns quite early. Nursery is the backbone of the fruit production and healthy planting material is the prerequisite for establishment of the orchard. Hence the poor establishment of the air-layers in the nursery is the major hindrance in obtaining optimum returns. This may be due to several factors namely, root thickness, genetic difference, insect and pathogen attack, unfavourable climatic conditions, low phosphorus uptake and other essential nutrients. The enhanced P uptake and phytohormones (IAA and iPAs) seemed to account for the changes in plant growth. However, information of AM fungal effect on rootstock seedlings is very rare. Photosynthesis is the basis of carbohydrate accumulation in plants and it improves photosynthesis together with increase the nutrient uptake by AM fungi contribute to the enhance the biomass of many plants ((Birhane *et al.,* 2012; Zhu *et al.,* 2012). the arbuscular mycorrhizal (AM) symbiosis affects plant hormone biosynthesis and plant metabolism (Torelli *et al.,* 2000; Bona *et al.,* 2010, 2011; Cicatelli *et al.,* 2012; Lingua *et al*., 2012; Baslam *et al*., 2013). Effects of the AM symbiosis are observed not only in colonized root systems but also in the above ground part of plants (leaves, flowers, and fruits) (Guerrieri *et al.,* 2004; Copetta *et al.,* 2006; Lingua *et al*., 2012). In fruit crops, AMF colonization stimulates growth (Hrselova *et al*., 1989; Gryndler *et al*., 2002), enhances photosynthesis (Burkowska 2002). Barea and Azcón-Aguilar (1982) reported that the presence of substances like auxin, gibberellins, and cytokinin have been found in *G. mosseae* extracts (Barea and Azcón-Aguilar 1982). While some studies have reported the lack of any effect of the AM symbiosis on auxin levels (Danneberg *et al.,* 1993), it is known that AMF colonization can increase the concentration in planta of molecules with auxinic activity (Jentschel *et al.,* 2007; Ludwig-Müller *et al*., 1997;Torelli *et al.,* 2000;Yao *et al.,* 2005). In addition, a synergistic effect of AMF and rhizobia on the production of IAA was shown in the roots and nodules of Vigna mungo (Chakrabarti *et al.,* 2010). Phosphorus is one of the important plant nutrients that involved and plays important role in in in plant Functions like photosynthesis, movement of nutrient within the plant, transformation of sugars and starches, and transfer of genetic characteristics from one generation to the next are mediated through phosphorus. The mycorrhizae thus increase the nutrient-uptake ability of the plant. The pigments are involved to the process of photosynthesis activity and increasing photosynthetic

activity enhances higher accumulation of synthesized organic compound which helps development of plant growth. The pigments which are involved in the process of photosynthesis are called photosynthesis pigment. The pigments are the coloured organic compounds that have the capacity to absorb a certain wavelength of light and reflect others (Kadam *et al.,* 2013; Kadam *et al.,* 2017). Chlorophyll (Chlorophyll- a and Chlorophyll-b) is a green pigment product which are found in cyanobacteria and the chloroplast of algae and plants. The plant forms chlorophyll in physiological process that occurs only in living cell (Momin and Kadam, 2011). The essential condition for chlorophyll formation is the presence of genetic factors (Anon., 1986). Chlorophyll is an extremely important biomolecule, critical in photosynthesis, which allows plants to adsorb energy from light most strongly in the blue portion of the electromagnetic spectrum followed by the red portion. There is a close relationship between photosynthesis with chlorophyll content in leaf. The Carotenoid occurs in photosynthetic tissue along with chlorophyll to protect them from photo oxidative damage. Carotenoids to protect their stem and leaves from the energy of sun. However, lesser information is available on flushing pattern and panicle emergence in litchi plants under subtropical conditions. However, the duration and interval of successive flushes in litchi appears to be strongly dependent on the vigour of the tree, irrigation, radiation and temperature. The photosynthetic rate also plays a key role for the energy availability in the plant, which is again control directly or indirectly by chlorophyll contents and its stability. Chlorophyll contents and its contribution towards photosynthetic activities have been reported in other fruits like apple. Considering the above facts, the present study was undertaken to determine the total chlorophyll (mg g^{-1}), carotenoid (mg g^{-1}), relative leaf water content (%), specific leaf weight (%) and flush length (cm) of the leaves of litchi of the samplings.

Materials and Methods

Plant materials and experimental design

The experiments were carried out at Bihar Agricultural University, Sabour during 2018-19 on uniform sized layered plants of litchi cultivar Purbi. The treatments were phosphorus (50 mg and 75 mg per kg of pot mixture) quantity of SSP , mycorrhiza (*G. mosseae* and *G. coronatum*) at 10 g per kg of pot mixture alone and in combination with phosphorus *viz.,*T_0 Control (Uninoculated), T_1 *G. mosseae* @10 g kg^{-1} of soil, T_2 *G. coronatum,* @10 g kg^{-1} of soil, T_3 Phosphorus @ 50 mg kg^{-1} of soil, T_4 Phosphorus @ 75 mg kg^{-1} of soil, T_5*G. mosseae* 10 g + Phosphorus 50 mg kg^{-1} of soil, T_6 *G. mosseae* 10 g +

Phosphorus 75 mg kg^{-1} of soil,T_7 *G. coronatum*10 g + Phosphorus50 mg kg^{-1} of soil, T_8*G. coronatum*10 g + Phosphorus 75 mg kg^{-1} of soil, Treatments were applied immediately after separation of litchi layers from their mother plant. Estimation of chlorophyll content, carotenoid content of leaf, relative water content and specific leaf weight, number of leaves per flush and length of flush were taken at 30 days interval till 120 days after inoculation.

The experiment was conducted on a completely Randomized Block Design (CRD) according to Gomez and Gomez (1984). The mean difference was tested by F-test at (5%) level of significance. Critical difference at 5% level of significance was used for comprising among the treatments.

Chlorophyll estimation

Chlorophyll contents a, b and total chlorophyll was estimated using acetone method with little modification as given by Arnon (1949). Leaf samples were collected at initial stage of flush emergence. Fully expanded leaf was used as materials for extraction and estimation of chlorophyll. 0.2 gram of freshly collected leaf material (devoid of mid- rib) were homogenized in 8 ml 80% acetone using mortar and pestle. The homogenate was then centrifuge at 4°C for 15 min at 15000 rpm. The supernatant collected carefully read the absorbance at 663 and 645 nm. Total Chlorophyll are determined by using the formula given below:

Total Chlorophyll = [(8.02*A663) + (20.2*A645) *V/1000*W

Carotenoids estimation

Estimation of carotenoids at continue 30 days interval *viz.,* 60, 90 and 120 DAI. Estimation of carotenoids was performed by the method of Hendry and Price (1993) with little modification. Leaf sample of 0.2 g was homogenized in 80% acetone. As mentioned in the chlorophyll estimation process, carotenoids were extracted and after centrifugation supernatant was used for spectrophotometric reading. An absorbance was recorded at three different wavelengths such as 663nm, 645 nm and 480 nm. Carotenoids content was calculated using.

Formula:

[A480 + (0.114*A663) – (0.638 – A645)] *V/1000*W

Here,

A = Absorption

V = Total volume,

W = weight of sample (gram)

Concentration of chlorophyll and carotenoids are expressed in mg g^{-1} fresh weight

Leaf relative water content (%):

The RWC of the recently mature leaves was determined following the method suggested by Weatherley (1950). According to this method, leaves were collected, and 8 mm diameter disc were made from those leaves. Fresh weights of these discs were measured and then they were floated over distilled water in petri dish for 4-6 hours. These discs were surface dried by placing them in between two sheets of Whatman No. 1 filter paper and saturated weight was recorded. After that the samples were dried in an oven dryer at 70°C for 24 h. The dry weights of the samples were recorded. The RWC was estimated using following formula**:**

$$\text{RWC (\%)} = \frac{\text{Fresh weight - Oven dry weight}}{\text{Turgid weight - Oven dry weight}} 100$$

Specific leaf weight - It is just reverse to specific leaf area and it was measured by using following

Formula:

$$\text{SLW} = \frac{\text{Leaf weight}}{\text{Leaf area}}$$

Results and Discussion

The litchi plants responded positively to the application of varying concentration of the AMF and inorganic phosphorus alone and in combination. All the mycorrhizal inoculated plants showed higher total chlorophyll, carotenoids, relative leaf water content, specific leaf weight, flush length and number of leaves per flush. Variation in the contents of chlorophyll was noticed amongst the treatments studied and also in flushes. Data depicted (Table 1) revealed that the highest total chlorophyll increased but treatment effect not performed after 60 days planting while after 90 days and 120 days total chlorophyll increased significantly under all the treatments.

Table 1: Effect of mycorrhiza and inorganic phosphorus on total Chlorophyll (mg g^{-1} fr.wt.) of litchi layers:

S. No	Treatments	Concentration	60 DAI	90 DAI	120 DAI
T_0	Control	No application	0.613	1.011	2.090
T_1	*G. mosseae*	10 g kg^{-1} of soil	0.675	1.477	2.413
T_2	*G. coronatum*	10 g kg^{-1} of soil	0.670	1.459	2.394
T_3	Phosphorus	50 mg kg^{-1} of soil	0.688	1.507	2.265
T_4	Phosphorus	75 mg kg^{-1} of soil	0.619	1.392	2.170
T_5	*G. mosseae* + Phosphorus	10 g +50 mg kg^{-1} of soil	0.739	1.603	2.474
T_6	*G. mosseae* + Phosphorus	10 g + 75 mg kg^{-1} of soil	0.688	1.507	2.401
T_7	*G. coronatum* + Phosphorus	10 g + 50 mg kg^{-1} of soil	0.726	1.588	2.411
T_8	*G. coronatum* + Phosphorus	10 g + 75 mg kg^{-1} of soil	0.685	1.457	2.398
CD (P=0.05)		**-**	**NS**	**0.128**	**0.154**
CV (%)		**-**	**-**	**5.183**	**3.872**

DAI-Date After Inoculation, fr.wt. – fresh weight

On second day of observation (90 DAI) the maximum chlorophyll (1.603 mg/g fr.wt.) was recorded in case T_5 *G. mosseae*10 g + P 50 mg which was significantly similar with T_7 *G. coronatum* 10 g + Phosphorus 50 mg (1.588 mg/g fr.wt.),T_6 *G. mosseae*10 g + Phosphorus 75 mg (1.507 mg/g fr.wt.), T_3 Phosphorus 50 mg (1.507 mg/g fr.wt.) and T_1 *G. mosseae* 10 g(1.477 mg/g fr.wt.). The minimum total chlorophyll (1.011 mg/g fr.wt.) was observed in T_0 Control.120 days after planting the highest level of chlorophyll content of 2.474 mg/g fr.wt. was found in treatment T_5 *G. mosseae* 10 g + Phosphorus 50 mg which was at par with T_6,T_7, T_8, T_1 and T_2 with respective values of (2.411,2.401, 2.398, 2.413 and 2.394 mg/g fr.wt.). Significantly lowest value of chlorophyll was noted in T_0 Control (2.090 mg/g fr.wt.). Gradual increase in chlorophyll content was noted under all the treatments with passes of time after treatment application. All the treatments significantly increased the chlorophyll content. In sour orange, Nemec and Vu (1990) observed increased chlorophyll on inoculation with Glomus spp. Inoculation of glass house grown apple seedlings with AM species increased chlorophyll content (Sharma and Bhutani, 1998).

Table 2: Effect of mycorrhiza and inorganic phosphorus on Carotenoids (mg g^{-1} fr.wt.) of litchi layers.

S. No	Treatments	Concentration	60 DAI	90 DAI	120 DAI
T_0	Control	No application	0.051	0.059	0.065
T_1	*G. mosseae*	10 g kg^{-1} of soil	0.102	0.118	0.130
T_2	*G. coronatum*	10 g kg^{-1} of soil	0.098	0.114	0.125
T_3	Phosphorus	50 mg kg^{-1} of soil	0.095	0.107	0.116
T_4	Phosphorus	75 mg kg^{-1} of soil	0.088	0.099	0.107
T_5	*G. mosseae* + Phosphorus	10 g + 50 mg kg^{-1} of soil	0.118	0.136	0.154
T_6	*G. mosseae* + Phosphorus	10 g + 75 mg kg^{-1} of soil	0.109	0.123	0.135
T_7	*G. coronatum* + Phosphorus	10 g + 50 mg kg^{-1} of soil	0.114	0.130	0.146
T_8	*G. coronatum* + Phosphorus	10 g + 75 mg kg^{-1} of soil	0.105	0.121	0.132
CD (P=0.05)		**-**	**NS**	**0.009**	**0.009**
CV (%)		**-**	**-**	**4.728**	**4.140**

DAI-Date After Inoculation.

The data (Table 2) revaled that the second day of observation (90 DAI) the significantly highest carotenoids (0.136 mg g^{-1} fr.wt.) was recorded in case T_5 *G. mosseae* 10 g + Phosphorus 50 mg which was followed by T_7 *G. coronatum* 10 g + Phosphorus 50 mg (0.130 mg g^{-1} fr.wt.), T_6 *G. mosseae*10 g + Phosphorus 75 mg (0.123 mg g^{-1} fr.wt.). The minimum carotenoids (0.059 mg g^{-1} fr.wt) was observed in T_0 Control. After 120 days inoculation the highest level of carotenoids content of (0.154 mg g^{-1} fr.wt) was found in treatment T_5 *G. mosseae* 10 g + Phosphorus 50 mg which was followed by T_7 *G. coronatum* 10 g + Phosphorus 50 mg (0.146 mg g-1 fr.wt), T_6 *G. mosseae*10 g + Phosphorus 75 mg (0.135 mg g^{-1} fr.wt). Significantly lowest value of carotenoids was noted in T_0 Control (0.065 mg g^{-1} fr.wt.). Gradual increase in carotenoids content was noted under all the treatments with passes of time after treatment application. All the treatments significantly increased the carotenoids content except T_0 control. The result also revealed an increasing concentration of carotenoids with increasing chlorophyll content. This may be due to the shielding activity of Carotenoids towards chlorophyll oxidation under high light. Present study supported by Neha *et al.*, (2018) reported that Carotenoid content was also measured maximum in Bedana (0.12 mg g^{-1}

fr. wt) followed by Shahi (0.11 mg g^{-1} fr. wt.), Dehrarose (0.087 mg g^{-1} fr. wt.), Purbi (0.079 mg g^{-1} fr. wt.) and China (0.056 mg g^{-1} fr. wt.).

Table 3. Effect of mycorrhiza and inorganic phosphorus on relative leaf water content (%) in litchi layers.

S. No	Treatments	Concentration	60 DAI	90 DAI	120 DAI
T_0	Control	No application	25.37	26.16	23.03
T_1	*G. mosseae*	10 g kg^{-1} of soil	32.89	36.41	29.28
T_2	*G. coronatum*	10 g kg^{-1} of soil	32.59	34.71	27.28
T_3	Phosphorus	50 mg kg^{-1} of soil	28.70	30.61	25.91
T_4	Phosphorus	75 mg kg^{-1} of soil	26.66	28.63	25.68
T_5	*G. mosseae* + Phosphorus	10 g +50 mg kg^{-1} of soil	37.78	39.09	31.43
T_6	*G. mosseae* + Phosphorus	10 g+ 75 mg kg^{-1} of soil	32.16	35.10	30.47
T_7	*G. coronatum* + Phosphorus	10 g+ 50 mg kg^{-1} of soil	36.62	38.71	31.14
T_8	*G. coronatum* + Phosphorus	10 g+ 75 mg kg^{-1} of soil	31.62	34.77	30.07
CD (P=0.05)		**-**	**2.26**	**2.42**	**1.63**
CV (%)		**-**	**4.21**	**4.22**	**3.40**

DAI-Date After Inoculation.

Relative leaf water content was significantly influenced by different treatments. The data depicted in (Table- 3) was recorded after 60 days inoculation maximum RWC in T_5*G. mosseae*10 g + Phosphorus 50 mg (37.78 %) that was at par with T_7*G. coronatum* 10 g + P 50 mg (36.62 %). Application of T_1 *G. mosseae* 10 g (32.89 %), T_2 *G. coronatum* 10 g (32.59 %), T_6 *G. mosseae* 10 g + Phosphorus 75 mg (32.16 %) andT_8 *G. coronatum* 10 g + Phosphorus 75 mg (31.62 %) were the next effective treatments and statistically equal to each other. Minimum RWC was recorded in untreated T_0 Control (25.37 %). At 90 days after inoculation same inclination was found while 120 days after inoculation highest relative water content was observed in T_5 *G. mosseae* 10g + Phosphorus 50 mg (31.43 %) which was statistically equal with T_7 *G. coronatum* 10 g + Phosphorus 50 mg (31.14 %), T_5 *G. mosseae* 10 g + Phosphorus 50 mg (30.47%), T_7 *G. coronatum* 10 g + Phosphorus 50 mg (30.07 %). It was followed by T_1 *G. mosseae* 10 g (29.28 %) andT_2 *G. coronatum* 10 g (27.28 %) and minimum was observed inT_0 Control (23.03 %). Present study supported by Sheng *et al.,*

(2008) represented that relative water content in the leaves was higher in mycorrhizal inoculated plant than non-mycorrhizal which supports the present finding.

Table 4: Effect of mycorrhiza and inorganic phosphorus on specific leaf wt. (%) in litchi layers

S. No	Treatments	Concentration	60 DAI	90 DAI	120 DAI
T_0	Control	No application	3.63	3.39	4.44
T_1	*G. mosseae*	10 g kg^{-1} of soil	5.31	4.20	6.34
T_2	*G. coronatum*	10 g kg^{-1} of soil	5.29	4.17	6.33
T_3	Phosphorus	50 mg kg^{-1} of soil	5.28	4.15	5.19
T_4	Phosphorus	75 mg kg^{-1} of soil	4.57	4.13	4.89
T_5	*G. mosseae* + Phosphorus	10 g +50 mg kg^{-1} of soil	6.22	5.77	7.28
T_6	*G. mosseae* + Phosphorus	10 g+ 75 mg kg^{-1} of soil	5.33	5.72	7.05
T_7	*G. coronatum* + Phosphorus	10 g+ 50 mg kg^{-1} of soil	6.18	5.75	7.18
T_8	*G. coronatum* + Phosphorus	10 g+ 75 mg kg^{-1} of soil	5.30	5.72	6.97
CD (P=0.05)		**-**	**0.13**	**0.35**	**0.54**
CV (%)		**-**	**1.51**	**4.21**	**5.11**

DAI-Date After Inoculation.

The data depicted in (Table - 4) pertaining to Specific leaf weight (SLW) clearly indicated that treatments differed significantly in influencing SLW at different date of observations. The performance was better found in T_5*G. mosseae*10 g + Phosphorus 50 mg (6.22 %) which was at par with T_7*G. coronatum*10 g + Phosphorus 50 mg (6.18 %) after 60 days inoculation followed by application of T_6*G. mosseae*10 g + Phosphorus 75 mg (5.33 %), T_1*G. mosseae* 10 g (5.31 %),T_8*G. coronatum*10 g + Phosphorus75 mg (5.30 %), T_2*G. coronatum* 10 g (5.29 %) and T_3Phosphorus 50 mg (5.28 %) which was statistically equal with each other. Minimum SLW of (3.63%) was recorded in control. After 90 days inoculation maximum specific leaf wt. observed in T_5*G. mosseae* 10 g + Phosphorus 50mg (5.77 %) which was statistically similar with T_7*G. coronatum* 10 g + Phosphorus 50 mg (5.75 %), T_6*G. mosseae* 10 g + Phosphorus 75 mg (5.72 %) and T_8 *G. coronatum* 10 g + Phosphorus 75mg (5.72 %).

Minimum found in Phosphorus 75 mg (4.13 %) which was at par with other treatments except control. After 120 days inoculation same inclination of treatments was noted with maximum SLW of (7.28 %) in case T_5 *G. mosseae* 10 g + Phosphorus 50 mg that was statistically similar to T_6, T_7 and T_8 with respective SLW of (7.05%, 7.18% and 6.97 %). Significantly minimum SLW of (4.44%) was noted under control. Present study supported by Sheng *et al.,* (2008) represented that relative specific leaf wight in the leaves of layered litchi was higher in mycorrhizal inoculated plant than non-mycorrhizal which supports the present finding

Table 5: Effect of mycorrhiza and inorganic phosphorus on flush length (cm) and number of leaves per flush in litchi layers.

S. No	Treatments	Length of flush			No. leaves/flus		
		60 DAI	90 DAI	120 DAI	60 DAI	90 DAI	120 DAI
T_0	Control	4.1	6.0	6.5	3-4	3-8	3-8
T_1	*G. mosseae*	6.0	7.7	8.5	4-5	4-7	4-7
T_2	*G. coronatum*	5.8	7.5	8.1	4-5	4-7	4-7
T_3	Phosphorus	5.3	6.9	7.7	4-5	4-5	4-5
T_4	Phosphorus	4.9	6.7	7.3	4-5	4-5	4-5
T_5	*G. mosseae* + Phosphorus	7.4	8.7	10.2	4-7	5-8	5-8
T_6	*G. mosseae* + Phosphorus	6.9	8.1	9.3	4-7	4-9	4-9
T_7	*G. coronatum* + Phosphorus	7.2	8.4	9.8	4-7	45-7	5-7
T_8	*G. coronatum* + Phosphorus	6.1	7.9	8.9	4-5	4-9	4-9

DAI-Date After Inoculation.

The data collected in (Table - 5) pertaining to Significant effect of treatments on leaf parameters of litchi layers pertaining to number of leaves per flush and flush length was also observed. T_5 (G. *mosseae* 10 g + Phosphorus 50 mg) is the longest of flush with 10.2 cm followed by T_7 (G. *coronatum* 10 g + Phosphorus 50 mg) treatment with 9.8 cm after 120 days of inoculation and Number of leaves per flush was also noted for the all treatments in which T_5 (G. *mosseae* 10 g + Phosphorus 50 mg) and T_7 (G. *coronatum* 10 g + Phosphorus 50 mg) has been noticed with maximum number of leaves i.e. (5 - 8) and (5 - 7). Present study supported by Singh and Kushwaha (2006), also reported that the importance and

contributions of leaf flushing towards litchi trees adaption under a strong seasonal subtropical climate. Increase in number of leaves might be due to better mobilization of nutrient and water from rhizosphere as the fungal hyphae of AM fungi goes up to (11 cm) even beyond the rhizosphere causing better exploitation of soil nutrients (Marschner and Dell, 1994). The increased level of cytokines as influenced with AM fungi inoculation might have caused higher leaf production and increased plant height as reported by (Rawat *et al*., 2013).

Conclusion

The influence of phosphorus (50 mg and 75 mg) mycorrhiza (*G. mosseae* and *G. coronatum*) alone and in combination. The treatment significantly influenced the changes in chlorophyll, carotenoids content, Relative water content, specific leaf weight, number of leaves per flush and length of flush in leaves of litchi saplings in nursery stage. After120 days of inoculation both the species of mycorrhiza combination with phosphorus application were very effective as evident from the results, highest Total chlorophyll content is (2.474 mg g^{-1}fr.wt.), Carotenoids (0.154 mg g^{-1} fr.wt.), RLW(31.43 %), SLW(7.28 %), number of leaves per flush and length of flush(5 - 8) and (10.2 cm) was analysed in case T_5 *G.mosseae*10 g + Phosphorus 50 mg kg^{-1} of soil. Hence, the treatment *G. mosseae* 10 g + Phosphorus 50 mg can be used as the best treatment to increase the healthy planting material and survival of litchi *cv.* Purbi without hampering the soil fertility status.

References

1 Anonymous. The Useful plants of India. CSIR, New Delhi, India. 1986.

2 Arnon DI. Copper enzymes in isolated chloroplast: Polyphenol oxidases in Beta vulgaris. Plant Physiology. 1949; 24:1-14.

3 Barea J, Azcón-Aguilar C. Production of plant growth-regulating substances by the vesicular-arbuscular mycorrhizal fungus *Glomus mosseae.* Applied Environment Microbiology.1982; 43:810–813.

4 Baslam M, Esteban R, García-Plazaola JI, Goicoechea N. Effectiveness of arbuscular mycorrhizal fungi (AMF) for inducing the accumulation of major carotenoids, chlorophylls and tocopherol in green and red leaf lettuces. Applied Microbiology Biotechnology. 2013; 3:119-3128.

5 Birhane E, Sterck FJ, Fetene M, Bongers F, Kuyper TW. Arbuscular mycorrhizal fungi enhance photosynthesis, water use efficiency, and growth of frankincense seedlings under pulsed water availability conditions. Oecologia. 2012;169: 895-904.

6 Bona E, Cattaneo C, Cesaro P, Marsano F, Lingua G, Cavaletto M, Berta G. Proteomic analysis of Pterisvittatafronds: two arbuscular mycorrhizal fungi differentially modulate protein expression under arsenic contamination. Proteomics. 2010; 10:3811–3834.

7 Bona E, Marsano F, Massa N, Cattaneo C, Cesaro P, Argese E, Sanità di Toppi L, Cavaletto M, Berta G. Proteomic analysis as a tool for investigating arsenic stress in Pterisvittata roots colonized or not by arbuscular mycorrhizal symbiosis. Journal Proteomics. 2011; 74:1338–1350.

8 Burkowska B. Growth and photosynthetic activity of micro propagated strawberry plants inoculated with endomycorrhizal fungi (AMF) and growing under drought stress. Acta Physiological Plant. 2002; 24:365–370.

9 Chakrabarti J, Chatterjee S, Ghosh S, Chatterjee NC, Dutta S. Synergism of VAM and Rhizobium on production and metabolism of IAA in roots and root nodules of Vigna mungo. Current Microbiology. 2010; 61:203-209.

10 Cicatelli A, Lingua G, Todeschini V, Biondi S, Torrigiani P, Castiglione S. Arbuscular mycorrhizal fungi modulate the leaf transcriptome of a *Populus alba* L. clone grown on a zinc and copper contaminated soil. Environment Experiment Botanical. 2012; 75:25–35.

11 Copetta A, Lingua G, Berta G. Effects of three AM fungi on growth, distribution of glandular hairs, and essential oil production in *Ocimum basilicum* L. var. *Genovese.* Mycorrhiza. 2006; 16:485–494.

12 Danneberg G, Latus C, Zimmer W, Hundeshagen B, Schneider-Poetsch HJ, Bothe H. Influence of vesicular-arbuscular mycorrhiza on phytohormone balance sinmaize (*Zeamays* L.). Journal Plant Physiology.1993; l141: 33–39.

13 Gomez KA, Gomez AA. Statistical Procedures for Agricultural Research. Wiley and Sons, New York. 1984.

14 Gryndler M, Vosatka M, Hrselova H, Catska V, Chvatalova I, Jansa J.Effect of dual inoculation with arbuscular mycorrhizal fungi and bacteria on growth and mineral nutrition of strawberry. Journal Plant Nutrition. 2002; 25:1341–1358.

15 Guerrieri E, Lingua G, Digilio MC, Massa N, Berta G. Do interactions between plant roots and the rhizosphere affect parasitoid behaviour? Ecological Entomology. 2004; 29:753–756.

16 Hendry GAF, Price AH. Stress indicators Chlorophyll and Carotenoids. In: Methods in Comperative Plant Ecology- A Laboratory Manual. Hendry GF, Grime J P.(eds.), Chapman and Hall, London. 1993;148-152.

17 Hrselova H, Grindler H, Vancura V. Influence of inoculation with VA mycorrhizal fungus *Glomus* sp. on growth of strawberries and runner formation. In: Agriculture Ecosystems and Environment. Elsevier Science Publishers B.V., Amsterdam. pp; 1989; 193–197.

18 Jentschel K, Thiel D, Rehn F, Ludwig-Müller J. Arbuscular mycorrhiza enhances auxin levels and alters auxin biosynthesis in *Tropaeolum majus* during early stages of colonization. Physiology Plant. 2007; 129:320–333.

19 Jiang Y, Gao H, Zhang M. Lychee (Litchi). In: Siddiq M (Ed). Tropical and Subtropical Fruit: Postharvest Physiology, Processing and Packaging, 1st edn. John Wiley & Sons, Inc., New Delhi, India, pp.2012; 241258.

20 Kadam VB, Deore SV, Kadam UB. Estimation of chlorophyll content in leaves of Trigonella foenum–graecum Linn. World. Journal Pharm. Pharmaceutical Science. 2017;6: 569-572.

21 Kadam VB, Tambe SS, Fatima S, Kadam UB, Wadikar MS. Biochemical analysis of leaves of some medicinal plants of Marathwada region in Maharashtra. Journal Biomed Pharmaceutical Research. 2013; 2: 27-30.

22 Kumari N, Nahakpam S, Rani R. Changing pattern of chlorophyll content and carotenoid in different flushes of five litchi varieties. Journal of Pharmacognosy and Phytochemistry. 2018; 1: 719-722.

23 Li LY, Li CS. An improved method of air-layering lychee tree. Fukien. Agriculture Journal. 1949; 11, 1-6.

24 Lingua G, Bona E, Todeschini V, Cattaneo C, Marsano F, Berta G, Cavaletto M. Effects of heavy metals and arbuscular mycorrhiza on the leaf proteome of a selected poplar clone: a time course analysis. PLoS ONE. 2012; 7:38662.

25 Ludwig-Müller J, Kaldorf M, Sutter EG, Epstein E. Indole-3butyric acid (IBA) is enhanced in young maize (*Zea mays* L.) roots colonized with the arbuscular mycorrhizal fungus *Glomus intraradices.* Plant Science. 1997; 125:153–162.

26 Marschner H, Dell B. Nutrient uptake in mycorrhizal symbiosis. Plant and soil.1994;65(3):89-102.

27 Menzel C. The lychee crop in Asia and the pacific. Bangkok, Thailand. 2002; FAO/RAP Publication.

28 Momin RK, Kadam VB. Biochemical analysis of leaves of some medicinal plants of genus *Sesbania.* journal Ecobiotechnology. 2011; 3: 14-16.

29 Nemec S, Vu JCV. Effect of soil P and *Glomus intraradices* on growth, non-structural carbohydrates and photosynthetic activity of *Citrus aurantium*. Plant Soil. 1990;128: 257-263.

30 Pandey, R.M. and Sharma, H.C. The Litchi. ICAR, New Delhi, India,1989. 80 pp.

31 Rawat A, Mishra N. K, Mishra DS, Kumar P, Rai RB, Damodaran T. Concentration mediated effect of Arbuscular Mycorrhizal Fungi (AMF) on growth and nutrition of air layered litchi plants. International Journal of Current Research. 2013;5(07):1730-1734.

32 Sharma SD, Bhutani VP. Response of apple seedlings to VAM, *Azotobacter* and inorganic fertilizers. Horticulture Journal.1998; 11(1): 1-8

33 Sheng M, Tang M, Chen H, Yang B, Zang F, Huang Y. Influence of arbuscular mycorrhizae on photosynthesis and water status of maize plants under salt strees. Mycorrhiza. 2008;18: 287-296.

34 Singh KP, Kushwaha CP. Diversity of flowering and fruiting phenology of trees in a tropical deciduous forest in India. 2006; 97(2):265-27.

35 Torelli A, Trotta A, Acerbi L, Arcidiacono G, Berta G, Branca C. IAA and ZR content in leek (*Allium porrum* L.) as influenced by P nutrition and arbuscular mycorrhizae, in relation to plant development. Plant Soil. 2000; 226:29–35.

36 Weatherley PE. Studies in water relations of the cotton plant. The field measurement of water deficits in leaves. New phytology. 1950; 49: 81-87.

37 Yao Q, Zhu HH, Chen JZ. Growth responses and endogenous IAA and iPAs changes of litchi (*Litchi chinensis* Sonn.) seedlings induced by arbuscular mycorrhizal fungal inoculation. Scientia Horticulture. 2005; 105:145– 151.

38 Zhu XC, Song FB, Liu SQ, Liu TD, Zhou X. Arbuscular mycorrhizae improve photosynthesis and water status of *Zea mays* L. under drought stress. Plant, Soil and Environment. 2012; 58:186-191.