INFLUENCE OF MYCORRHYZA 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. 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 inoculationboth 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) in case T_5*G. mosseae*10 g + Phosphorus 50 mg. Significantly lowest value of chlorophyll was noted in T_0Control (2.090 mg/g).Carotenoid content was also measured maximum in T_5*G. mosseae*10 g + Phosphorus 50 mg(0.118 mg g-1 fr. wt.) as compare to T_0 Control with (0.065g/mg fr. wt.). Relatve water content (RWC) initially measured non-significant but 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 in influencing specific leaf weight of different date of observations. The performance was maximum found in T_5*G. mosseae* 10 g + Phosphorus 50mg (6.22 %) as compare toT_0 control (4.44 %). Significant effect of treatments on leaf parameters of litchi layers pertainingnumber of leaves per flush and length of flushis 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.), a member of the Sapindaceae, is an important fruit crop that is widely cultivated in tropical and subtropical areas of the world. The fruit are fleshy drupes with an edible aril surrounded by the pericarp.Phosphorus is one of the important plant nutrients that involved and plays important role in in in plantFunctionslike 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 mycorrhizal symbiosis significantly improved plant growth performance, such as plant height, stem diameter, shoot, root or total dry weight (Wu *et al.*, 2011).The beneficial effect

of AM fungi includes enhanced seedling growth, reduced phosphate requirementsincreased resistance to fungal root pathogens and abiotic stresses consequently increased fruit production.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 [8].Considering the above facts, the present study was undertaken to determine and compare chlorophyll-a (g/mg),chlorophyll-b (g/mg), total chlorophyll (g/mg)and carotenoid content of the leaves of litchiof 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) , mycorrhiza (*G. mosseae* and *G. coronatum*) at 10g 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_3Phosphorus @ 50 mg kg^{-1} of soil, T_4Phosphorus @ 75 mg kg^{-1} of soil, T_5*G. mosseae* 10 g + Phosphorus50 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 was 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 15000rpm. 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 areexpressed in mg g^{-1} freshweight

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**:**

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

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

Formula:

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

Results and Discussion

Generally, three flushes are produced in litchi between fruit harvest to panicle emergence. Time of emergence of different flushes has a profound influence on the floriferousness of the shoot. It was clearly shown by the previous report that the flush maturity earliest (before the winter period) produce floral shoots, while flushes maturing quite late produce vegetative shoots. Flush emergences time and duration of flushing and its influence towards the fruit is variety specific. Therefore, to investigate the role of flushing in

the litchi varieties and patterns in chlorophyll contents and also in carotenoids, experiment has been designed.

Variation in the contents of chlorophyll was noticed amongst the treatments studied and also in flushes. Data depicted (Table 1) revealed that the highesttotal 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 mycorrhizae and inorganic phosphorus on total Chlorophyll (g/mg) 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**

*- Showed that the mean values of three replications.

On second day of observation (90 DAI) the maximum chlorophyll (1.603 mg/g) 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),T_6*G. mosseae*10 g + Phosphorus 75 mg (1.507 mg/g), T_3Phosphorus 50 mg (1.507 mg/g) and T_1 *G. mosseae* 10 g(1.477 mg/g). The minimum total chlorophyll (1.011 mg/g) was observed in T_0Control.120 days after planting the highest level of chlorophyll content of 2.474 mg/g was found in treatment T_5 *G. mosseae* 10g + 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). Significantly lowest value of chlorophyll was

noted in T_0 Control (2.090 mg/g).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 mycorrhizae and inorganic phosphorus onCarotenoids (g/mg) 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**

Carotenoids are generally studied for its light harvesting ability and photoprotection activity. Under adverse stress conditions their roles become more pronounce. Carotenoids contents in plants also provide complementary information on plant canopy physiological status. Data presented in (Table - 2) revealed that a trend of carotenoids content with that of chlorophyll content in all the studied nine treatment. Maximum carotenoids were measured in T_5 *G. mosseae* 10g + Phosphorus 50 mg (0.12 mg g^{-1} fr. wt.) followed by T_7 *G. coronatum* 10 g + Phosphorus 50 mg (0.118 mg g^{-1} fr. wt.), and then T_6 *G. mosseae* 10 g + Phosphorus 75

mg(0.114 mg g^{-1} fr. wt.) as compare toT_0 Control(0.051 mg g^{-1} fr. wt.) measured the lowest carotenoids concentration amongst nine treatments taken in the present study (Table - 2). 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 (0.056mg g^{-1} fr. wt.).

Table 3. Effect of mycorrhizae and inorganic phosphorus on relative leaf water content

S. No	Treatments	Concentration	Initial	60 DAI	90 DAI	120 DAI
T_0	Control	No application	24.14	25.37	26.16	23.03
T_1	*G. mosseae*	10 g kg^{-1} of soil	24.56	32.89	36.41	29.28
T_2	*G. coronatum*	10 g kg^{-1} of soil	25.16	32.59	34.71	27.28
T_3	Phosphorus	50 mg kg^{-1} of soil	23.11	28.70	30.61	25.91
T_4	Phosphorus	75 mg kg^{-1} of soil	25.24	26.66	28.63	25.68
T_5	*G. mosseae* + Phosphorus	10 g +50 mg kg^{-1} of soil	25.61	37.78	39.09	31.43
T_6	*G. mosseae* + Phosphorus	10 g+ 75 mg kg^{-1} of soil	25.64	32.16	35.10	30.47
T_7	*G. coronatum* + Phosphorus	10 g+ 50 mg kg^{-1} of soil	24.15	36.62	38.71	31.14
T_8	*G. coronatum* + Phosphorus	10 g+ 75 mg kg^{-1} of soil	24.19	31.62	34.77	30.07
CD (P=0.05)		**-**	**NS**	**2.26**	**2.42**	**1.63**
CV (%)		**-**	**-**	**4.21**	**4.22**	**3.40**

(%) in litchi layers.

Relative leaf water content was significantly influenced by different treatments. The data depicted in (Table- 3) was recorded after 60 days inoculation maximum RWCin 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* 10g + 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 mycorrhizae 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)		**-**	**NS**	**0.13**	**0.35**
CV (%)		**-**	**-**	**1.51**	**4.21**

The data depicted in (Table - 4) pertaining to Specific leaf weight (SLW) clearly indicated that treatments differed significantly in influencing SLW of different date of observations. The performance was better found in T_5*G. mosseae*10 g + Phosphorus 50mg (6.22 %) which was at par with T_7*G. coronatum*10 g + Phosphorus 50mg (6.18 %) after 60

days inoculation followed by application of T_6*G. mosseae*10 g + Phosphorus 75mg (5.33 %), T_1*G. mosseae*10 g(5.31 %),T_8*G. coronatum*10 g + Phosphorus75mg (5.30 %), T_2*G. coronatum* 10 g (5.29 %) and T_3Phosphorus 50mg (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 75mg (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 caseT_5 *G. mosseae* 10g + 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 mycorrhizae and inorganic phosphorus on flush length (cm) and number of leaves per flush inlitchi layered

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

The data depicted 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 carotenoid content, Relative water content, specific leaf weight, number of leaves per flush and length of flushin leaves of litchi saplings in nursery stage. After120 days of inoculationboth the species of mycorrhiza combination with phosphorus application werevery effectiveas evident from the results,highest Total chlorophyll content is (2.474 mg/g), Carotenoids (0.118mg g^{-1} fr. wt.),RLW(31.43 %), SLW(6.22 %), number of leaves per flush and length offlush(5 – 8) and(10.2 cm) was analysedincase 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.

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