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PIRIFORMOSPORA INDICA EFFECT ON LETTUCE (LACTUCA SATIVA): THE MOST NEEDED AGRICULTURE CROP

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ABSTRACT

Piriformospora indica is a miracle endophytic fungus which benefits the number of plants in their growth by establishing a symbiotic relationship between the plants roots usually with the vascular plants which enhance the plant properties. *Lactuca sativa* (lettuce) is an agriculture crop used in food industry at boom, plants grown with the *P.indica* shows healthier plant biomass with more leaves surface. The fungus interact with plants roots forms a symbiotic relationship with them and benefit the plant in all ways from increasing the uptake of more nutrition from the roots to the huge leave surface and length. These plants were grown from the seeds and treated with *Piriformospora indica* under controlled conditions and were compared with the control plants of same species under certain parameters like dry cell weight, protein content, spore count, root and shoot length, interaction with fungus. Excellent results were obtained with significant difference in both plants. This suggest *Lactuca sativa* (lettuce) grow much faster and healthier with *P.indica.* Which overcome the concern of efficient and fast growth of *Lactuca sativa.*

KEYWORDS: *Lactuca sativa* (Lettuce), *P. indica*, endophytic fungus, cultivation, Plant interaction.

INRODUCTION

Root mutualistic fungus, *Piriformospora indica* is a basidiomycota was first originated from Indian thar desert, it was taken as symbiont fungus. It was discovered from the plant species orchid. This fungus benefit the plant by developing thesymbiotic relationship with the plant growth and is capable to increase the plant immunity from certain pathogens, increase tolerance to stress environment such as high salt and low water content in soil (Gosal SK, Gosal SS, et.al, 2010). *Lactuca sativa* (lettuce) is considered to be a salad crop under leafy vegetable which belongs to daisy family, Asteraceae which is grown all over the world, constitutes many healthy properties such as bone strength as it is excellent source of vitamin K,improves vision as it have ample amount of vitamin A, promotes sleep and many more other benefits (Monsees H, Suhl J, 2019). It is widely used in salads, vegetable curries, soups and various other roles in food industry. Lettuce is full of many anti oxidants, vitamins, fibers and minerals. With its vast uses in food industry it was selected for experiment with the point of concern to find a new efficient and effective way to grow the crop faster and healthier (Day J A, Diener c, 2021). An experiment was performed to study and analyse the effect of *Piriformaspora indica* on the growthof *Lactuca sativa* . Under certain parameters like spore count, dry cell weight, shoot length, root length, protein content of plant with different methods. *P.indica* cultivated on cost effective jaggery medium and was recorded that *P.indica* significantly increased the uptake of P, Zn and Cu (Raklami A and Bechtaoui N 2019). This was possible by producing hyphae that penetrate inside the soil and thereafter creating a network of mycelium (Garg N and Aggarwal N 2011).

The mycelium played the role of a biological medium for transferring some macro and micro nutrients from soil to the roots of the plant (Oliveira and Duarte 2010) and thereby showed a evident increase in the crop quality (Smith and Reed, 2008).

It increased photosynthesis and also showed rise in yield of crop plants by reducing translocation of heavy metals to host plants (Varma A and Sherameti 2012).

It was recorded that colonizing lettuce with *P.indica* simultaneously increased the accumulation of copper, iron anthocyanin and caretonoid contents (Baslam Marouane et.al, 2013). Net photosynthesis and chlorophyll content was seen to be higher in *P.indica* inoculated lettuce plants as compared to the control plants. Also the root and leaf tissues of *P.indica*

inoculated plants displayed enhancement in nitrogen, phosphorus and potassium contents than in control plants. Various measures were calculated for comparison between plants with *P.indica* and without *P.indica*.

FUNGAL CULTURE CULTIVATION

Fungal spores were taken from the previous culture ready plate and was streaked fromit on new plates having jaggery media containing agar, initial pH of media was 6.5.

DRY CELL WEIGHT

Dry cell weight is a measure for the growth measurement of plants. It is taken out as DCW in per liters of broth cultures. Whatmann filter paper is taken and culture is extracted from broth after 6-8 days, this culture is allowed to pass through the whatmann filter paper(Attri M.K and Varma.A, 2018). The wet filter paper is dried overnight in hot air oven at 80^0C for moisture removal from the surface of paper.

Grown mycelium on inoculated membrane is measured at every one day gap by removing flask.

SPORE COUNT

P. *indica* was grown on jaggery medium with specific pH. It plays a vital role in culture growth as in much acidic medium the growth of culture was not flourished. For optimum growth pH should be maintained at 6.5. Spores of *P.indica* were grown in petriplates and inoculated in jar bottles which were put on shaker for 3-5 days to obtain optimum growth of culture at 30^0C temperature (YANG, Liu & CAO, Jin-Li & Ying-Ning, et.al 2020). As sporulation starts after 6 days, spore yield was taken for sample after 8-9 days for maximum yield and dry cell weight was obtained after 42 hrs of growth. (Kumar V, Sahai V et.al, 2011). Sporulation occurs when carbon is depleted and glucose is consumed by the culture .(Hart, M.M. and Trevors, J.T, 2005).

SEED GERMINATION

Seeds of lettuce were first sterilized with distilled water for 5 minutes then soak them in tween 20 for 5 minutes, again wash it with distilled water for 1 minutes. After sterilizing the seeds sow them in moist soil in different pots one with the fungus inoculated soil and other without the fungus present in soil. After sowing them in upper most layer of soil sparkle some water on it and observe it after interval of 3-4 days. Leaves of plant grown can be seen first in the pot where soil was inoculated with fungus while the shoot and leaves of plants was seen a week later in pot withoutthe fungus inoculated in soil.

INTERACTION OF PLANT WITH FUNGUS

P. indica is a miracle endophyte fungus which help in nutrient uptake of plants, helps in root colonization, seed germination.When plants are grown with fungus, they result in better growth with measurable difference in leaves biomass, fruits as compare to other non fungus associate plants (Liu, W.; Tan, M. et.al, 2022). *P.indica* interacts with plants and helps in overcoming the drought or extreme conditions such as high temperature, high salinity in soil. It also protects from pathogens. (Kumar Bhuyan,S. Bandyopadhyay et.al,2015).

In this experiment two lettuce plants were taken (Table 1). One was grown with *P.indica*, where culture was mixed in the soil as sandwich layer method and other was grown without fungus in soil. Both were grown in same environment condition such as same temperature was provided, watering alternative days. After few days their growth was measured from seed grown to healthy plant.(Attri M.K and Varma.A,2018).

Table 1: Effect of *P.indica* on different growth parameters of Lettuce.

Parameters	Plant with *P.indica*	Plant without *P.indica*
Biomass growth	Healthier as compared	Normal
Shoot elongation	Taller	Shorter
No. of leaves	More than nine	More than five

FRESH AND DRY ROOT AND SHOOT LENGTH

The length and weight of shoots and roots were measured after they were weigh fresh. The samples are then subsequently dried for 2 days in a forced draught oven at 70°C to ascertain their dry weights. After 2 days of drying the samples the relative water content (RWC) was calculated by soaking 1 g of fresh roots and shoots in 50 ml of distilled water for 6 hours. After washing samples are dried again overtime approx 48 hours at same temperature of 70°C before the dry weight was calculated. (Li, Qiansheng, Li. Xiaoqiang et.al, 2018).

Root and shoot length ratio determines the parameter for the plant growth , if the shoot length is more it is said that nitrogen role is viable in plant absorbance of nutrients (S.K. Verma,P.K. Sahu et.al, 2015), while if the root length is more it is said phosphorus is more absorbed by the plant hence nitrogen promotes the shoot length while the phosphorous stimulates the root growth and development.(Bagheri A.A, Saadatmand et.al, 2013) Table 2.

Table 2: Effect of *P.indica* on growth of Lettuce.

S.NO	Parameters	Control	Treated
1	Germination(%)	74 +/- 0.54	88 +/- 0.16
2	Shoot length (cm)	5.9 +/- 0.76	7.6 +/- 0.065
3	Root length (cm)	0.4 +/- 0.48	0.7+/- 0.55
4	Dry shoot weight(g)	3.1+/- 0.01	4.2+/- 0.05
5	Dry root weight	1.1+/- 0.06	1.7+/- 0.16

RT-PCR ANALYSIS

RT-PCR analysis is used for mapping and reading the genetic material of the *p.indica* treated plants and controls. RT-PCR helps in observing weather the reactions with specific markers are taking place. These markers are specific to the genetic material ofthe *p.indica* genome which are known for benefiting the plants. If the plants do not have the specific markers reaction will not take place. This method is also used for comparing control and treated plants genetic material and observing the changes or the mutants in treated plants which can be the new gene discovery which can be a beneficial discovery for agriculturally important plant. For the analysis RNA was isolated from RNA extraction kit through TRIZOL mixture where after adding trizol, chloroform is added and when the three distant phases are observed take the aqueous phase and add isopropanol. Put the RNA pellet dissolved in nuclease free water for checking its purity. RNA purity was checked under nanodrop machine which will be used for preparing cDNA samples.(Gill,Sarvajeet, Gill Ritu et.al, 2016).

RT- PCR was performed on thermal cycler of thermofisher where machine was setup on specific time set and was run. For the experiment master mix was made readywhere the specific dNTPS (specific primers), TaqMan polymerase, SYBR green dye and treated Cdna libraries are mixed. Cdna libraries are prepared using Qiagen Cdna preparation kit. In PCR reactions are as followed 10 min at 95 °C, 40 cycles while annealing reaction at 95 °C and 30 sec at 60 °C, 90 s at 72 °C. To ensure that the most efficient single production was produced, a melting curve study was performed. The Realplex Software 2.2.10 was used to determine Ct values. The 2Ct technique was used to calculate the relative gene expression levels (Ghahfarokhi R, Goltapeh M.et.al, 2010). For proper error free ct value quantitative PCR was performed at least twice, with three replicates in each well. (Bryan, G.T., Daniels et.al, 1995).***Four fungal growth regulators genes i.e hexose transporter (Hex T5), urease (Ure A), Glutamate synthase (Glut N) and glutamine synthatase (Glut S) were selected*** **(Table 3).**

Table 3: Effect on Genome of *P.indica.*

S.No.	Genes	Increase in fold
1.	Hex T5	10 folds
2.	Ure A	14 folds
3.	Glut N	17 folds
4.	Glut S	14 folds

PROTIEN CONTENT BY MS-MS SPECTROMETRY

MS spectrometry is done for the plants proteomics analysis of plants and comparison between them. For this analysis sample preparation is done where extraction of sample tissue is isolated in liquid nitrogen with help of mortar.(Hayet Beltayef, Mongi Melki et al, 2021). For removal of soluble organic substances in sample acetone or TCA precipitation extraction method is used which leaves proteins and insoluble materials as precipitation. Pure protein is extracted by using LC separation buffer (phenol extraction method). The frozen tissue sample was removed two times with methanol/water, 80/20 (v/v) and reconstituted in methanol/water, 30/70 (v/v) preceding LC/MS-examination (Erin Gemperline, Caitlin Keller et.al, 2016).

After extraction of protein as sample plants metabolites, phytohormones are analyzed for biological interpretation. The results of the MS spectrometry is depicted in Table 4.

Table 4: Effect on protein content of *P.indica.*

S.No.	Protein	Increase in fold
1.	Eno F	4.3 folds
2.	Ure D	3.4 folds
4.	Glut S	5.3 folds

CONCLUSION

Lettuce plant grown with *P.indica* shows excellent positive results in all parameters from seed germination to more healthier biomass. *P.indica* can be grown in much effective and cheap method by using jaggery medium which is used for flourishing development of lettuce. Hence *P.indica* enhances the plants nutrient uptake capacity which can be used to grow agriculture crop in more cost effective manner in order to over come the shortage of food and cost cutting.

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