A plausible mechanism of biosorption in dual symbioses by vesicular-arbuscular mycorrhizal in plants

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Abstract: Dual symbioses of vesicular-arbuscular mycorrhizal (VAM) fungi with growth of *Momordica charantia* were elucidated in terms of plausible mechanism of biosorption in this article. The experiment was conducted in green house and mixed inoculum of the VAM fungi was used in the three replicates. Results demonstrated that the starch contents were the main source of C for the VAM to builds their hyphae. The increased plant height and leaves surface area were explained in relation with an increase in the photosynthetic rates to produce rapid sugar contents for the survival of plants. A decreased in protein, and amino acid contents and increased proline and protease activity in VAM plants suggested that these contents were the main bio-indicators of the plants under biotic stress. The decline in protein may be due to the degradation of these contents, which later on converted into dextrose where it can easily be absorbed by for the period of symbioses. A mechanism of C chemisorption in relation with physiology and morphology of plant was discussed.

Keywords: Dual symbioses, VAM fungi, Biosorption, C, degradation.

INTRODUCTION

An interaction of large microbial activity in the region of soil surrounding the plants roots always exist which sometime results in the disorganization of host tissues. There is much evidence that soil microorganisms can affect plant growth, morphology, and physiology, and that some of these effects can be explained by their production of growth-regulating substances. Microbial production of hormones in the root zone could provide an additional supply of these biologically active substances, since they are taken up and used by the plant (Barea and Azcon-Aguilar 1982). The assistances of VAM to the plants are considerable against disease and drought resistance through increases in nutrients and water quantity including excessive fixing of soil bits into bulky combinations with the roots of plants (Smith and Read 1997).

VAM (Vesicular-arbuscular mycorrhizae) takes its most of carbon from the roots of the host plant (Jennings, 1995). The most visible VAM structure is hyphal network. The host plant is essential for the VAM survival (Barea & Jeffries 1995). The main source of VAM for up taking of carbon by active and passive sugar transport systems have no direct evidence of its operation in plant root system (Blumenthal, 1976). Smith and Read studied in 1997 and reported that currently approximately 95% terrestrial calm sustain certain mycorrhizal fungal link therefore it is not “Mycorrhizas”, roots which are the leading periodicals for nutrient taking via soil. The investigation on VAM has vital significance in horticulture, agronomy, reforestation, environmental biology and root biology with bionetwork organization (Bethlenfalvay and Schepp 1994).

VAM hyphal network develop a multifaceted advancement of exchange among plant roots and fungal hyphae that primary useful jointly for both fungus and plants state. It was reported that host plant act as energy to the VAM hyphal progress and also the pre-establishment period of VAM foundation (Jennings (1995). The motivation of fungal progression is related with the quantity as compared to the existence of some precise compounds in the root exudates, primarily the carbon comprising compound. Other studies, however, showed no relationship between root exudation and VAM infection. There is no sign that they need any exact mass issues. Though, carbohydrates compounds of host plants root or plant cells may act as indication of C provider for persuading hyphal development or diverging. The fungal life cycle activate when fungal propagules start to growth. The VAM now grows widely among and inside host roots exodermal and cortical cells and create intra radical structures containing arbuscules and lipid rich vesicles (Frey & Schüepp 1992).

The aims and objective of this research was to propose a mechanism corresponds to utilization of C by VAM from the roots of plants and enhancement of nutrients in the plant for survival. This study will reveal that how C from the sugar and nitrogen contents get perturb in the inoculation of VAM fungi through bio-sorption in duel symbiosis. The proposed mechanism of adsorption for the transformation is the possible way by which C is taken up by VAM and nutrients by plant.

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MATERIAL AND METHODS

The experiments were conducted in a green house. Surface sterilized seeds of *Momordica charantia* were used for this investigation as a widely used important plant. Each experiment were laid out in a complete randomize block design with VAM containing soil treatment in three replicates plus control plants. The soil was then put into the 20cm diameter 18 clay pots. The mixed VAM fungi inoculants consisting of spores, mycorrhizal fragments, and infested soil collected from the field where plants have been grown inoculated with VAM. In each pot five seeds were sowed, percent germination was 90%. 125gram of VAM soil was inoculated after 15 days. Root and shoot length was recorded individually for each plant by measuring total length of all roots and shoots from base to maximum, length were measured using standard centimeter scale. The carbohydrate, sucrose and total soluble sugars were determined by the method defined by Riazi *et al* (1985) and Buysse and Merckx (1993) respectively in 80% ethanol through spectrophotometer.

Proline contents: Proline was extracted from 0.5 g of fresh shoot and root into 10 ml of 3% sulfosalicylic acid and sieved over Whatman No.2 filter paper. Proline was estimated by ninhydrine method as reported by Bates *et al* (1973) in Schimadzo UV/Visible Spectrophotometer 180 A, through pure proline as a usual.

Protein contents were estimated using the method of Bradford (1976). Protease activity was determined by the method described by Ainous (1970) using phosphate buffer and absorbance was recorded on Spectrophotometer at 570nm. Amino acid determined at 566nm by the action of extract with ninhydrin solution. protein anticipated by the copper tartrate action with plant extract, protease projected at 570nm by the incubation of extract in casein solution for 1 hour then treated with TCA solution(40%) and finally with folin phenol reagent.

RESULTS

Morphological features of plants

The results of this work were very fascinating. It was observed that all of the VAM plants of *Momordica charantia* were larger in the length as compared to non-treated plants (table 1) after 30 days. At this time, no significant difference between the dry weights of the plants was observed. However, fresh weight of MP (mycorrhizal plants) was higher as compared to NMP (non- mycorrhizal plants) (table 1). In contrast, leaf area of VAM plants was considerably greater than those of non-VAM plants. It was observed that there was higher water content in MP as compared to NMP and reduced ratio of fresh weight and dry weight (table 1).

Chemical biology of plants

Estimation of Carbohydrates

The maximum starch content was recorded in leaves of seedling of VAM inoculated *Momordica chianti* plants in 15 days. There was an increase of 1.911% which was decreases 8.95% in 30 days old seedlings over control plants, while in shoot and root they were decreases in both phases as reported in the table 2.

Estimation of sucrose contents

The maximum increase in sucrose contents was recorded in leaves of seedling of VAM inoculated *Momordica charantia* in 15 days (6.19%) which were non significantly decreased (3.13%) in 30 days old seedlings in comparison of control plants, while in shoot there was decrease in both phases (table 3) but in case of roots it was higher (3.68%) in MP as compared to NMP.

Estimation of total soluble sugars

The total soluble sugars in the leaves, shoots and roots of two phases of *Momordica charantia* showed a decrease in mycorrhizal seedlings than NMP seedlings. The total soluble sugar content was recorded as 6.077, 6.365 and 6.117mg/g for MP plants in 15 d, while at 30 days it was 6.132, 6.36 and 5.218mg/g respectively in leaves, shoot, and roots (Table 2).

Estimation of proteins

Results showed that the total proteins content in the leaves, increases at 15 d significantly (20.53% in leaves and 24.18% in shoot) while considerable decrease (20.26%) at 30 d whereas in shoots for second phase analysis of *Momordica charantia* showed 5.138 % decrease in mycorrhizal seedlings than NMP seedlings. The total proteins content in roots was recorded as 0.691, 0.281 in 15d and 30 days respectively over control and showed 37.36% decline in 30 d (table 3).

Estimation of total amino acids

The total amino acids in leaves, shoot and roots are 0.135, 0.096 and 0.095 respectively in control plants, which were reduced in leaves and shoot while increases in roots of MP in 15d. On the other hand there was non-significant decrease in leaves, shoot and root after 30 d as shown in the table 3.

Estimation of protease

Results of protease activity in MP showed that protease activity significantly increases in the leaves in both phases (33.33% and 10.51%), whereas deceases in the shoot and in the roots. It was decreases non-significantly in comparison over control in two phases analysis of MP (table 3).

Estimation of proline

The proline contents in present study recorded the maximum increase in leaves (46.55%) in 15 d and

11.31% in 30 d over control while non-significant increase in shoots and roots of MP plants (table 3).

DISCUSSION

The function of soil is to provide the physical support for enlargement of progression of the plant root system via as the reservoir of water and nutrients, which are essential for plant growth. The VAM Fungi (AMF) is popular to increasing the root length of plant species by transferring the insoluble soil nutrients.

Carbohydrate and N metabolism

Various carbohydrate contents like, sucrose, total soluble sugars and total carbohydrate were analyzed in the plants grown in absence and presence of VAM spores (table 2). Carbon uptake by VAM is a common well-known phenomenon, which was observed in this investigation during analysis of carbohydrates contents. As VAM firmly fixed with the roots of plants therefore it was suggested that C uptake may probably through carbohydrate or dextroses take place from cell wall through penetration (Douds *et al*., 2000; Berta *et al*., 2000).

Results reported in the table 2 showed that sucrose, total soluble sugars and total carbohydrate were affected by VAM during dual symbioses between VAM and plants. This indicated that enlargement of VAM hypahe was on the cost of C from carbohydrate contents due to which these contents were decline in the roots (Berta *et al*., 2000). It may be attributed for the plants survival where rapid transfer of these contents from areal part to the underground part due to which it was higher in shoot in 2nd phase analysis. This non-significant decrease in carbohydrate, sucrose, and total soluble sugars contents may be the consequence of an increase in the surface area of leaf of VAM treated plants for increase in the photosynthetic processes that may overcome the continuous uptake of C by VAM through roots (Azmat 2013a). It was strongly suggested that the increase in the surface area of leaves were the survival strategy under VAM stress which was adopted by the plants for increases in the rate of formation of carbohydrate contents to provide energy consciously (Azmat 2013b).

Soluble carbohydrate accumulation, amino acid, proline, proteins, protease enzyme activity are usually biochemical factors commonly applied in researches on plant defensive alteration. The difference in these biochemical parameters in between MP and NM plants usually recommend greater drought resistance in the VAM plants. Like, decline accretion of total soluble sugars (Table 2) shows that plants evade lack conditions more effectively and therefore it will need less osmotically adjustment or symplasm or osmoprotect enzymes, or displays fewer stress or harm (Azcón-Aguilar *et al*., 1999). Total soluble sugar (TSS) content is the chief aid of the plants to regulate the carbohydrate metabolism and temporary storage in relation to processes of synthesis of sugar molecule production. The quantity of TTS content was a symbol of the source capability of plants leaves and reproduced alteration and ability of particles to use integrates. The non-significant decrease in TSS indicates that the larger leaf area responsible for increase in photosynthesis, which in turns related with the regulation of plants under stress through continuous production of TSS.

Sucrose is a key final product of photosynthesis that functions is a major transference of sugar and indirect regulator of gene expression in certain circumstances. The increase in the sucrose contents in roots of VAM plants was established as a support of the growth regulation of plants under stress (Winter & Huber 2000). Proline is an amino acid produced in stress condition to overcome the stress. In VAM plants proline contents increases in leaves, shoots and roots. It may be related to that proline prevents oxidation of cells from inside and also normalizes osmotic pressure of plant under drought stress for absorbing water (Frey & Schüepp 1992). The decreases in protein contents showed that proteins are highly sensitive towards stress or it may be hydrolyzed and converted into carbohydrates for survival of plants. This fact is also supported by an increase in protease activity in the leaves while in shoot and root activity is less which may be due to autolysis or cleaved in stress condition (table 3).

Mechanism of probable chemisorption from dextroses

Results reported in this article suggest that interaction of VAM with the plant root is probably due to the exist biosorption mechanism where the mycorrhiza physically sorbed at the surface of root and developed their hyphae by taking C from the surface of roots which was made up of cellulose (a polymer, glucose in nature). Fig. 1 showed a possible picture of adsorption.

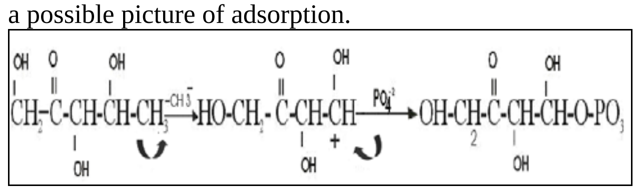

Fig. 1: A probable proposed mechanism for the exchange of Carbon and PO4 by VAM

And can be explained by two ways. It might be debated that growth differences in the non-exodermal and exothermal species reflects dependence of species on absorption of mineral nutrients with the help of fungal partner (Azmat 2013a). Mycorrhizas are an extensive link among plant roots and VAM neighborhood to the soil. The best common morphotype are the vesicular arbuscular mycorrhiza (VAM) that looks to have been substantial in the entrance of plants to the land probable attending as adsorptive structures formerly the expansion of true roots in host plants (figs. 2-3) (Azmat 2013b).

Table 1: Effect of VAM on physicomorphological parameters of *Momordica charantia* (30d)

Sample	Total length of plsnt (cm)	Total Fresh Weight of plant. (mg)	Total Dry weight (mg)	Total Water content	Relative growth Rate	Fw/Dw
Control	156.00±21.23	81.60±14.2	22.343±8.56	59.257±21.01	0.265	4.679
VAM	158.30±19.31	109.20±16.5	23.335±6.23	85.865±25.25	0.0863	3.652

Table 2: Effect of VAM on sugars contents of *Momordica charantia*

Days	Sample	Concentration in Leaves 102 (µg/0.5gm leaves)	Concentration in Shoots 102 (µg/0.5gm shoot)	Concentration in Roots 102 (µg/0.2gm root)
Carbohydrates				
15	CONTROL	6.262±0.089	5.105±0.089	5.412±0.058
	VAM	6.384±0.079	4.998±0.056	5.112±0.056
	%	1.91	-2.14	-6.02
30	CONTROL	5.693±0.087	5.381±0.026	5.177±0.024
	VAM	5.225±0.087	5.515±0.045	5.015±0.045
	%	8.95	2.42	3.23
Sucrose				
15	CONTROL	5.287±0.099	6.718±0.058	5.162±0.058
	VAM	5.636±0.047	6.151±0.023	5.329±0.087
	%	6.19	-9.21	3.13
30	CONTROL	5.779±0.082	5.806±0.036	5.043±0.096
	VAM	5.682±0.023	5.703±0.045	5.236±0.098
	%	1.707	1.806	3.68
Total soluble Sugar				
15	CONTROL	6.408±0.087	6.896±0.096	6.248±0.054
	VAM	6.077±0.085	6.365±0.085	6.117±0.056
	%	-5.446	-8.342	-2.141
30	CONTROL	6.391±0.089	6.068±0.087	5.493±0.054
	VAM	6.132±0.026	6.136±0.088	5.218±0.025
	%	-4.223	1.1206	-5.270

Table 3: Effect of VAM inoculation on nitrogen metabolism of *Momordica charantia* under VAM

Days	Sample	Concentration in Leaves 10^2 (µg/0.5gm leaves)	Concentration in Shoots 10^2 (µg/0.5gm shoot)	Concentration in Roots 10^2 (µg/0.2gm root)
Protein				
15	CONTROL	4.891±0.059	1.006±0.045	0.831±0.021
	VAM	6.155±0.098	1.327±0.098	0.691±0.023
	%	20.53	24.18	-20.26
30	CONTROL	1.270±0.069	0.532±0.059	0.386±0.065
	VAM	1.034±0.058	0.506±0.063	0.281±0.059
	%	-22.82	-5.138	-37.36
Amino Acid				
15	CONTROL	0.135±0.023	0.096±0.089	0.095±0.069
	VAM	0.122±0.059	0.076±0.059	0.103±0.012
	%	-10.65	-26.31	7.766
20	CONTROL	0.160±0.012	0.151±0.099	0.152±0.020
	VAM	0.155±0.069	0.150±0.069	0.150±0.012
	%	-3.225	-0.666	-1.333
Proline				
15	CONTROL	0.0079±0.012	0.0069±0.015	0.0041±0.058
	VAM	0.0130±0.059	0.0074±0.013	0.0041±0.012
	%	46.55	6.756	0
20	CONTROL	0.0870±0.013	0.0670±0.059	0.0735±0.014
	VAM	0.0981±0.014	0.0826±0.06	0.0798±0.059
	%	11.31	18.886	7.984
Protease				
15	CONTROL	0.675±0.023	0.479±0.041	0.425±0.025
	VAM	1.012±0.042	0.401±0.031	0.384±0.031
	%	33.33	-19.45	-10.67
20	CONTROL	1.132±0.014	1.489±0.021	0.903±0.021
	VAM	1.265±0.036	0.981±0.01	0.868±0.021
	%	10.51	-51.78	-4.032

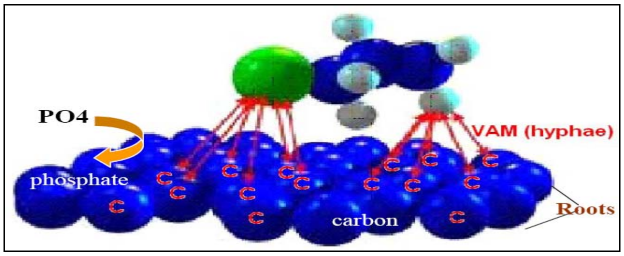

Fig. 2: Exchange of carbon and PO4 by VAM during adsorption

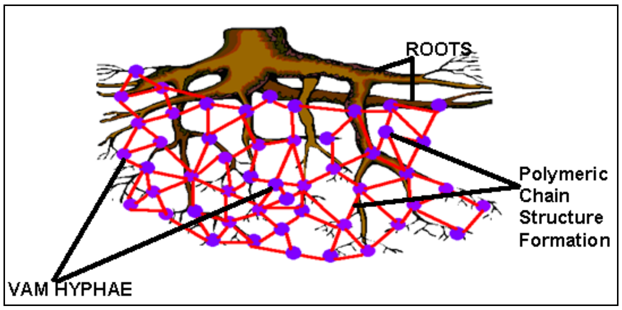

Fig. 3: A polymeric Network formation around a root sphere by a VAM.

Vesicles and arbuscules which are present within the plants roots showed links of VAM and possible building and secretion of some metabolites (fig. 3). The primary mechanism for growth stimulation in VAM plants is the enhancement of uptake of phosphorus and other nutrients by fungal hyphae. A greater rate of nutrient absorption by the plant is well explained by the adsorption of C and in place of C the other nutrients may take part or fix at the place of C which results in the increase leaf area and the development of lateral roots, as observed earlier (Finlay & Read 1986; Blumenthal 1976). However, mycorrhizal assistances may not be restricted to enriched nutrient endorsement and synthesis of plant hormones by dual symbiosis but also effects on plant metabolic process. The morphological and physiological changes that microbial plant hormones can induce in the host plant may favor the establishment of VAM symbiosis and its activity, thus leading to enhancement in growth. In fact, it is known that gibberellins that cytokinins are involved in many basic processes of plant growth, including improvement of photosynthetic rate, and that auxins control root formation and increase elasticity of the cell wall (figs. 2-3). The absorption of these metabolites indicates that *Momordica charantia* does not accumulate more metabolites or degradation of these metabolites in presence of VAM because an increase in proline and protease content showed that plant is in stress because protease hydrolyzed protein and other contents and proline increases to absorb hydroxyl radical but the (table 2 and fig. 2) estimated that, in roots all the metabolites increasing with decreased in protein. This was definitely related with VAM C up taking through the roots that disturb normal metabolic pathway of the plant. Finally on the basis of the above results we suggest that VAM has a symbiotic relation with other plants but not with *Momordica charantia* because it utilizes the carbon at the cost of sugars and proteins and the results in the enhanced proline and protease activity. This utilization of C by VAM from sugar contents weakens the plant instead strengthen the plant via altered metabolic processes. It was suggested that C uptake by fungus through root surface involve the process of biosorption (Adsorption). It is a surface phenomenon by which “atoms, molecules or ions are retained on the surfaces of solids by chemical or physical bonding and desorbed also”. The desorption of C from cell wall surface may be referred as C up taking by VAM. As Blumenthal and Lagunas (1976) reported that there is no evidence how carbon transform in VAM through plant, we strongly recommend that the only way that how this transformation mechanism is taking place was through the “live or kinetic Biosorption or chemisorption which is type of “ADSORPTION”.

CONCLUSION

It was concluded that the C uptake by the VAM fungi was mainly from sugar compounds for building up of their hyphal network through phenomena of adsorption where C is attached to the fungal hyphae via exchange of nutrients or desorption of C from root surface and empty place covered by other important nutrient ions

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