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Biochemical Assessment on Three Medicinal Plants with Inoculation of Arbuscular Mycorrhiza and Growth Regulators

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Abstract: The interactive potential benefits of inoculation with arbuscular mycorrhizal fungi (AMF) *Glomus fasciculatum* (Thax. Sensu Gerd.) Gerd. And Trappe to three medicinal plants such as Nirgandi (*Vitex negundo* L), Henna (*Lawsonia inermis* L) and Copper leaf (*Acalypha wilkesiana* L)., with IBA in the presence of NAA (250 ppm) conducted in earthen pots in completely randomized block design with three replications and the plants were uprooted periodically 30, 60 and 90 days. Root samples for each harvest were analyzed for total protein and sugar concentration.

Key words: Arbuscular Mycorrhizal Fungi (AMF) · Indol-3 butyric acid (IBA) · 1 naphthalene acetic acid (NAA) · Protein

INTRODUCTION

Successful adventitious rooting during cutting propagation depends on several factors, including the physiological condition of the propagation stock plants and the environmental conditions during adventitious root formation [1].

Many changes in metabolism are known to occur during adventitious root formation including changes in proteins and carbohydrate [2]. The response to exogenous application of auxins role in metabolic changes like (viz., specific enzyme, carbohydrate, RNA, DNA, protein metabolism etc) during the initiation, emergence and development of root primordial in the cuttings rooting zone. Attempt have been made by a number of researchers to envisage the macro molecular changes during adventitious roots in different plant species in order to better understand the underlying physiological and biochemistry [3-8].Carbohydrate concentration in cuttings were play a role in regulating part of the response of cutting to AMF, differences to AMF or hormone application.

The photosynthates of the plant (apparently not specific metabolites) are absorbed by the AM fungus in the root, especially in arbuscles where the surface contact between the fungus and the host plant is very large. The still unidentified possible physiological mechanisms of carbohydrate movement from the host plant to the fungus and the carbohydrate transport in the fungus structure are discussed by Dexheimer [9].

Proteins are the most abundant biological macromolecules occurring in all parts of the cells and it also occur in great variety. Thousands of different kinds, ranging in size from relatively small peptides to huge polymers with molecular weights in the millions may be found in single cell. Moreover, proteins exhibit enormous diversity of biological function and are the most important final product of the pathways. Proteins are the molecular instruments through which genetic information is expressed. Hydrolyzing proteins and estimating the amino acids alone will give the exact quantification.

MATERIALS AND METHODS

The experiment was conducted under mist chamber using sterile soil to know the effect of arbuscular mycorrhizal fungi on the growth and yield of three medicinal plants such as Nirgandi (*Vitex negundo* L), Henna (*Lawsonia inermis* L) and Copper leaf (*Acalypha wilkesiana* L). Root cuttings were collected in plastic bags early in the morning from UAS Saidapur nursery, Dharwad Karnataka India. The cuttings selected for the experiments are free from disease and pest immediately placed them in coolers containing ice, while collecting cuttings it was taken carefully and they can be stored in a refrigerator until needed. Cuttings were sorted for

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uniformity (based on length), 20 cm long stem cuttings were used in present study, planted in earthen pots measuring 27 cm diameter, pots containing sterilized 5 kg soil and sand (3:1 v/v).

Arbuscular mycorrhizal fungi (*Glomus fasciculatum*) was multiplied by using Jowar (Sorghum vulgare pers) as host plant. The pot measuring 34 cm diameter containing 8.5 kg soil. 15 g of air dried AM fungi inoculum of *Glomus fasciculatum* was given to each pot as a thin layer, 2 cm bellow the soil surface except uninoculated (control*)*. The inoculum consisted of 8g rhizospheric soil (100 Chlamydospore / 50 g soil approximately) and 2g of root bits of host plant with hyphae and sporocarps.

Experimental Set up

Uninoculated (Control)
Glomus fasciculatum
IBA (250 ppm)
IBA (250 ppm) + *Glomus fasciculatum*
NAA (250 ppm)
NAA (250 ppm) + *Glomus fasciculatum*
IBA (125 ppm) + NAA (125 ppm)
IBA (125) ppm + NAA (125 ppm)+ *Glomus fasciculatum*

The basal portion of the stem cuttings were dipped for 1 min in 250 mg/l (IBA and NAA) [10], then placed into pots containing the same soil sand (3:1 v/v). The layout of the experiment was randomized complete blocks design with three triplicate for each treatment in mist chamber.10 ml of Hoagland solution [11] without P was treated for each plant at a interval of 15 days. There were treated i.e inoculated and non-inoculated (control) to a three varieties of medicinal plants, experimental pots were kept free of weeds. The observations were recorded at a period of 60,120 and 180 days after planted.

Root samples for each harvest were analyzed for total protein and sugar concentration. Total soluble protein was determined by UV spectrophotometer (Coomassie brilliant blue [12] and the wavelength is 496nm. Total soluble sugar content of samples was determined by UV spectrophotometer using phenol sulphuric method [13], and the wavelength is 596nm.

Chemicals and Glassware: The chemicals used during the course of study were of analytical grade. Glucose, phosphate buffer, sulphuric method, bovine serum albumin (BSA) solutions, Bradford reagent, growth regulators like Indole-3- butyric acid (IBA), 1 naphthalene acetic acid (NAA). Glass wares wer procured from Borosil, Mumbai, India and they were autoclaved before use.

Estimation of Carbohydrate: The carbohydrate was estimated by Phenol sulphuric method.

- Weigh 1 g of sample; macerate the sample in pestle mortar in 5ml of phosphate buffer and transfer the homogenate at 8000 rpm for 20 min.
- Collect the supernatant and repeat the extraction 4-5 times, combine the supernatants and make the volume to 50 ml with distil water.
- To 2ml of the standard solution (containing 10-100ìg of sugar) or appropriately diluted unknown sugar sample solution add 1ml of phenol reagent, followed by rapid addition of 5 ml of concentrated sulphuric acid, using a burette or a pro-pipette and vortex.
- After 30 min read the yellowish-orange colour developed against reagent blank at A 490 nm in a UV-spectrometer and recorded the absorbance.
- Constructed a calibration curve on a graph paper, by plotting the sugar concentration (10-100ig) on the y-axis.

Calculation: On the basis of standard graph, the amount of protein in mg/g or 100g of sample was calculated as shown in (Table 1, 2, 3).

Estimation of Protein: The protein was estimated by Bradford method.

- Weigh 1 g of sample; macerate the sample in pestle mortar in 5ml of phosphate buffer and transfer the homogenate at 8000 rpm for 20 min.
- Collect the supernatant and repeat the extraction 4-5 times, combine the supernatants and make the volume to 50 ml with phosphate buffer.
- Take 0.1 ml of sample solution and make the volume to 1 ml with 0.1 m Phosphate buffer (PH 7.5).
- Pipette appropriate aliquots of bovine serum albumin (BSA) solutions containing 0-100 ìg protein.
- Make the volume to 1 ml with 0.1 m Phosphate buffer (PH 7.5) in all the tubes. Add 5ml of Bradford reagent to all the tubes and mix thoroughly.
- Record the absorbance at 595 nm against the reagent blank. Plot a standard curve of A 595 versus ìg of protein in the standard.
- Determine the protein content in the sample extract from the standard curve. Calculate the amount of protein per ml of the sample preparation.

Table 1: Showing an effect of AM fungi *Glomus fasciculatum* (Taapp Gerd andTrapp). Gerd with hormone treatment on biochemical reactions in *Vitex negundo* L., for 60,120 and 180 days

	Protein in root (mg/g)	Soluble sugars in root (mg/g)
60 days		
CN	0.15±0.02e	0.83±0.10e
Gf	0.47±0.01b	1.70±0.11bc
IBA	0.35±0.01c	1.20±0.11d
IBA+ *Gf*	0.44±0.02b	1.53±0.08c
NAA	0.23±0.00d	0.52±0.00f
NAA+ *Gf*	0.26±0.00d	1.61±0.00bc
IBA+NAA	0.32±0.01c	1.81±0.00b
IBA+NAA+*Gf*	0.65±0.01a	2.51±0.00a
120 days		
CN	0.95±0.01f	1.44±0.01f
Gf	1.52±0.01b	3.10±0.04b
IBA	1.05±0.01b	1.64±0.01e
IBA+ *Gf*	1.56±0.00b	1.74±0.01de
NAA	0.97±0.00f	1.51±0.00f
NAA+ *Gf*	1.24±0.00d	1.85±0.00d
IBA+NAA	1.34±0.02c	2.04±0.01c
IBA+NAA+*Gf*	2.23±0.01a	3.71±0.09a
180 days		
CN	0.77±0.01f	0.50±0.00g
Gf	0.96±0.00e	0.60±0.00f
IBA	1.55±0.01c	0.57±0.01f
IBA+ *Gf*	1.19±0.09d	1.82±0.00c
NAA	0.90±0.00ef	0.64±0.01e
NAA+ *Gf*	0.95±0.00e	0.91±0.00d
IBA+NAA	1.14±0.06b	2.52±0.00b
IBA+NAA+*Gf*	3.25±0.03a	4.03±0.02a

CN - Control, *Gf- Glomus fasciculatum,* IBA - Indol-3 butyric acid,IBA+ *Gf-* Indol-3 butyric acid + *Glomus fasciculatum,* NAA -1 naphthalene acetic acid+ *Glomus fasciculatum*, NAA+ *Gf*-1 naphthalene acetic acid+ *Glomus fasciculatum,* IBA+NAA - Indol-3 butyric acid+ 1 naphthalene acetic acid + *Glomus fasciculatum*, IBA+NAA+ *Gf-* Indol-3 butyric acid+1 naphthalene acetic acid+ *Glomus fasciculatum.* Data represents means ± SE of 3 replicates; each experiment was repeated thrice. Mean separation within column by Duncan's multiple range test at $P < 0.05$.

Table 2: Showing an effect of AM fungi *Glomus fasciculatum* (Taapp Gerd andTrapp). Gerd with hormone treatment on biochemical reactions in *Lawsonia inermis* L., for 60,120 and 180 days

	Protein in root (mg/g)	Soluble sugars in root (mg/g)
60 days		
CN	0.08±0.08dc	0.36±0.01g
Gf	0.70±0.05dc	0.67±0.01e
IBA	0.12±0.00b	0.42±0.01f
IBA+ *Gf*	0.27±0.00c	0.85±0.01c
NAA	0.05±0.00e	0.36±0.01g
NAA+ *Gf*	0.05±0.00e	0.73±0.02d
IBA+NAA	0.16±0.00d	0.95±0.01b
IBA+NAA+*Gf*	0.97±0.00a	1.75±0.02a
120 days		
CN	0.54±0.01h	0.83±0.02h
Gf	0.96±0.00e	1.07±0.02b
IBA	0.75±0.02f	0.95±0.01g
IBA+ *Gf*	1.56±0.01b	2.44±0.02f
NAA	0.67±0.01g	1.26±0.01e
NAA+ *Gf*	1.26±0.00d	1.69±0.00d
IBA+NAA	1.43±0.02c	2.07±0.02c
IBA+NAA+*Gf*	2.74±0.01a	3.26±0.01a
180 days		
CN	0.95±0.01f	1.08±0.04f
Gf	1.35±0.01d	1.92±0.02d
IBA	1.08±0.02e	1.27±0.01e
IBA+ *Gf*	1.96±0.00b	2.55±0.02c
NAA	1.05±0.01e	1.07±0.03f
NAA+ *Gf*	1.86±0.01c	1.86±0.01d
IBA+NAA	1.97±0.01b	2.84±0.01b
IBA+NAA+*Gf*	3.55±0.01a	4.54±0.02a

CN - Control, *Gf- Glomus fasciculatum,* IBA - Indol-3 butyric acid,IBA+ *Gf-* Indol-3 butyric acid + *Glomus fasciculatum,* NAA -1 naphthalene acetic acid+ *Glomus fasciculatum*, NAA+ *Gf*-1 naphthalene acetic acid+ *Glomus fasciculatum,* IBA+NAA - Indol-3 butyric acid+ 1 naphthalene acetic acid + *Glomus fasciculatum*, IBA+NAA+ *Gf-* Indol-3 butyric acid+1 naphthalene acetic acid+ *Glomus fasciculatum.* Data represents means ± SE of 3 replicates; each experiment was repeated thrice. Mean separation within column by Duncan's multiple range test at $P < 0.05$.

Table 3: Showing an effect of AM fungi *Glomus fasciculatum* (Taapp Gerd andTrapp). Gerd with hormone treatment on biochemical reactions in *Acalypha wilkesiana* L., for 60,120 and 180 days

	Protein in root (mg/g)	Soluble sugars in root (mg/g)
60 days		
CN	0.16±0.01c	0.55±0.01bc
Gf	0.25±0.01b	0.38±0.31a
IBA	0.17±0.01c	0.75±0.01abc
IBA+ *Gf*	0.24±0.02b	0.94±0.01a
NAA	0.16±0.00c	0.47±0.01bc
NAA+ *Gf*	0.24±0.02b	0.65±0.00abc
IBA+NAA	0.23±0.02b	0.82±0.01ab
IBA+NAA+*Gf*	0.36±0.00a	0.94±0.02a
120 days		
CN	0.15±0.02f	0.84±0.01h
Gf	0.27±0.01e	1.66±0.01d
IBA	0.25±0.02e	1.06±0.02f
IBA+ *Gf*	0.56±0.01h	2.95±0.02b
NAA	0.35±0.02d	0.94±0.00g
NAA+ *Gf*	0.45±0.01c	1.55±0.02e
IBA+NAA	0.56±0.00b	1.94±0.01c
IBA+NAA+*Gf*	0.96±0.02a	3.26±0.01a
180 days		
CN	0.27±0.00f	1.35±0.02f
Gf	0.94±0.01d	3.07±0.01c
IBA	0.94±0.02d	1.95±0.01e
IBA+ *Gf*	1.61±0.01b	2.24±0.02d
NAA	0.95±0.02d	0.94±0.01f
NAA+ *Gf*	0.74±0.00e	1.96±0.00e
IBA+NAA	1.36±0.02b	3.27±0.01b
IBA+NAA+*Gf*	1.69±0.01a	4.16±0.01a

CN - Control, *Gf- Glomus fasciculatum,* IBA - Indol-3 butyric acid,IBA+ *Gf-* Indol-3 butyric acid + *Glomus fasciculatum,* NAA -1 naphthalene acetic acid+ *Glomus fasciculatum*, NAA+ *Gf*-1 naphthalene acetic acid+ *Glomus fasciculatum,* IBA+NAA - Indol-3 butyric acid+ 1 naphthalene acetic acid + *Glomus fasciculatum*, IBA+NAA+ *Gf-* Indol-3 butyric acid+1 naphthalene acetic acid+ *Glomus fasciculatum.* Data represents means ± SE of 3 replicates; each experiment was repeated thrice. Mean separation within column by Duncan's multiple range test at $P < 0.05$.

Standard error of the mean was calculated and the 'mean' values were assessed for their significance.

Calculation: On the basis of standard graph, the amount of protein in mg/g or 100g of sample was calculated as shown in (Table 1, 2, 3).

RESULT

Protein content of roots accounted for less than 10% of the total protein in cuttings, but cuttings inoculated with AMF had a higher proportion of total protein in roots than non-inoculated treatments. The inoculation of *Glomus fasciculatum* was higher protein content compare to single hormone treatment.

In all the experimental plants, treatment of IBA with inoculation of *Glomus fasciculatum* had a higher proportion of protein content compare to treatment of IBA alone. IBA had a higher proportion of protein content compare to NAA. Treatment of IBA and NAA had a higher proportion of protein content compare to treated with single hormone IBA or NAA. Inoculation with *Glomus fasciculatum* had a higher proportion of protein content compare to NAA with *Glomus fasciculatum,* IBA and NAA. NAA with *Glomus fasciculatum* had a higher proportion of protein content compare to NAA and, IBA and NAA. The maximum protein content was recorded in cuttings treated with IBA, NAA with inoculation *Glomus fasciculatum.*

After 60 days minimum protein content was recorded in *vitex negundo* L., IBA with inoculation of *Glomus fasciculatum* had a higher proportion of protein content (0.65 mg/g) compare to treatment of IBA alone (0.15 mg/g). IBA had a higher proportion of protein content (0.23 mg/g) compare to NAA(0.15 mg/g). Treatment of IBA and NAA had a higher proportion of protein content (0.44 mg/g) compare to treated with single hormone IBA (0.23 mg/g) or NAA (0.15 mg/g). NAA with *Glomus fasciculatum* had a higher proportion of protein content (0.26 mg/g) compare to NAA. The maximum protein content was recorded in cuttings treated with IBA, NAA with inoculation *Glomus fasciculatum* (0.47 mg/g).

After 120 days the unioculated (control) cuttings exhibited minimum protein content (0.67 mg/g) in the Rooting zone. Treatment with IBA or NAA in combination with *Glomus fasciculatum* the highest level of protein content was recorded compare to single hormone treatment. IBA with inoculation of *Glomus fasciculatum*

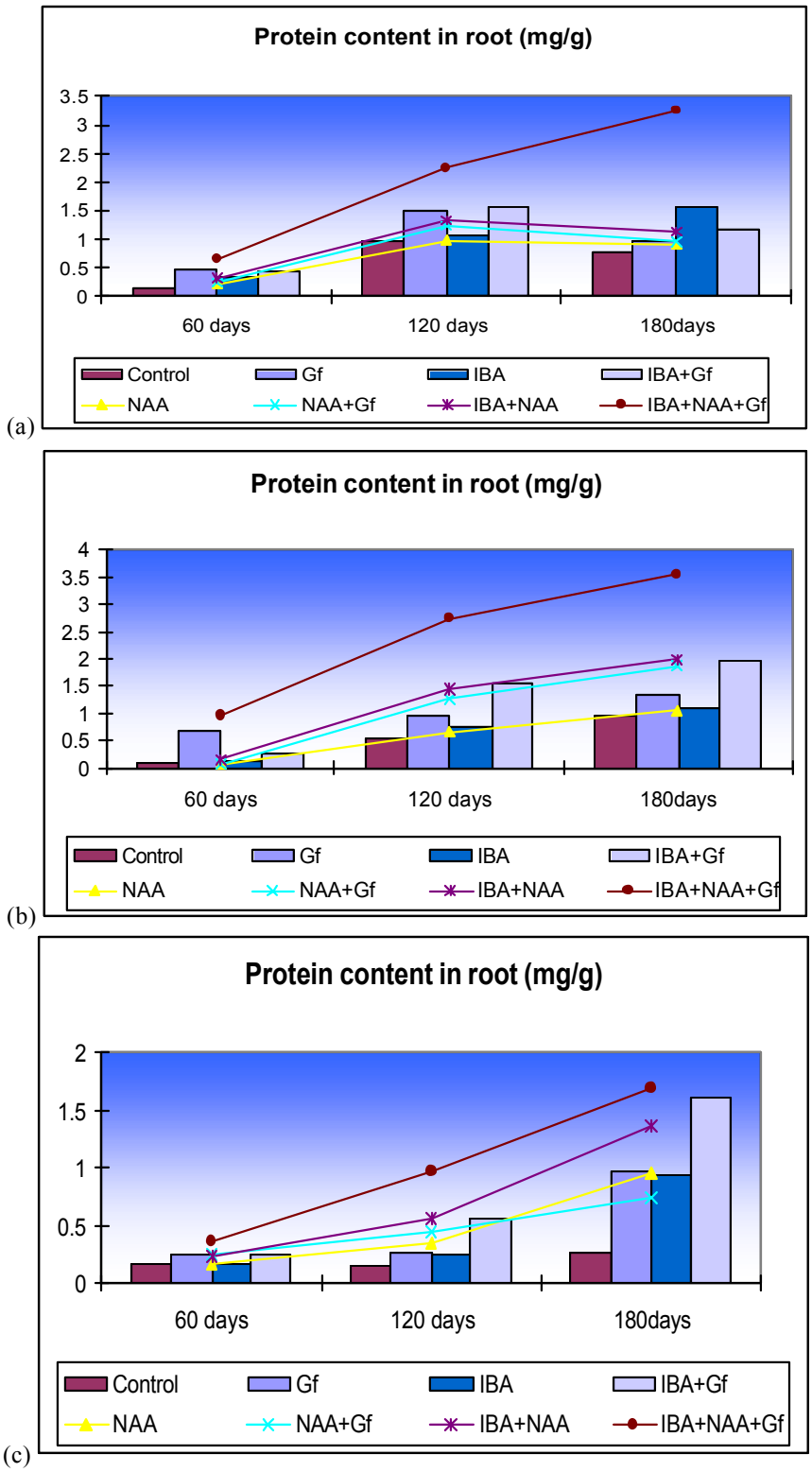

Fig. 1: Showing the effect AMF (*Glomus fasciculatum*) and hormone treatment of protein content in (a).*Vitex negundoL.*, (b).*Lawsonia inermis* L., and (c).*Acalypha wilkesiana* L.

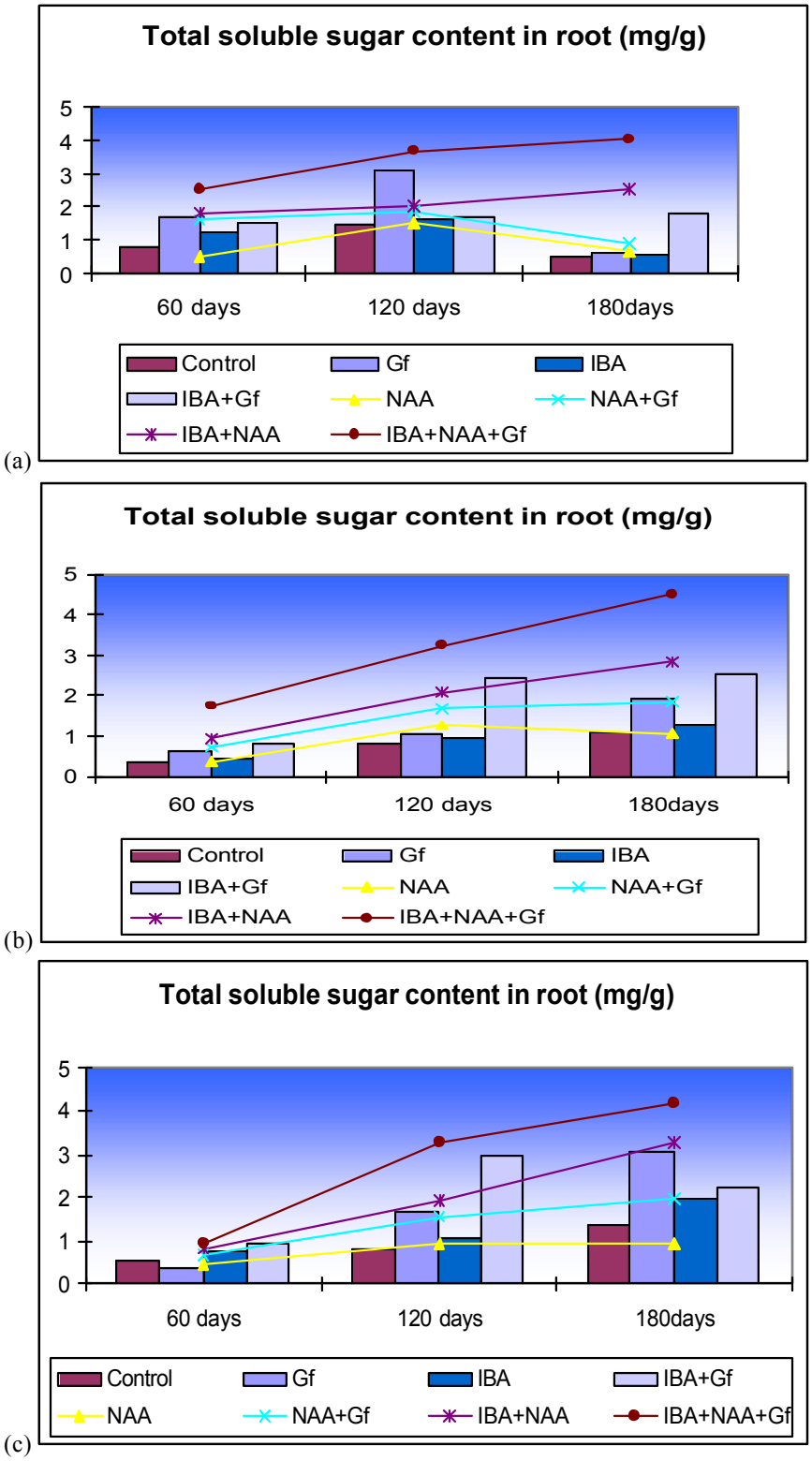

Fig. 2: Showing the effect AMF (*Glomus fasciculatum*) and hormone treatment of total soluble sugar content in (a).*Vitex negundoL.*, (b).*Lawsonia inermis* L., and (c).*Acalypha wilkesiana* L.

had higher protein (1.24 mg/g) content compare to NAA with inoculation of *Glomus fasciculatum* (1.05 mg/g), treated with combination of hormone IBA, NAA and treated with hormone alone (Table 1).

After 180 days the maximum protein content was recorded in *vitex negundo* L., plant cuttings. The highest level of protein content were recorded in treated with combination of hormone with inoculation of *Glomus fasciculatum* compare to all the treatments,*Glomus fasciculatum* (0.77 mg/g), IBA(1.19 mg/g), IBA with *Glomus fasciculatum*(1.55 mg/g), NAA(0.90 mg/g), NAA with *Glomus fasciculatum* (0.96 mg/g), IBA and NAA (1.14 mg/g) (Table 1).

After 60 days minimum protein content was recorded in *Lawsonia inermis* L., IBA with inoculation of *Glomus fasciculatum* had a higher proportion of protein content (0.70 mg/g) compare to treatment of IBA alone(0.12 mg/g). IBA had a higher proportion of protein content (0.12 mg/g) compare to NAA (0.05 mg/g). Treatment of IBA and NAA had a higher proportion of protein content (0.16 mg/g) compare to treated with single hormone IBA or NAA (Fig. 1). NAA with *Glomus fasciculatum* had a higher proportion of protein content (0.18 mg/g) compare to NAA (0.05 mg/g) and, IBA and NAA(0.16 mg/g). The maximum protein content was recorded in cuttings treated with IBA, NAA with inoculation *Glomus fasciculatum* (0.97 mg/g).

Even after 120 days the uninoculated (control) cuttings exhibited minimum protein content (0.54 mg/g) in the Rooting zone. Treatment with IBA or NAA in combination with *Glomus fasciculatum* the highest level of protein content was recorded compare to single hormone treatment. IBA with inoculation of *Glomus fasciculatum* had higher protein (1.56 mg/g) content compare to NAA with inoculation of *Glomus fasciculatum* (1.26 mg/g), treated with combination of hormone IBA, NAA and treated with hormone alone shown in (Table 2).

After 180 days the maximum protein content was recorded in *Lawsonia inermis* L., plant cuttings. The highest level of protein content were recorded in treated with combination of hormone with inoculation of *Glomus fasciculatum* (3.55 mg/g) compare to all the treatment such as *Glomus fasciculatum* (1.35 mg/g), IBA(1.08 mg/g), IBA with *Glomus fasciculatum*(1.96 mg/g), NAA(1.05), NAA with *Glomus fasciculatum* (1.86 mg/g), IBA and NAA (Table 2).

After 60 days minimum protein content was recorded in *Acalypha wilksiana* L., IBA with inoculation of *Glomus fasciculatum* had a higher proportion of protein content (0.24 mg/g)compare to treatment of IBA alone(0.17 mg/g). IBA had a higher proportion of protein content compare to NAA(0.14 mg/g). Treatment of IBA and NAA had a higher proportion of protein content (0.23 mg/g) compare to treated with single hormone IBA or NAA. NAA with *Glomus fasciculatum* had a higher proportion of protein content (0.20 mg/g) compare to NAA and, IBA and NAA. The maximum protein content was recorded in cuttings treated with IBA, NAA with inoculation *Glomus fasciculatum* (0.36 mg/g).

After 120 days the unioculated (control) cuttings exhibited minimum protein content (0.15 mg/g) in the Rooting zone. Treatment with IBA or NAA in combination with *Glomus fasciculatum* the highest level of protein content was recorded compare to single hormone treatment. IBA with inoculation of *Glomus fasciculatum* had higher protein (0.56 mg/g) content compare to NAA with inoculation of *Glomus fasciculatum* (0.45 mg/g), treated with combination of hormone IBA, NAA and treated with hormone alone shown in (Table 3).

After 180 days the maximum protein content was recorded in *Acalypha wilkesiana* L., plant cuttings. The highest level of protein content were recorded in treated with combination of hormone with inoculation of *Glomus fasciculatum* (1.69 mg/g) compare to all the treatments such as *Glomus fasciculatum* (0.14 mg/g), IBA(0.94 mg/g), IBA with *Glomus fasciculatum*(1.61 mg/g), NAA(0.74 mg/g), NAA with *Glomus fasciculatum* (0.95 mg/g), IBA and NAA (Table 3).

Concentration of soluble sugars in roots were significantly lower in control at 60 days. The inoculation of *Glomus fasciculatum* was higher carbohydrate content compare to single hormone treatment.

In *Vitex negundo* L., AMF treatments had higher proportion of total soluble sugars in roots (1.70 mg/g) than non inoculated cuttings. IBA had a higher proportion of total soluble sugars (1.20 mg/g) compare to NAA (0.52 mg/g). IBA and NAA treated cuttings exhibited higher total soluble sugar content (1.81 mg/g) than the treated with IBA or NAA alone. IBA with inoculation *Glomus fasciculatum* had higher soluble sugar content (1.53 mg/g) compare to IBA, and NAA with inoculation of *Glomus fasciculatum*(1.61). NAA with inoculation of *Glomus fasciculatum* had higher total soluble sugar content(1.61 mg/g) compare to IBA with NAA(1.81 mg/g) and NAA alone. The total soluble sugars content was observed in the treatment of IBA and NAA with inoculation of *Glomus fasciculatum* (2.51 mg/g).

After 120 days the variation in total soluble sugar content. The highest total soluble sugar content the higher total sugar to content was recorded in cuttings.

Inoculated with *Glomus fasciculatum* (3.10 mg/g) followed by those of uninoculated cuttings (1.44 mg/g) and treated with IBA (1.64). Treated with IBA and NAA with inoculation of *Glomus fasciculatum* had a maximum total soluble sugar content (3.71 mg/g) compared to the treated IBA with inoculation of *Glomus fasciculatum* (1.74 mg/g) and NAA with inoculation of *Glomus fasciculatum* (1.85 mg/g).

The maximum total soluble sugar was recorded in all the cuttings at 180 days treated IBA and NAA with inoculation of *Glomus fasciculatum* (4.03 mg/g) had a higher proportion of total soluble sugar compare to all the treatment and the lowest (0.50 mg/g) in uninoculated cuttings shown in (Tables 1).

In *Lawsonia inermis* L., AMF treatments had higher proportion of total soluble sugars in roots (0.67 mg/g) than non inoculated cuttings. IBA had a higher proportion of total soluble sugars (0.42 mg/g) compare to NAA (0.36 mg/g). IBA and NAA treated cuttings exhibited higher total soluble sugar content (0.95 mg/g) than the treated with IBA or NAA alone. IBA with inoculation *Glomus fasciculatum* had higher soluble sugar content (0.85 mg/g) compare to IBA (0.42 mg/g), and NAA with inoculation of *Glomus fasciculatum* (0.73 mg/g). NAA with inoculation of *Glomus fasciculatum* had higher total soluble sugar content (0.73 mg/g) compare to IBA with NAA and NAA alone. The total soluble sugars content was observed in the treatment of IBA and NAA with inoculation of *Glomus fasciculatum* (1.75 mg/g).

After 120 days the variation in total soluble sugar content. The highest total soluble sugar content the higher total sugar to content was recorded in cuttings. Inoculated with *Glomus fasciculatum* (1.07 mg/g) followed by those of uninoculated cuttings (0.83 mg/g) and treated with IBA (0.95 mg/g). Treated with IBA and NAA with inoculation of *Glomus fasciculatum* had a maximum total soluble sugar content (3.26 mg/g) compared to the treated IBA with inoculation of *Glomus fasciculatum* (2.44 mg/g) and NAA with inoculation of *Glomus fasciculatum* (1.96 mg/g).

The maximum total soluble sugar was recorded in all the cuttings at 180 days treated IBA and NAA with inoculation of *Glomus fasciculatum* had a higher proportion of total soluble sugar (4.54 mg/g) compare to all the treatment and the lowest (1.08 mg/g) in uninoculated stem cuttings shown in (Tables2).

In *Acalypha wilkesiana* L., AMF treatments had higher proportion of total soluble sugars in roots (0.55 mg/g) than non inoculated cuttings(0.38 mg/g). IBA had a higher proportion of total soluble sugars (0.75 mg/g) compare to NAA(0.47 mg/g). IBA and NAA treated cuttings exhibited higher total soluble sugar content (0.82 mg/g) than the treated with IBA or NAA alone. IBA with inoculation *Glomus fasciculatum* had higher soluble sugar content (0.88 mg/g) compare to IBA, and NAA with inoculation of *Glomus fasciculatum*. NAA with inoculation of *Glomus fasciculatum* had higher total soluble sugar content (0.65 mg/g) compare to IBA with NAA and NAA alone (Fig. 2). The total soluble sugars content was observed in the treatment of IBA and NAA with inoculation of *Glomus fasciculatum* (0.94 mg/g).

After 120 days the variation in total soluble sugar content. The highest total soluble sugar content the higher total sugar to content was recorded in cuttings. Inoculated with *Glomus fasciculatum* (1.66 mg/g) followed by those of uninoculated cuttings (0.84 mg/g) and treated with IBA (1.06 mg/g). Treated with IBA and NAA with inoculation of *Glomus fasciculatum* had a maximum total soluble sugar content (3.26 mg/g) compared to the treated IBA with inoculation of *Glomus fasciculatum* (2.95 mg/g) and NAA with inoculation of *Glomus fasciculatum* (1.55 mg/g).

The maximum total soluble sugar was recorded in all the cuttings at 180 days treated IBA and NAA with inoculation of *Glomus fasciculatum* (4.16 mg/g) had a higher proportion of total soluble sugar compare to all the treatment and the lowest (1.35 mg/g) in uninoculated stem cuttings (Tables 3).

DISCUSSION

Analysis of proteins and carbohydrate in mycorrhizal and hormone treated roots represents a useful tool to insights on those mechanisms. In present study, the protein and carbohydrate differences between AMF and treated with hormone root extracts proteins and carbohydrate content was much higher in inoculation of *Glomus fasciculatum* than in non-mycorrhiza and treated with hormones (Tables 6,7&8), in agreement with [14] for tobacco and onion.

Other reports have not shown such a difference in mycorrhizal, treated with hormone and uninoculated roots as have been described so far and further deep studies of this aspect are needed. Perhaps this difference is a consequence of factors such as higher metabolic activity in AM-colonized root cells and the presence of internal and external fungal mycelium. A particular type of auxin is effective in enhancing rooting in a particular species [15,16]. A number of workers have shown that rooting of cuttings is facilitated when carbohydrates and growth promoters are in abundance [17, 18].

The fungus may induce plant metabolic changes through the release of fungal metabolites [19], there by increasing rooting on cuttings inoculated with AMF. The concentrations and contents of metabolic reserves in cuttings have been related to rooting ability [20, 21]. However, propagating concentrations of carbohydrates in cuttings did not limit rooting. Increased rooting was associated with higher sucrose, starch ratio in cuttings, reflecting an increased assimilates export needed for rooting and in the miniature roses it was found that nitrogen containing compounds appear to play a primary role in adventitious root formation while initial carbohydrate concentrations may play a smaller, yet interactive, role [7]. Mycorrhizal colonization can also increase carbon sink strength in roots of plants sugars in larger concentrations of reducing sugars in roots [22].

IBA and NAA with inoculation *Glomus fasciculatum* were recorded maximum similar results for roots on miniature rose cuttings treated with hormone were reported by Scagel [23]. There are no mechanisms to explain this recognition, although it has been demonstrated that important biochemical changes were treated with hormones of inoculated with AMF generally produced and accumulated more proteins and carbohydrates in roots compared to cutting that received no hormones or inoculum. Although many reports described change in plant composition during colonization by AMF few studies have reported changes in stem and root composition prior to colonization.

The biochemical studies on root initiation clearly indicate that the root inducing effects of the treatment, i.e., auxins and inoculations of AMF were related to the variation of total carbohydrate and protein. Our findings suggested that stored carbohydrates are utilized during adventitious root formation in shoot cuttings. Value of protein content has generally been observed at the time of induction of root primordial and it has been related to increased synthesis of enzymatic protein during the root regeneration process [2, 24].

The kinetic of carbohydrate in the cuttings during storage provides evidence that AM in stock plants can greatly influence post harvest carbohydrate fluxes. Considering that high sugar availability is crucial for both survival and the adventitious root formation in cuttings [25], the present results suggest that an altered carbohydrate balance can contribute to improved rooting capacity of AM-conditioned cuttings.

Similar result indicating the effect of reserved carbohydrates in cutting root ability were found in Chrysanthemum [7]. Also Kozlowski [26] recorded the correlation between reserved carbohydrate of cuttings and rooting. They stated that accumulation of carbohydrate during the growth season of evergreen trees play an important role in early season growth, transplants survival and rooting.

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