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RESEARCH ARTICLE

Effects of Inoculation of *ArbuscularMycorrhiza* and Growth Regulators on Reducing Sugar contents of Three Medicinal Plants

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ABSTRACT

The interactive potential benefits of inoculation with arbuscularmycorrhizal 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 (0, 1000, 15000 and 2000 mg L^{-1}) conducted in earthen pots in factorial based completely randomized design with four replications and the plants were uprooted periodically 90 days. Leaf samples for each harvest were analyzed for reducing sugar concentration. The results showed remarkably high reducing sugars content in Acalife plant at IBA concentration of 2000, 1000 and 1500 mg L^{-1}, 111.56, 98.88 and 98.61 mg L^{-1}, respectively. The lowest reducing sugars content was in the extract of Henna plant (at 2000 ppm) with 31.17 mg L^{-1}. The highest rate of reduced sugar in three medicine plants *Vitex negundo*, *Lawsonia inermis* and *Acalypha wilkesiana* was related to *Acalypha wilkesiana* plant that had higher reduced sugar compared to two other plants. With increasing in IBA hormone concentration up to 1500 mg L^{-1} the rate of reduced sugar significantly increased in *Vitex negundo* plant, but it was decreased in 2000 mg L^{-1} concentration. 1500 mg L^{-1} treatment with the highest rate of reduced sugar in *Vitex negundo* plant was significantly different from control treatment. The highest rate of reduced sugar in 1000, 1500 and 2000 mg L^{-1} IBA belonged to *Acalypha wilkesiana* plant.

KEYWORDS

Arbuscular Mycorrhizal Fungi, Indol-3 Butyric Acid, Reducing Sugar.

INTRODUCTION

It has been well discussed that arbuscularmycorrhizal (AM) association as agents in biological control will be acting by more than one mechanism. The activation of specific plant defense mechanisms as response to AM colonization is an obvious basis for the protective capacity of AMF.

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The elicitation by an AM symbiosis of specific plant defense reactions could predispose the plant to an early response to attack by a root pathogen[10]. During their life cycle, plants evolve a number of defense responses elicited by various signals, including those associated with pathogen attack.

Among the compounds involved in plant defense studied in relationship to AM formation are phytoalexins, enzymes of the phenylpropanoid pathway, β-1,3-glucanases,

chitinases, peroxidases, pathogenesisrelated (PR) proteins, callose, hydroxylproline- rich glycoproteins (HRGP) and phenolics[4, 10, 11]. Proteins associated with mycorrhizal associations have been well studied and showed that mycorrhization increases protein production in the roots[3,5].

Symbiotic association between AMF and plants is based on the exchange of carbohydrates and other nutrients between both the partners. Plants roots become a strong sink for sugars during mycorrhization which in turn increases the photosynthethic ability of the phototroph to compensate this usage of sugars[29].

There are many works showing that amino acid concentration is more in mycorrhizal than non mycorrhizal plants. Baltruschat and Schonbeck[2] showed increase in both arginine and citrulline in mycorrhizal plants over non mycorrhizal ones, which inhibit the propagation of *Thielaviopsisbasicola*. There are many other reports mentioning the change in amino acid concentration due to AMF colonization[1, 24].

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[12]. Many changes in metabolism are known to occur during adventitious root formation including changes in proteins and carbohydrate[13]. The response to exogenous application of auxins has a role in metabolic changes like viz., specific enzyme, carbohydrate, RNA, DNA, protein metabolism, 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[20, 22]. Carbohydrate concentration in cuttings was playing 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[6].

This research was aimed to investigate the changes arbuscularmycorrhizal fungi (AMF) and Indol-3 butyric acid effects on reducing sugarof three medical plants.

MATERIALS AND METHOD

The experiment was conducted under mist chamber using sterile soil to know the effect of arbuscularmycorrhizal fungi on the growth and yield of three medicinal plants such as Nirgandi (*Vitexnegundo*L), Henna (*Lawsoniainermis*L) and Copper leaf (*Acalyphawilkesiana*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 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 sand.

Arbuscularmycorrhizal fungi (*Glomusfasciculatum*) were multiplied by using Jowar (Sorghum vulgarepers) as host plant. The pot had 34 cm diameter containing8.5 kg soil. 15 g of air dried AM fungi inoculum of *Glomusfasciculatum*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 2 g of root bits of host plant with hyphae and sporocarps.

The basal portion of the stem cuttings were dipped for 5 Second in (0, 1000, 1500 and 2000 mg L^{-1}) IBA [Swamy et al., 2001], then placed

into pots containing the same sand. The layout of the experiment was randomized complete design (CRD) with four replication for each treatment in mist chamber.10 ml of Hoagland solution [Hinasyed et al., 2002] without P was treated for each plant at an 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 180 days after planted.

Leaf samples for each harvest were analyzed for reducing sugarsconcentration. Reducing sugarscontent of samples was determined by UV spect Nirgandi (*Vitex negundo* L.), Henna (*Lawsonia inermis* L.) and Copper leaf (*Acalypha wilkesiana* L.), rophotometer using Somogy method[25], and the wavelength is 600 nm.

Estimation of Reducing Sugars

Glucose and fructose containing aldehyde and ketone groups can be oxidized by some materials. Sugars containing free anomeric carbons are called reducing sugars. In this experiment, presence of reducing sugars reduced Cu^{+2} to Cu_2O. Cu_2O reduces phosphomolybdic acid which produces blue color formation. Severity of produced color which is positively correlated with reducing sugars concentration can be evaluated by spectrophotometer. Somogy method [25] was used to determine the concentration of reducing sugars. 0.02 g of aerial part was pulverized with 10ml of distillated water. The mixture was transferred in to a small beaker and heated on electrical stove. Heating was stopped when the mixture reached boiling point; content of the beaker was filtrated by whatman filter paper no.1 to obtain plant extract. 2 ml of the plant extracts was transferred to test tube, 2 ml of copper sulphate was added, the tubes were sealed with cotton and incubated for 20 min in water bath 100°c. in this step, Cu^{+2} is transformed in to Cu_2O by reduced aldehyde monosaccharide and a brick red color is observed. When the tubes were cooled, 2 ml of phosphomolybdic acid was added and blue color appeared. The test tubes were thoroughly agitated until the color was evenly distributed in the tube. Absorbance was determined in 600 nm by spectrophotometer and concentration of the reducing sugars was calculated by drawing standard curve. The results were calculated and reported as mg per g of fresh weight.

Drawing Standard Curve

To draw standard curve, concentrations of 5, 10, 20, 40, 60 and 100 mg L^{-1} of glucose were prepared and 2 ml of each concentration was poured in clean test tube. Other steps were performed as for unidentified samples and solution absorbance was read by spectrophotometer in 600 nm. Absorbance curve was drawn against concentration and the line equation was achieved.

Preparation of Copper Sulphate Solution

40 g of anhydrous sodium carbonate was dissolved in 400 ml of distillated water and added to 7.5 g of tartaric acid. After dissolving in acid, 4.5g of $CuSO_4.5H_2O$ was added and final volume was increased to 1 liter.

Preparation of Phosphomolybdic Acid Solution

70 g of phosphomolybdic acid and 10 g of sodium tungstate were dissolved in 700 ml of 5% hydroxide sodium and heated for 40 min. when the solution was cooled, 250 ml of 85% phosphoric acid was added and the final volume was increased to1 liter.

Statistical Analysis

All these experiments were replicated four times, and the average values are reported. The effects of Inoculation of ArbuscularMycorrhiza and Growth Regulators of IBA on Reducing Sugars of Three Medicinal Plants [Nirgandi (*Vitex negundo* L.), Henna (*Lawsonia inermis* L.) and Copper leaf (*Acalypha wilkesiana* L.)] were determined using the analysis of variance (ANOVA) method, and significant differences of means were compared using Duncan's test at 5 % significant level using the SAS software (2008) program.

RESULTS AND DISCUSSION

According to results of figure (1), reduced sugar significantly was affected by IBA hormone. The highest rate of reduced sugar with 62.23, 111.56 and 58.35 mg L^{-1} belonged to control, 2000 and 1500 mg $L^{-1,}$ respectively between three medicinal plants *Vitex negundo*, *Lawsonia inermis* and *Acalypha wilkesiana*. The lowest rate of reduced sugar with 40.28, 57.58 and 31.17 mg.l^{-1} belonged to 2000 and control treatment, respectively in three medicine plants *Vitex negundo*, *Lawsonia inermis* and *Acalypha wilkesiana*. According to results (fig 1), the highest rate of reduced sugar in three medicine plants *Vitex negundo*, *Lawsonia inermis* and *Acalypha wilkesiana* was related to *Acalypha wilkesiana* plant that had higher reduced sugar compared to two other plants. With increasing in IBA hormone concentration up to 1500 mg L^{-1} the rate of reduced sugar significantly increased in *Vitex negundo* plant, but it was decreased in 2000 mg L^{-1} concentration. 1500 mg L^{-1} treatment with the highest rate of reduced sugar in *Vitex negundo* plant was significantly different from control treatment. The highest rate of reduced sugar in 1000, 1500 and 2000 mg L^{-1} IBA belonged to *Acalypha wilkesiana* plant. Analysis of reducing sugars in mycorrhizal and hormone treated leaf represents a useful tool to insights on those mechanisms. In present study, the reducing sugars differences between AMF and treated with hormone leaf extracts proteins and reducing sugars content was much higher in inoculation of Glomus fasciculatumthan in non-mycorrhiza and treated with hormones (Table 1), in agreement with[9] 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[26, 21]. A number of workers have shown that rooting of cuttings is facilitated when carbohydrates and growth promoters are in abundance [15, 28]. The fungus may induce plant metabolic changes through the release of fungal metabolites[19], thereby increasing rooting on cuttings inoculated with AMF. The concentrations and contents of metabolic reserves in cuttings have been related to rooting ability [14, 16]. However, propagating concentrations of reducing sugars 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[8]. Mycorrhizal colonization can also increase carbon sink strength in roots of plants sugars in larger concentrations of reducing sugars in roots[30].

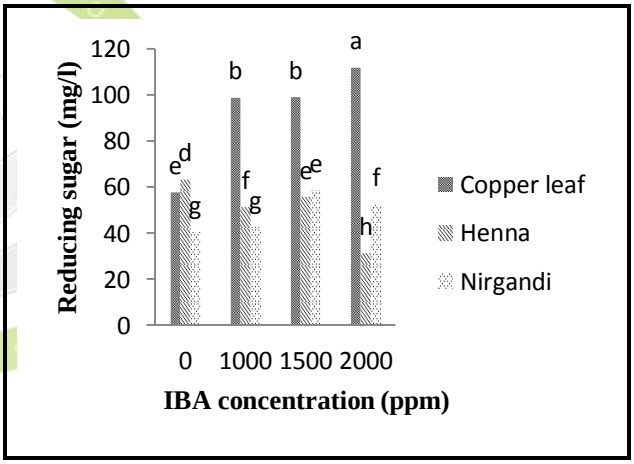

Figure 1: Effects of Inoculation of ArbuscularMycorrhiza and Growth Regulators of IBA on Reducing Sugars of Three Medicinal Plants [Nirgandi (*Vitex negundo* L.), Henna (*Lawsonia inermis* L.) and Copper leaf (*Acalypha wilkesiana* L.)] (Means with same superscripts had no significant difference with each other (P >0.05).

The biochemical studies on root initiation clearly indicate that the leaf 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. The kinetic of carbohydrate in the cuttings during storage provides evidence that AM in stock plants can greatly influence postharvest carbohydrate fluxes. Considering that high sugar availability is crucial for both[7], the present results suggest that an altered carbohydrate balance can contribute to improved rooting capacity of AM-conditioned cuttings. Similar results indicating the effect of reserved carbohydrates in cutting root ability were found in Chrysanthemum[8]. Also Kozlowski[18] 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. Among studied plants, Copper leaf contained the highest amount of reducing sugars. In this plant, amount of reducing sugar increased with increasing IBA concentration. Results showed that reducing sugars of Henna extracts decreased by increase of IBA concentration and no significant differences ($P < 0.05$) observed between Henna and Nirgandi plants at 1500 mg L^{-1} in case of reducing sugars. IBA 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 reducing sugars in leaf 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.

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