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ORIGINAL ARTICLE

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Effects of plant growth regulators on the carbohydrate accumulation in *Simarouba glauca* seedlings

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A study was conducted to assess the accumulation of carbohydrate in germinating seeds as well as leaves, stem and roots of *Simarouba glauca* in response to various Plant Growth Regulators (PGRs). Field experiment was carried out to investigate effect of foliar application of PGRs like 6-benzylaminopurine (6-BA), gibberellic acid (GA), chlormequat (CCC), salicylic acid (SA), cysteine and methionine with 5 and 20 ppm concentration on carbohydrate content of *Simarouba glauca* DC whereas Seeds were subjected to 100 ppm solutions of various PGRs, which include GA, 6-BA, CCC, SA, Cysteine, and Methionine. It could be concluded that application of PGRs affect the carbohydrate metabolism or synthesis. Thus, the application of growth regulators in present study will be beneficial for induction of synthesis of primary metabolic products followed by synthesis of secondary metabolites of *S. glauca*.

Key words: Carbohydrates, Plant growth regulators, Simarouba glauca, secondary metabolites

Carbohydrates occupy very important place in the primary metabolism of all green plants because these are major products of photosynthetic carbon assimilation and used as substrates for respiration. The level of carbohydrate indicates metabolic status of plant tissue and also the energy content of plant tissue. Carbohydrate provide carbon skeleton for variety of carbon compounds present in plant tissue. These compounds are various secondary metabolites having medicinal potential. Starch is storage carbohydrate mostly found to be present in seeds and various tubers. The main carbohydrate reserve in potato is hemicelluloses amyloids and the raffinose series of oligosaccharides. Both sucrose and starch represent major products of steady state photosynthesis although starch synthesis occurs in plastid while sucrose biosynthesis takes place in cytosol. The main product of photosynthetic carbon assimilation in higher plants is oligosaccharides-sucrose as the most important non reducing sugars which are utilized as source of energy for growth of plant tissue.

Simarouba glauca is an evergreen edible oil tree, commonly known as 'Laxmitaru' or 'paradise tree' belonging to family *Simaroubaceae*. This plant is endemic to Florida, Lesser Antilles, South America, and the United States. The plant contains many essential phyto constituents of major pharmacological significance.

MATERIALS AND METHODS

The sugars were analyzed by using the method of Nelson (1944). Five hundred mg oven dried powder of germinated seeds, leaves, stem and roots were extracted with 80% alcohol. The extract was filtered through Buchner's funnel using Whatman No. 1 filter paper. The filter paper with residue was saved for starch estimation. The filtrate was condensed to 5 ml on water bath and to this 2 g lead acetate and potassium oxalate (1:1) were added for decolourization, 40 ml distilled water was added and aliquot was filtered through Buchner's funnel. The volume of filtrate (a) was measured and it served as an extract for determination of reducing sugars. For the estimation of starch, the insoluble residue along with the filter paper obtained at the beginning after filtering the alcoholic extract was transferred to a 100ml conical flask. To this 50ml distilled water and 5ml concentrated HCl were added and the contents were hydrolyzed at 15lbs pressure for half an hour. These conical flasks were cooled to room temperature, and the contents were neutralized by addition of anhydrous sodium carbonate and filtered through Buchner's funnel. The volume of filtrate (b) was measured and this contains reducing sugars (glucose) formed as a result of hydrolysis of starch. The amount of glucose so formed is equivalent to the starch content in the residue.

For estimation of reducing sugars and starch, 0.4 ml (a) and 0.1 ml (b) filtrates were taken in a set of other test tubes respectively. Different concentrations of glucose (0.1 mg ml-1) were taken in other test tubes. In each test tube requisite amount of distilled water was added to make final volume 1 ml. In case of blank 1 ml distilled water was taken instead of filtrate or standard glucose. To this 1 ml Somogyi's alkaline copper tartarate reagent (4 g $CuSO_4.5H_2O$, 24 g anhydrous Na_2CO_3, 16 g Na-K-tartarate and 180 g anhydrous Na2SO4 dissolved in 1 liter distilled water) was added and then the tubes were kept in boiling water bath for 10 minutes and cool to room temperature. Nelson's Arsenomolybdate reagent prepared by mixing (25 g Ammonium molybdate dissolved in 450 ml distilled water, 3 g sodium arsenate dissolved in 25 ml distilled water, 21 ml concentrated HCl) these ingredients were mixed well and digested for 48 hours at 37°C in dark.1ml of this reagent was carefully added and reaction mixtures were further diluted to 10 ml with distilled water. The absorbances of these samples were measured on a double beam spectrophotometer (Shimadzu, 190) at 560 nm. The amount of reducing sugars was calculated with the help of calibration curve of standard glucose (0.1 mg ml^{-1}) and expressed as g $100g^{-1}$ dry tissue.

The extract prepared earlier for reducing sugars was used for the estimation of soluble sugars. The soluble sugars were estimated following the method of Dey (1990) (Phenol-sulphuric acid) with slight modification. For the estimation, 0.2 ml plant extract was poured in a test tube and to this 1 ml 0.5% phenol was carefully added and mixed thoroughly. Five ml of analytical grade

sulphuric acid were added very slowly and carefully to the above test tubes. This was mixed thoroughly by vertical agitation with a glass rod with a broadened end. The contents were cooled in air and the absorbance was measured at 485 nm. The amount of soluble sugars was estimated with the help of standard glucose (0.1 mg ml $^{-1}$). The values are expressed in g 100 g^{-1} dry tissue.

The Nelson-Somogyi method is one of the classical and widely used methods for the quantitative determination of reducing sugars. This method utilizes the reducing properties (because of the presence of a potential aldehyde or keto-groups) of certain types of carbohydrates. Determination of reducing sugars using Somogyi-Nelson is based on the absorbance at 520 nm of a coloured complex between a copper oxidized sugar and arsenomolybdate.

RESULTS

Effect of growth regulators on carbohydrate content in germinating seeds of S. *glauca* is shown in fig. 1. It is evident that the amount of total soluble sugars is higher than the starch content. The total soluble sugars, reducing sugars and starch content increases significantly after presowing soaking treatments in one month old seedlings. Effect of foliar application of growth regulators on carbohydrate fractions is shown in fig. 2-3. The content of total soluble sugars is higher than the starch content in root, stem and leaves. In leaf tissue after the application of 6-BA and GA the level of starch is elevated than the total soluble sugars. In root and stem tissue the amount of total soluble sugar is higher than the starch level. The total soluble sugars and reducing sugars are found to be increased considerably in root and stem tissue in response to all growth regulators, while in leaf tissue the total soluble sugars and reducing sugars are decreased in response to 5 and 20 ppm 6-BA, GA, 20 ppm CCC and SA, 5 ppm cysteine and 20 ppm methionine. The sugar content is elevated in leaf tissue only in response to 5ppm CCC, 20 ppm cysteine and 5 ppm methionine. 160 The starch content of root, stem and leaves of S. *glauca* is shown in fig. 4. It is evident that the starch content of root and stem tissue is slightly altered showing decreasing trend in response to foliar applications of growth regulators while in leaf tissue, 6-BA, GA, 20 ppm CCC and 5 ppm SA exhibits accumulation of starch content and in other treatments it is slightly decreased.

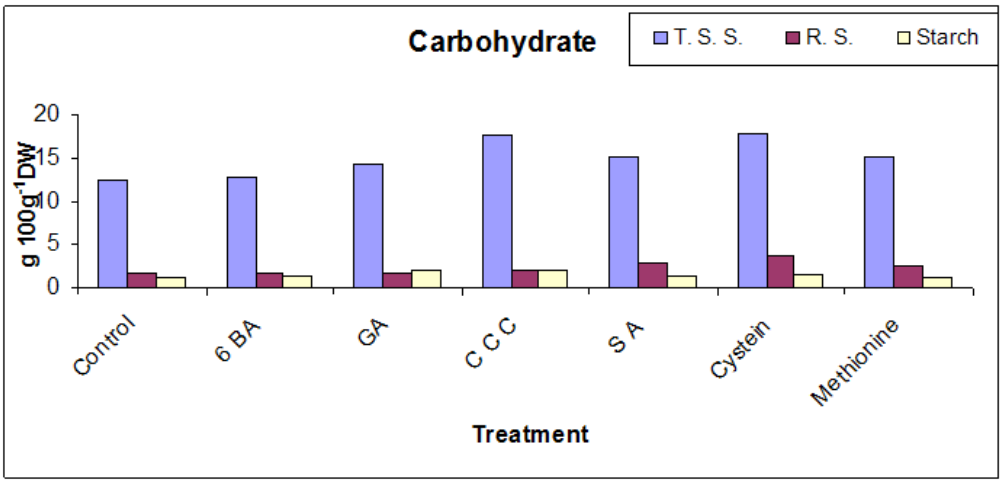

Figure 1 Effect of presowing soaking treatments of PGRs on the content of total sugars, reducing sugars and starch in 30 days old seedlings of *S. glauca*

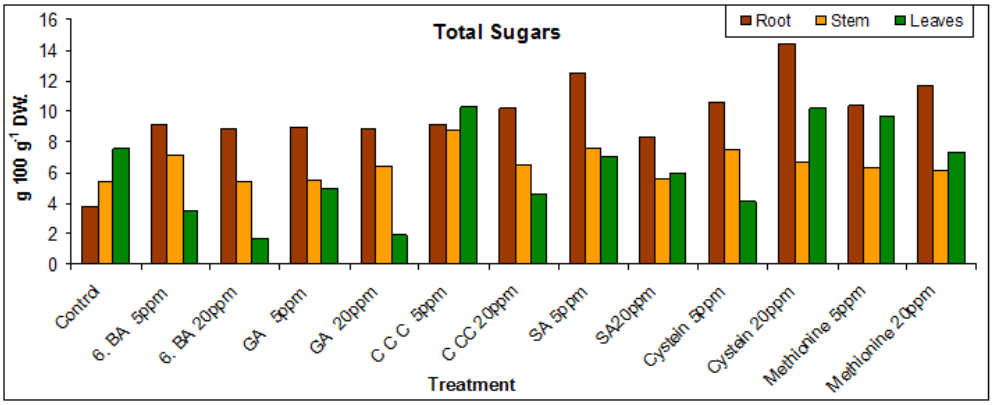

Figure 2 Effect of foliar spray of PGRs on the content of total soluble sugars in the root, stem and leaves of *S. glauca*

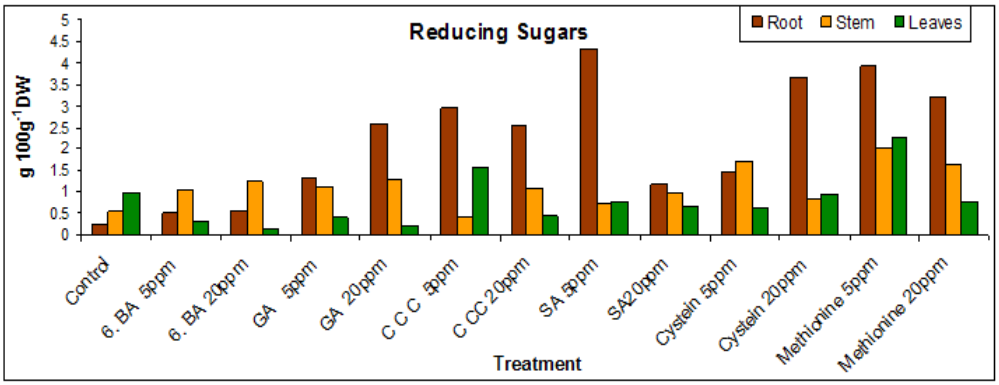

Figure 3 Effect of foliar spray of PGRs on the content of reducing sugars in the root, stem and leaves of *S. glauca*

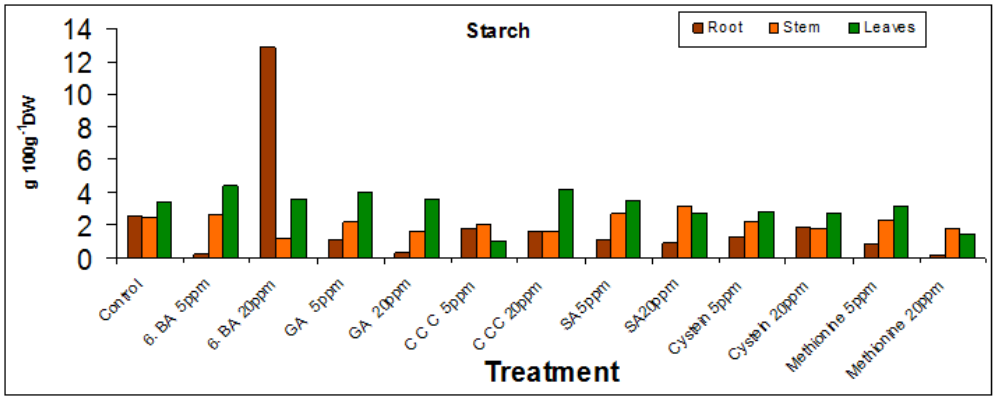

Figure 4 Effect of foliar spray of PGRs on the content of starch in the root, stem and leaves of *S. glauca*

DISCUSSION

Effect of gibberellic acid on sucrose accumulation and sucrose biosynthesizing enzymes activity during banana ripening was studied by Rossetto *et al*. (2003). They observed the starch content started to decreaseon day 12 in the control group and 2 days later in GA treated slices further they reportedthat the amount of starch in both groups was similar on day 16. GA also affects sugaraccumulation by delaying the increase in SPS (Sucrose - Phosphate synthase) during banana ripening. Increase in carbohydrates content (soluble, insoluble and total) level was recorded in *Balanitesa egyptiaca* plants when treated with GA (Mostafa and Abou-Alhamd, 2011). This accumulation of carbohydrates due to GA treatment might be linked with the efficiency of photosynthetic apparatus, which leads

to increase in plant productivity and dry matter production (Azooz*et al*., 2004). Response of croton plants to gibberllic acid, 6-BA and ascorbicacid was studied by Eid and Abou- Leila (2006). They observed GA-treated leaves of plants showed greatest carbohydrate percentage. Application of GA increased the total carbohydrates of *Amaranthus caudatus* plants (Bialecka and Kepezynski, 2010). Salicylic acid treatments significantly decreased the contents of soluble sugars than control plants and increased the polysaccharide contents in maize plants (Khodary, 2004). He further suggested that SA might activate the metallic consumption of soluble sugars to form new cell constituents to stimulate growth whereas it might also be involved to inhibit polysaccharide hydrolyzing enzyme system or accelerate the incorporation of soluble sugars into polysaccharides. Increase in soluble and insoluble sugars in barley grains treated with SA noticed by EI-Tayeb (2005). Fang and Wei (2009) reported dipping the bulblet of lily into 5mM l^{-1} SA before planting increases the carbohydrate contents of bulb, starch content and the soluble sugar content also increased. Effect of drought stress and SA on soybean plants was studied by AI-Hakimi (2006). He reported that application of SA increased soluble sugars of roots on the other hand decreased soluble sugars of shoots. Foliar application of SA distinctly increased total carbohydrates of groundnut (Jayalakshmi *et al*., 2010). Singh *et al*. (2010) observed in presence of external NO_3 - 50µM SA increased sugar content in cucumber while 500µM SA reduced sugar levels. Similar trends were recorded for starch contain except at 100 and 50 µM concentration of SA where starch content was sharply reduced as compared to control. Effect of 6-BA application on roselle seeds was studied by Mostafa *et al*. (2005). They found 6-BA and mixture of 6-BA + GA stimulated the soluble sugar, polysaccharide and total carbohydrate accumulation. According to Smolen and Sady (2008), foliar application of 6-BA enhanced concentration of soluble sugars in storage roots of carrot. Study of Smolen and Sady (2009) revealed that application of foliar nutrition treatment (combination of Urea+Mo+BA+sucrose) significantly increased soluble sugars in radish roots. Further they reported roots of control plants and plants sprayed with only BA showed more soluble sugars. Wang *et al*. (2009) reported soluble sugars and starch accumulated in roots and stolon rather than leaves and stem of potato after treatment with CCC. Further they reported increase in starch and sucrose content in potato tubers by CCC treatment. Application of CCC causes decrease in sugars in melon fruits (Ouzounidou *et al*., 2008). According to Ouzounidou *et al*. (2010), CCC inhibited total soluble solids (TSS) in chilli (*Capsicum annuum* L.).

CONCLUSION

It was noticed that 11 to 15 % soluble sugars in germinating seeds and 2 to 10 % in root, stem and leaves. The higher levels of soluble sugars than the starch level may play important role in osmoregulation followed by improved water content during seed germination. Thus, the higher level of soluble sugars might be beneficial during seed germination of *S. glauca*. While, the lower levels of starch content of leaf tissue followed by higher levels of soluble sugars might be due to the synthesis of variety of carbon compounds from primary metabolites to secondary metabolites with medicinal potential which was also reflected in the accumulation of polyphenols, flavonoids and anthocyanins as a secondary metabolites. Thus, the application of growth regulators in present study will be beneficial for induction of synthesis of primary metabolic products followed by synthesis of secondary metabolites of *S. glauca*.

CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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