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EFFICACY OF MEDIA AND GROWTH REGULATORS ON BIOCHEMICAL COMPONENTS OF BLACK PEPPER (*PIPER NIGRUM*) CUTTINGS

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Abstract

The present investigation clearly indicates that the reducing sugars, non reducing sugars and total sugars content was minimum in cuttings treated with IBA 1000 ppm (0.56%, 1.10% and 1.66%, respectively) compared to other concentrations of growth regulators. Cuttings grown in the treatment soil + sand + FYM + vermicompost (1:1:1:1 v/v) recorded the maximum chlorophyll-a, chlorophyll-b and total chlorophyll content was 1.39mg/100g, 0.59mg/100g and 1.99mg/100g, respectively.

Key words : Black pepper, media, growth regulator, sugars and chlorophyll.

Introduction

India is the land of spices. The flavour and fragrance of Indian spices had magic spell in human civilization and culture. India contributes about 40-50 per cent of world's production of spices (Parthasarathy and Madan, 2010). Black pepper (*Piper nigrum* L., Family : Piperaceae) popularly known as "king of spices" is the oldest and most important spice crop grown in India. It is native to Western Ghats and it is grown in 26 countries including India, Indonesia, Srilanka, Thailand, China, Vietnam, Cambodia, Brazil, Mexico and Guatemala.

Black pepper is widely used spice in the world and it is an indispensable item in the preparation of processed meat, sauces, soups, curry powders and pickles. From medicinal point of view it is used as a carminative, stomachic and febrifuge. The major economic products from pepper are black pepper and white pepper. Other value added products like pepper oil and oleoresin find increasing use in food industries of developed countries. Besides some new forms of pepper like preserve green pepper in brine, vinegar, dehydrated green pepper etc. are becoming more popular. Hence the black pepper is also called as 'Black gold' on account of its economic importance. (Devasahayam *et al.*, 2010).

Black pepper can be propagated through seeds and vegetative methods. Owing to its heterozygous nature, seedlings do not breed true to type and known to have long pre-bearing period. Hence, vegetative propagation through cuttings is commercially adopted. Besides this grafting, budding and layering are also practiced. But propagation through cuttings is easier hence it is preferred for large scale multiplication. Cuttings taken from runners shoots (Creeping shoots on the ground) and orthotropic shoots (erect growing shoots) are used commercially for vegetative propagation.

The effect of reducing sugar (fructose or glucose) was found that extension of the shoots and development of their root system were better in media with reducing sugar than in the medium supplemented with non-reducing sugar (Bondarev *et al.,* 2003). Rooting rate was negatively correlated with total sugars and reducing sugar contents whereas negatively correlated with starch content in prunus mume (Zhang and Masaki, 1994). And higher contents of nitrogen and various sugars were associated with bette rooting in case of peach (Gill, 1995).Therefore, the main objective is to study the effect of biochemical parameters on rooting of black pepper

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Table 1 : Effect of growth regulators on sugar content of black pepper cuttings.

Treatment	Reducing sugar (%)	Non reducing sugar(%)	Total sugar (%)
T_1 - Control	0.74	1.44	2.18
T_2 – IBA 500 ppm	0.60	1.66	1.75
T_3 - IBA 1000 ppm	0.56	1.10	1.66
T_4 - IBA 1500 ppm	0.63	1.22	1.85
T_5 – NAA 250 ppm	0.60	1.16	1.76
T_6 - NAA 500 ppm	0.62	1.19	1.81
T_7 – NAA 1000 ppm	0.69	1.33	2.02
T_8 –IAA 1000 ppm	0.61	1.17	1.78
T_9 – NAA + IBA 250 ppm	0.65	1.27	1.92
T_{10} – NAA+ IBA 500 ppm	0.67	1.30	1.98
T_{11} – NAA+ IBA 1000 ppm	0.70	1.36	2.06
F –test	**	**	**
S. Em. ±	0.01	0.02	0.02
C. D. 5%	0.02	0.05	0.07
Initial value	0.83	1.63	2.46

** Highly significant.

cuttings is essential.

Materials and Methods

The present investigations were carried out in naturally ventilated polyhouse with completely randomized design at Regional Horticultural Research and Extention Centre, Mudigere during the period from February 2012 to June 2012. The healthy cuttings of Panniyur-1 variety were procured from the RHREC, Mudigere. The cuttings having two nodes with thickness of 0.8-1.0 cm diameter and 10 cm length were selected. For rooting media a potting mixture consists of jungle soil, sand and FYM in the ratio of 2:1:1 was filled into 20 × 12 cm sized perforated polythene bag of 200 micron thickness. Before planting the cuttings, media was drenched with Copper-oxy chloride (0.3 %) as a prophylactic measures against fungal diseases.

There were eleven treatments including control in which several growth regulator formulations were used at different concentrations. Each treatment was replicated thrice, with 100 cuttings per replication. The growth regulator formulations are T_1- Control (dipped in tap water), T_2-IBA 500 ppm, T_3-IBA 1000 ppm, T_4-IBA 1500 ppm, T_5-NAA 250 ppm, T_6-NAA 500 ppm, T_7-NAA 1000 ppm, T_8- IAA 1000 ppm, T_9- IBA + NAA 250 ppm, T_{10}- IBA + NAA 500 ppm, T_{11}- IBA + NAA 1000 ppm. Where, IBA = Indole Buteric Acid, IAA = Indole Acetic Acid and NAA = Naphthalene Acetic Acid. Before preparing the stock solutions, IAA, IBA and NAA were dissolved in 0.1 N NaOH solutions. Required concentration of the growth regulator solution was taken and the cuttings were dipped upto 1.5–2.0 cm deep in the solution for one minute. Then they were air dried subsequently for few seconds and immediately two cuttings per polythene bag were planted and placed in naturally ventilated poly house.

Table 2: Effect of rooting media on chlorophyll content of black pepper cuttings.

Treatment	Chlorophyll content (mg/100 g fresh weight)		
	Chlorophyll ' a'	Chlorophyll ' b '	Total chlorophyll
T_1- Soil : sand : FYM (2:1:1)	0.35	0.13	0.49
T_2- Soil : sand : FYM: SD (1:1:1:1)	0.89	0.40	1.29
T_3 - Soil : sand : FYM: SD (2:1:1:1)	0.92	0.28	1.20
T_4 - Soil : sand : FYM: SD (3:1:1:1)	0.77	0.32	1.09
T_5- Soil : sand : FYM: CD (1:1:1:1)	1.22	0.46	1.75
T_6 - Soil : sand : FYM: CD (2:1:1:1)	1.06	0.41	1.47
T_7 - Soil : sand : FYM: CD (3:1:1:1)	0.84	0.35	1.19
T_8 - Soil : sand : FYM: CC (1:1:1:1)	0.89	0.30	1.19
T_9 - Soil : sand : FYM: CC (2:1:1:1)	0.75	0.35	1.10
T_{10}- Soil : sand : FYM: CC (3:1:1:1)	0.55	0.20	0.75
T_{11}- Soil : sand : FYM: VC (1:1:1:1)	1.39	0.59	1.99
T_{12}- Soil : sand : FYM: VC (2:1:1:1)	1.26	0.53	1.79
T_{13}- SOIL: SAND: FYM: VC (3:1:1:1)	1.02	0.49	1.51
F- test	**	**	**
S Em ±	0.07	0.03	0.06
C D 5%	0.21	0.09	0.16

FYM – Farm Yard Manure **SD**- Saw Dust **CD**- Coir Dust
CC- Coffee pupl Compost **VC** – Vermicompost
** Highly Significant.

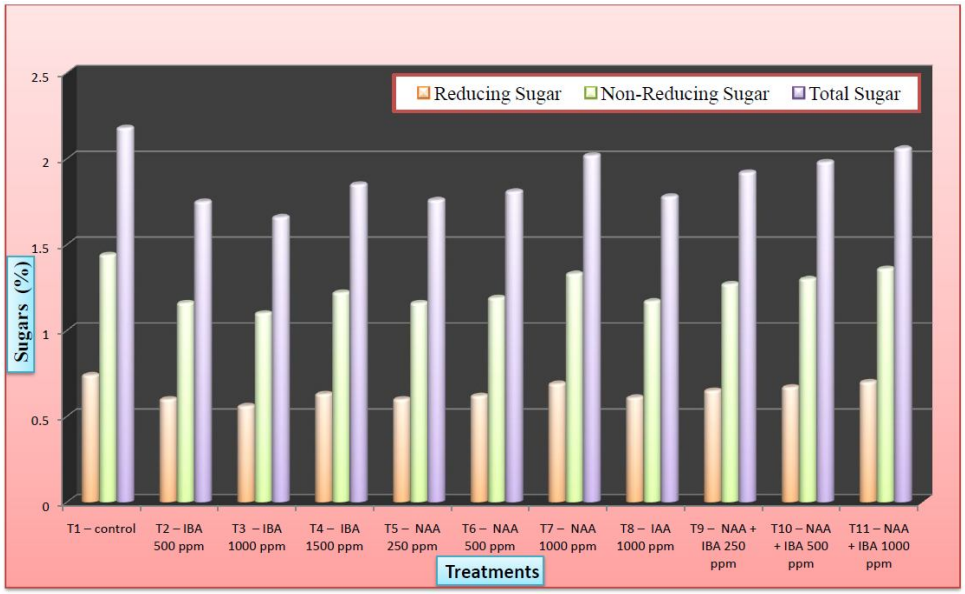

Fig. 1 : Effect of growth regulators on sugar content of black pepper cuttings.

A sample of 100 g each of the plant material from basal 1.0-1.5 cm portion of semi-hard wood cuttings was collected and oven dried then ground to fine powder for the purpose of all biochemical estimations. For estimation of total sugars and reducing sugars 500 mg of oven dried finely ground sample was extracted successively thrice using 80 per cent ethanol. A known volume (1 ml) of this extract was taken in a test tube and alcohol was evaporated on a boiling water bath. Distilled water was added and volume made upto 10 ml. The total sugar content in the alcohol free extract with 1N HCl on a hot water bath for 20 minutes at 50°C and after neutralizing it with 1N NaOH, the total sugars were estimated by Di nitro salicylic acid (DNSA) method (Miller, 1972). Non-reducing sugars were computed by deducting the value of reducing sugars from the amount of total sugars. And all the results were expressed in percentage on dry weight basis.

Chlorophyll content of leaf was analyzed by collecting the healthy, fully matured leaves from fifth node from the tip of stem at peak growth stage. Chlorophyll-a, chlorophyll-b and total chlorophyll content of leaf tissue were determined by using Di methyl Sulfoxide (DMSO) as suggested by Shaof and Lium (1976). A known weight of sample (100 mg) was incubated in 7.0 ml of DMSO at 65^{0}C for 120 minutes. After the incubation, supernatant was collected by decanting and leaf tissue was discarded. Then the volume of the supernatant was made up to 10 ml using DMSO. The absorbance of the extract was measured at 645 nm and 663 nm using DMSO as blank in spectrophotometer (Elico India). The chlorophyll-a, chlorophyll-b and total chlorophyll contents were calculated by using the formulae given below.

$$\text{Chlorophyll ``a''} = [12.7(A663) - 2.69(A645)] \times \frac{V}{1000 \times W \times a}$$

$$\text{Chlorophyll ``b''} = [22.9(A645) - 4.68(A663)] \times \frac{V}{1000 \times W \times a}$$

$$\text{Total chlorophyll} = [20.2(A645) \times 8.02(A663)] \times \frac{V}{1000 \times W \times a}$$

Where,

A = Absorbance at specific wave lengths (645 nm and 663 nm)

V = Volume of the extract (10 ml)

W = Fresh weight of the sample (100 mg)

a = Path length of light in cuvette (1 cm)

Results and Discussion

The physiological state of cuttings exerts a strong influence on development of roots and shoots from cuttings. This may be mainly related to sugars. Reducing sugars, non reducing sugars and total sugars marginally changed during the process of rooting in both untreated and treated cuttings (fig. 1). IBA 1000 ppm treated cuttings had least total sugars (1.66%), reducing sugar (0.56%) and non reducing sugars (1.10%) (table 1 and fig. 1). This may be due to enhanced depletion of sugars by hydrolysis and their utilization during root initiation as reported by Hegde (1981) in black pepper. The steady decline of sugars during the initiation and growth of roots indicates breakdown of carbohydrates during root development. Arslonov (1979) noted a rise in catalase and peroxidase activity which accompanied break down

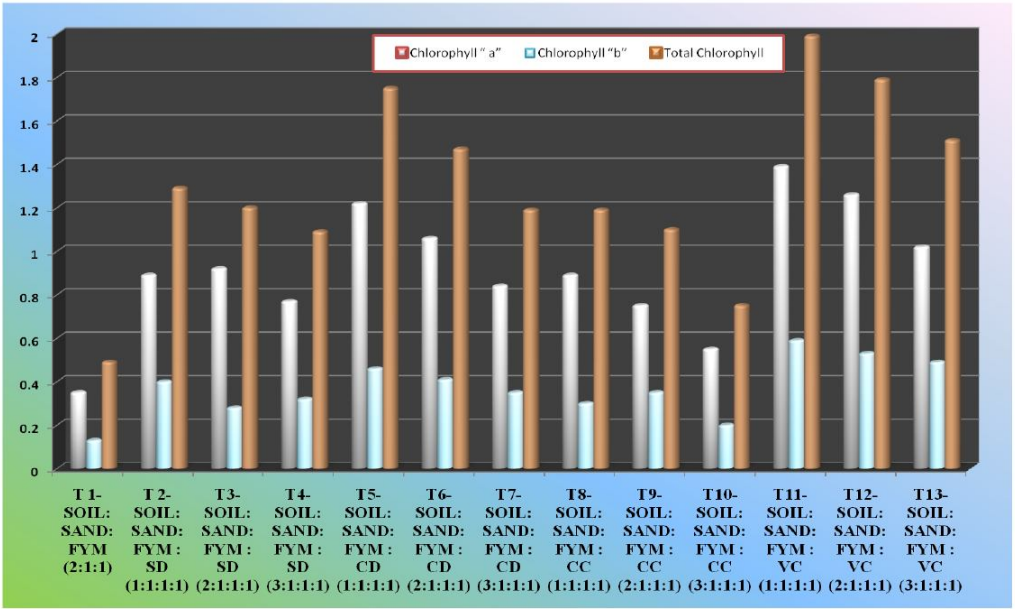

Fig. 2 : Effect of rooting media on chlorophyll content of black pepper cuttings.

of carbohydrates in lemon cuttings. According to Somappa (1979), exogenous application of auxins produced changes in the redox regime and this resulted in utilization of stored food substances for quicker root formation in Indian lavender cuttings. Similar trend was noticed by earlier workers *viz*., Patil and Shirol (1991) in oleander and Singh (1992) in *Callistemon lanceolatus*. The fact that relatively higher total sugar and lower starch content of cuttings was essential for good rooting was reported by earlier workers (Nanda *et al.*, 1971; Reuveni and Adato, 1974).

There was only a marginal variation in chlorophyll-a, chlorophyll-b and total chlorophyll content of the cuttings raised in all the treatments (fig. 2). Cuttings grown in the treatment soil + sand + FYM + vermicompost (1:1:1:1 v/v) recorded the maximum chlorophyll-a, chlorophyll-b and total chlorophyll content (1.39 mg/100 g, 0.59 mg/100 g and 1.99 mg/100 g, respectively) (table 2 and fig. 2). The superiority of vermicompost can be explained by its ability to supply nutrients like N, P, K, Ca and Mg in available form (Edwards and Neuhauser, 1988). As N and Mg are the important constituents of chlorophyll, higher availability and uptake of these nutrients resulted in higher chlorophyll synthesis in turn leading to higher photosynthesis and growth rate of black pepper cuttings.

Conclusion

The present investigation shown the minimum content of reducing sugar, non reducing sugar and total sugar in cuttings treated with IBA 1000 ppm compares to other concentration of growth regulators indicates that negatively correlated with rooting rate and initials. Nitrogen and magnesium are the important constituents of chlorophyll, higher availability and uptake of these nutrients resulted in higher chlorophyll synthesis in turn leading to higher photosynthesis and growth rate of black pepper cuttings.

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