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Effect of Biofertilizers and Auxin on Total Chlorophyll content of Leaf and Leaf Area in Pomegranate (*Punica granatum* L.) Cuttings

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ABSTRACT

Studies on the Influence of bio-fertilizers and auxin on total chlorophyll content of leaf and leaf area on pomegranate (Punica granatum L.) cuttings cvs. Bhagwa and Ruby was conducted at the Department of Fruit Science, College of Horticulture, Bengaluru during 2015-2016. The results revealed that significantly highest total chlorophyll content in leaves (5.13 and 5.66 mg/g), and leaf area (41.31 and 42.49 cm^2) in cvs. Ruby and Bhagwa respectively, recorded with application of IBA1500 ppm + NAA 1500 ppm + Biomix and the least total chlorophyll content of leaves (2.36 and 2.62 mg/g), and leaf area (33.54 and 34.35 cm^2) in cvs. Ruby and Bhagwa respectively, recorded in control.

Key words: *Pomegranate, Biofertilizer, Chlorophyll, Growth regulators*

INTRODUCTION

The pomegranate (*Punica granatum* L.) belongs to the family *punicaceae* which is one of the favourite table fruits of the tropical and subtropical regions. Pomegranate is commercially propagated by cuttings. Multiplication of plants through stem cutting is the most convenient method and by this method a stronger plant can be developed considerably in less time. The rooting capability of cuttings varies from cultivar, location, season and age of the branch. Besides, growth regulators and bio-fertilizers also play an important role in rooting and growth of pomegranate cutting. Therefore in order to improve rooting ability and success per cent, techniques have been improved with the use of synthetic root promoting growth regulator and biofertilizers[3]. The application of indole butyric acid (IBA) and naphthalene acetic acid (NAA) induce rooting in stem cuttings and in air layers due to their ability to activate cambium regeneration, cell division and cell multiplication[8]. The combined effect of biofertilizers and growth regulator have showed better root and shoot parameters which could be attributed to increased levels of growth promoting substance available due to synergistic effect of both biofertilizer and growth regulators in various ways[3].

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Chlorophylls are a dominant factor controlling leaf optical properties of healthy green vegetation and are thus an essential part of the photosynthetic process. They harness light energy from the sun to store it as chemical energy[7]. Within the cross-section of healthy green leaves, chlorophyll pigments are located in the chloroplasts of palisade parenchyma cells. Leaf area is an important variable for most ecophysiological studies in terrestrial ecosystems concerning light interception, evapotranspiration, photosynthetic efficiency, fertilizers, and irrigation response and plant growth[2].

MATERIAL AND METHODS

The present investigation was carried out during 2015-16, in the Department of Fruit Science, College of Horticulture, UHS, Campus GKVK, Bengaluru -560065, Karnataka, India. The experiments were laid out in Randomized Block Design (RBD), and the stem cuttings of pomegranate cultivars Bhagwa and Ruby were taken into study. The experiments consist of eleven treatment combination auxin and biofertilizers were taken into study viz.T_1 - IBA 1000 ppm + NAA 1000 ppm, T_2 - IBA 1500 ppm + NAA 1500 ppm, T_3 - IBA 1000 ppm + NAA 1000 ppm + Biomix, T_4 - IBA 1000 ppm + NAA 1000 ppm + PSB, T_5 - IBA 1000 ppm + NAA 1000 ppm + PGPR, T_6 - IBA 1500 ppm + NAA 1500 ppm + Biomix, T_7 - IBA 1500 ppm + NAA 1500 ppm + PSB, T_8 - IBA 1500 ppm + NAA 1500 ppm + PGPR, T_9 - IBA 1000 ppm + NAA 1000 ppm + PSB + PGPR, T_{10} - IBA 1500 ppm + NAA 1500 ppm + PSB + PGPR and T_{11} - Control. Twenty five cuttings were used for each treatment which was replicated thrice. The stem cuttings were treated to different concentration of auxin solution for 6 hours and planted in a media of sand, soil and fym (1:1:1) equal proportion along with the biofertilizers (PGPR, PSB and Biomix) 10 g/pot. The cutttings were kept under shade net condition.

Total chlorophyll content of leaf

Chlorophyll was extracted by immersing of leaf discs (100 mg) collected randomly from five leaves of each treatment using Dimethyl Sulphoxide (DMSO) 10 ml each overnight. The extract was used for measuring optical density. The optical of the extract was used for measuring at two wavelengths i.e., 645 and 663 nm by using spectrophotometer. Chlorophyll fraction a, b and total were calculated as per the following formula, Hiscox and Israelstam[4].

$$\text{Chlorophyll a} = 12.7(A_{663}) - 2.69(A_{645}) \times \frac{V}{1000 \times W}$$

$$\text{Chlorophyll b} = 22.9(A_{645}) - 4.68(A_{663}) \times \frac{V}{1000 \times W}$$

$$\text{Total chlorophyll (Mg/g of leaf)} = \text{Chlorophyll a} + \text{Chlorophyll b}$$

W = weight of leaf sample

V = volume made up

Leaf area (cm2)

All the leaves were collected from labeled plants at 120 days after planting and leaf area was measured with the help of leaf area meter and mean was computed and expressed in centimeter square.

RESULTS AND DISCUSSION

Total chlorophyll content of leaf (mg/g)

Study on the total chlorophyll content of leaves in two cvs. Bhagwa and Ruby stem cuttings as influenced by biofertilizers and auxin are presented in Table 1. There was significant dereference recorded with respect

to chlorophyll content of leaves. The highest chrorophyll content (5.13 mg/g) in cv. Bhagwa and (5.66 mg/g) in cv. Ruby recorded with 1500 ppm + NAA 1500 ppm + Biomix (T_6) followed by (T_{10}) IBA 1500 ppm + NAA 1500 ppm + PSB + PGPR, with chrorophyll content of (4.79 mg/g) in cv. Bhagwa, and (5.10 mg/g) in cv. Ruby, which was on par with (T_7) IBA 1500 ppm + NAA 1500 ppm + PSB with chrorophyll content of (4.52 mg/g) in cv. Bhagwa. While the lowest chlorophyll content of leaves was recorded in control with chlorophyll content of (2.36 mg/g) in cv. Bhagwa, and (2.62 mg/g) in cv. Ruby. According to Kaur *et al.*[6], the total chlorophyll content of leaves in grape vine stem cuttings enhanced after IBA treatment. Similarly, Sivaci and Yalcin[10], reported that total leaf chlorophyll content in stem cuttings of three apple kinds ('Golden Delicious, Starkrimson Delicious' and Misket Delicious) were significantly increased by treatment of 2000 and 3000 mg/l IBA. Sharma and Bhutani[9], reported that the inoculation of *Azotobactor chrocooccum* produced higher chlorophyll content in apple seedling.

Leaf area (cm^2)

The data on leaf area as influenced by different treatments of bio fertilizers and auxin in two cultivar of pomegranate are presented in (Table 1.). The leaf area of cuttings treated with different treatment of bio-fertilizers and auxin showed significant difference. The highest leaf area (41.31cm^2) in cv. Bhagwa and (42.49 cm^2) in cv. Ruby, recorded with 1500 ppm + NAA 1500 ppm + Biomix (T_6) followed by (T_{10}) 1500 ppm + NAA 1500 ppm + PSB + PGPR (40.70cm^2) in cv. Bhagwa and (41.59cm^2) in cv. Ruby, which was on par with (T_7) IBA 1500 ppm + NAA 1500 ppm + PSB, with leaf area of (39.15 cm^2) in cv. Bhagwa, and (40.73 cm^2) in cv. Ruby, and (T_8) IBA 1500 ppm + NAA 1500 ppm + PGPR, with leaf area of (39.62 cm^2) in cv. Bhagwa, and (40.34 cm^2) in cv. Ruby, and (T_9) IBA 1000 ppm + NAA 1000 ppm + PSB + PGPR (38.68 cm^2) in cv. Bhagwa, and (39.34 cm^2) in cv. Ruby respectively. While the least leaf area recorded in control with a leaf area of (33.54 cm^2), in cv. Bhagwa, and (34.35 cm^2) in cv. Ruby. Bhat *et al*.[1], reported that, that treatment of hard wood cuttings of pomegranate with a mixture of IBA 3000 ppm and 2 per cent Borax for 15 minutes, gave the highest mean total leaf area. Similarly, Ismail and Asghar[5], reported that cuttings of *Ficus Hawaii* showed maximum leaf area (19.33 cm2) when cuttings were treated with IBA 4000 ppm. In another study, Vinayak and Bagyaraj[12], reported that application of *Glomus macrocarpum*, *Glomus caledonium*, *Glomus velum*, *Glomus munosporum* and *Gigaspora margarita* increase the leaf area as well as increase the phosphorous, zinc and copper content in the root and shoot of Troyer citrange which would result in better development of the plant. Sonawane and Konde[11], found that use of *Azospirrillim* in combination with the VAM culture enhanced the *Mycorrhizal* root colonization and spore count, and increased the leaf area and reduced the time to sprout of grapevine.

Table 1: Influence of bio-fertilizers and auxin on total chlorophyll content of leaf and leaf area in pomegranate cultivars

Treatments	Bhagwa		Ruby	
	Total chlorophyll content of leaf mg/g	Leaf area (cm^2)	Total chlorophyll content of leaf mg/g	Leaf area (cm^2)
T_1 = IBA 1000 ppm + NAA 1000 ppm	2.61	34.36	2.91	35.52
T_2= IBA 1500 ppm + NAA 1500 ppm	2.87	35.31	3.53	36.43
T_3= IBA 1000 ppm + NAA 1000 ppm + Biomix	3.46	37.71	4.36	38.50
T_4 = IBA 1000 ppm + NAA 1000 ppm + PSB	3.09	36.25	3.94	37.35
T_5 = IBA 1000 ppm + NAA 1000 ppm + PGPR	3.26	36.71	4.07	37.75
T_6 = IBA 1500 ppm + NAA 1500 ppm + Biomix	5.13	41.31	5.66	42.49
T_7 = IBA 1500 ppm + NAA 1500 ppm + PSB	4.52	39.15	4.58	40.73
T_8 = IBA 1500 ppm + NAA 1500 ppm + PGPR	4.32	39.62	4.73	40.34
T_9 = IBA 1000 ppm + NAA 1000 ppm + PSB + PGPR	3.84	38.68	4.43	39.34
T_{10} = IBA 1500 ppm + NAA 1500 ppm + PSB + PGPR	4.79	40.70	5.10	41.59
T_{11}= Control	2.36	33.54	2.62	34.35
SE.m±	0.24	1.11	0.29	1.09
CD at 5%	0.70	3.27	0.85	3.23
CV	8.76	9.23	8.78	6.91

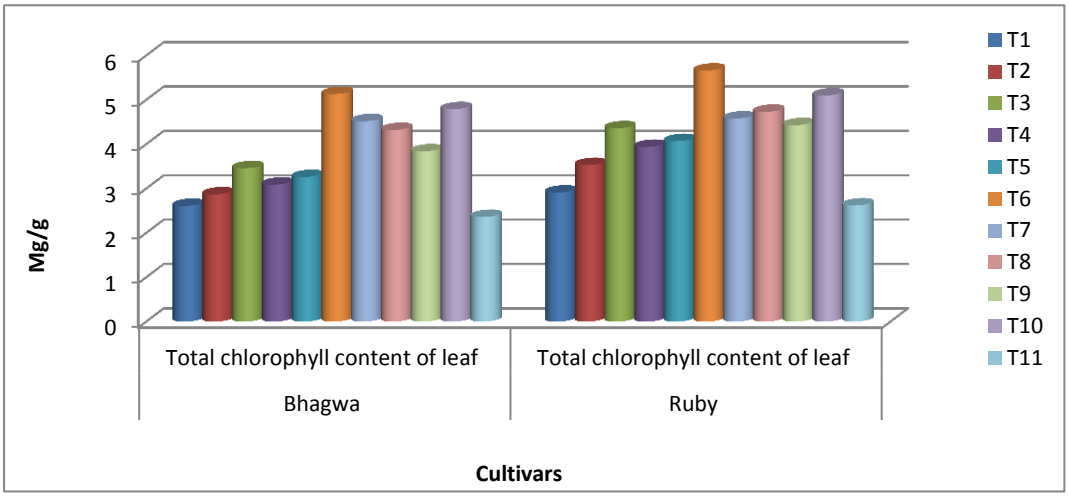

Fig. 1: Influence of bio-fertilizer and auxin on total chlorophyll content of leaf in pomegranate cultivars

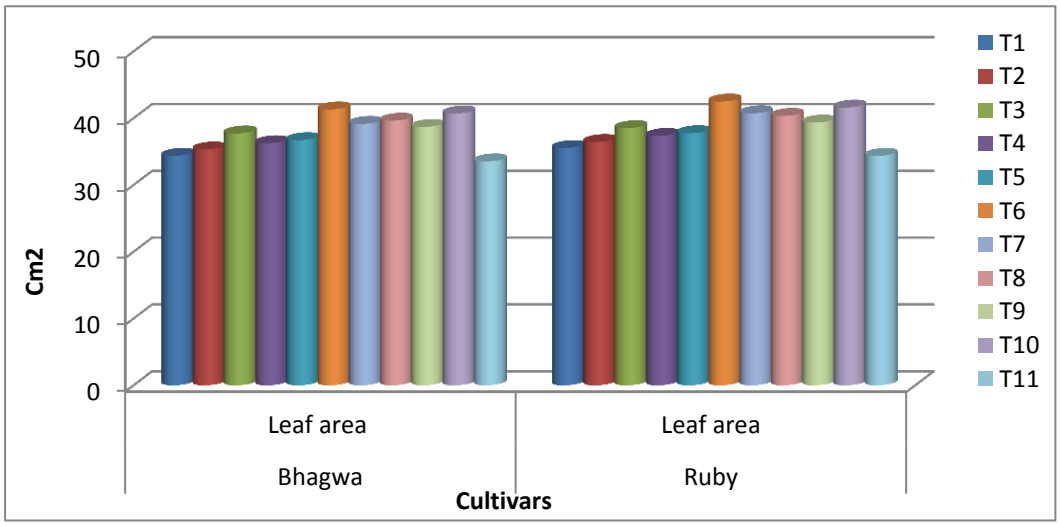

Fig. 2: Influence of bio-fertilizer and auxin on leaf area in pomegranate cultivars

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