Research Article

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Effect of plant growth regulators on leaf area, chlorophyll content, carotenoids, stomatal count and yield of cashew (*Anacardium occidentale* L.) var. Bhaskara

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Abstract

An experiment was conducted to study the effect of exogenous application of growth regulators at three important growth stages (flushing, flowering and fruiting) on leaf area, chlorophyll content, carotenoids, stomatal count and yield of cashew var. Bhaskara. Irrespective of growth stages, foliar application of GA_3 @ 50 ppm and ethrel @ 50 ppm was found to be superior in all the parameters and on par with each other compared to other growth regulators. Out of nine treatments of different growth regulators; the highest leaf area was recorded in trees sprayed with GA_3 @ 50 ppm and ethrel @ 50 ppm. At flushing stage, spraying with GA_3 @ 50 ppm resulted in highest stomatal number (21.9) and carotenoids (0.41) whereas unsprayed (control) trees recorded least stomatal number (11.6) and carotenoids (0.19). Thus, leaf area, chlorophyll content, carotenoids and stomatal count increased in trees sprayed with growth regulators than unsprayed trees. Spraying of ethrel @ 50 ppm recorded highest nut yield (14.3 kg tree^{-1}) followed by NAA @ 25 ppm + GA_3 50 ppm (12.9 kg tree^{-1}). This study demonstrated the potential of ethrel as well as GA_3 in improving various biochemical parameters *viz.*, chlorophyll 'a', chlorophyll 'b', carotenoids and leaf area in cashew which are important determinants in increasing nut production.

Keywords: Cashew, growth regulators, chlorophyll, carotenoids, stomatal count, yield

Introduction

Cashew nuts are globally consumed for their desirable sensory and nutritional attributes. Among the dry fruits, cashew nuts are very popular due to their characteristic odour and taste. They are a good source of proteins (20%), carbohydrates (23%) and fat (45%) (Bhattacharjee *et al.,* 2003). The global production of cashew is around 37,13,467 MT from a total of 60,37,313 hectares (FAO, 2014). India is the second largest producer of raw cashew nut in the world. India produces about 0.78 million MT of cashew from an area of 1.04 million hectares with a productivity of 0.75 MT ha^{-1} (DCCD, 2017).

Pigments in general and chlorophyll in particular are most important for green plants to effectively harvest solar radiation and to convert it into chemical energy. Chlorophyll 'a' plays an important primary role in the photosynthetic process. The status of chlorophyll pigments in the leaf tissue is thus a major determinant of overall photosynthetic efficiency of the plants. The photosynthetic efficiency directly influences the growth, development and yield of the crops. The carotenoids, are organic pigments and act as passive light filters that would reduce light intercepted by chlorophyll (Williams *et al*., 2003) and thereby provide protection from reactive oxygen species (ROS) (Steyn *et al*., 2002).

Plant growth regulators can improve the physiological efficiency including photosynthetic ability and thereby helping in effective flower formation, fruit and seed development and ultimately enhance productivity of the crops (Solaimalai *et al.,* 2001). Role of growth regulators on pigment concentration has been documented in

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herbaceous crops (Kavina *et al.,* 2011). Gibberellic acid increases the vegetative growth and pigment concentration in maize (Kaya *et al.,* 2006). Foliar application of GA_3 improved the chlorophyll levels in salinity stressed maize plants (Tuna *et al.,* 2008). However, in a perennial fruit tree like cashew, such reports are not available. Prolonged flowering, poor production of hermaphrodite flowers, low fruit set and premature fruit drop are some major problems plaguing cashew cultivation across the country (Bhat *et al*., 2010). Foliar application of plant growth regulators can be resorted for improving growth, photosynthesis, flowering and fruiting in cashew. Hence, it is worthwhile to find out the effect of foliar application of growth regulators on the concentration of pigments like chlorophyll 'a' and 'b' during the plant growth as they are vital components for photosynthesis. Hence, an investigation was taken up to study the influence of foliar application of growth regulators on leaf area, photosynthetic pigments and stomatal density in cashew.

Materials and methods

The experiment was conducted at ICAR-Directorate of Cashew Research, Puttur, Karnataka. The experimental site situated in a cashew growing belt, has typical lateritic soils of the west coast, located 87 m above mean sea level with latitude of 12.77° N and longitude of 75.22° E. The climate is hot and humid throughout the year with an average annual rainfall of 3,500 mm, distributed mainly from June to September. The mean annual temperature is 27.6 °C and mean maximum and minimum temperature are 36 °C and 20 °C, respectively. The study was carried out on 10 years old plantation (variety Bhaskara) by adopting randomized block design (RBD) with 9 treatments and 3 replications. The treatments were control (T_1), ethrel @ 50 ppm (T_2), 2,4-D @ 10 ppm (T_3), NAA @ 25 ppm (T_4), IAA @ 10 ppm (T_5), BA @ 1000 ppm (T_6) , GA_3 @ 50 ppm (T_7), NAA @ 25 ppm + GA_3 @ 50 ppm (T_8) and IAA @ 100 ppm + GA_3 @ 50 ppm (T_9). The plant growth regulators were sprayed during flushing, flowering and fruiting stage using foot pump paddle sprayer covering the entire canopy. Leaf area was measured using CI-202 Portable Laser Area Meter.

Leaf chlorophyll content

Chlorophyll 'a', chlorophyll 'b' and total chlorophyll content of the leaves were measured by following the method of Arnon (1949) and carotenoids by Goodwin's method (1954). One gram leaf sample was taken and chlorophyll was extracted in 80 per cent acetone by grinding in a clean mortar. The resulting green liquid was filtered through whatman No. 1 filter paper. The grinding and filtration were repeated 3-4 times for each sample with fresh aliquots of 80 per cent acetone for ensuring the complete extraction of chlorophyll. The final volume was made to 100 ml with 80 per cent acetone. The absorbance of chlorophyll was recorded with spectrophotometer (Spectronic 20) at 645 and 663 nm against 80 per cent acetone solvent as blank. The entire procedure was carried out in a dark room to avoid the loss of chlorophyll with direct contact of light. Chlorophyll 'a' and chlorophyll 'b' contents were calculated. In contrast to the chlorophylls, which absorb light in two regions of the visible spectrum, the carotenoids exhibit intense absorption only in 350-500 nm.

Calculations

Arnon's equation to convert absorbance measurements to mg chl g^{-1} leaf tissue is given below.

Chl a (mg g^{-1}) = $[(12.7 \times A_{663}) - (2.6 \times A_{645})] \times$ ml acetone / mg leaf tissue

Chl b (mg g^{-1}) = $[(22.9 \times A_{645}) - (4.68 \times A_{663})] \times$ ml acetone / mg leaf tissue

Total Chl = Chl a + Chl b.

$C_{x+c} = 1000\ A_{470} - 1.90$ Chl a $- 63.14$ Chl b/ 214, (x = xanthophylls and c = carotenes).

Stomatal frequency

Stomatal frequency of the leaf under microscopic field (10 x 100 magnifications) was determined, as per the procedure of Beakbane and Mujumder (1975).

Statistical analysis

Data generated from the experimental plots were analyzed using SAS 9.3 version of the statistical package (SAS Institute Inc, 2011). Analysis of variance (ANOVA) was performed using SAS PROC ANOVA procedure. Means were

separated using Fisher's protected least significant difference (LSD) test at a probability level of $p<0.05$.

Results and discussion

Leaf chlorophyll content

Plant growth regulators sprayed at various stages of crop growth exerted significant differences on leaf chlorophyll a and b contents (Table 1). At flushing stage, spraying of ethrel @ 50 ppm recorded highest value of chlorophyll a (1.62), followed by GA_3 @ 50 ppm (1.61), NAA @ 25ppm + GA_3 @ 50 ppm (1.43), IAA @ 100 ppm + GA_3 @ 50 ppm (1.30), BA @ 1000 ppm (1.11), IAA @ 100 ppm (1.06), NAA @ 25 ppm (0.92) and 2,4-D @ 10 ppm (0.73) while unsprayed trees (control) registered least chlorophyll a content (0.67). At flowering stage also, spraying of ethrel @ 50 ppm recorded highest chlorophyll a (1.32) whereas control recorded least chlorophyll a value (0.55). However, spraying of GA_3 @ 50 ppm at fruiting stage registered highest chlorophyll a (0.84). The chlorophyll content increased gradually with leaf expansion (Balasimha, 1991; Palanisamy *et al*., 1993). GA_3 significantly increased the total chlorophyll contents in *Mentha piperita* (Kavina *et al.,* 2011).

At flushing stage, spraying of GA_3 @ 50 ppm recorded highest chlorophyll b (0.86) content, followed by Ethrel @ 50 ppm (0.84), NAA @ 25ppm + GA_3 @ 50 ppm (0.76), IAA @ 100 ppm + GA_3 @ 50 ppm (0.74), BA @ 1000 ppm (0.41), IAA @ 100 ppm (0.32) and NAA @ 25 ppm (0.32), 2,4-D @ 10 ppm (0.31) while lowest (0.29) chlorophyll b value was recorded in unsprayed trees (control). Highest chlorophyll b (0.35) was recorded with spraying of GA_3 @ 50 ppm and ethrel @ 50 ppm at flowering stage whereas the lowest chlorophyll b (0.15) was recorded with control. At fruiting stage, chlorophyll b content was not influenced by the growth regulators.

Carotenoids

Carotenoids content in the leaves of cashew var. Bhaskara significantly differed with foliar application of growth regulators during flushing, flowering and fruiting stages. At flushing stage, spraying of GA_3 @ 50 ppm recorded highest carotenoids (0.41) content which was on par with IAA @ 100 ppm + GA_3 @ 50 ppm (0.40). However, spraying of NAA @ 25 ppm + GA_3 @ 50 ppm during flowering stage and ethrel @ 50 ppm during fruiting stage recorded highest carotenoids content (0.38) and (0.19), respectively. Irrespective of growth stages, lowest carotenoids content was recorded in unsprayed (control) trees (Table 2).

Stomatal number

Stomatal number and density can vary within leaves, plants, and individuals of a single species.

Table 1. Effect of growth regulators on chlorophyll contents in cashew

Treatment	Chlorophyll a			Chlorophyll b			Total chlorophyll		
	Flushing	Flowering	Fruiting	Flushing	Flowering	Fruiting	Flushing	Flowering	Fruiting
Control	0.67 ^{F}	0.55 ^{D}	0.39 ^{C}	0.29 ^{B}	0.15 ^{C}	0.12	0.98 ^{D}	0.69 ^{F}	0.52 ^{C}
Ethrel@50 ppm	1.62 ^{A}	1.32 ^{A}	0.74 ^{AB}	0.84 ^{A}	0.35 ^{A}	0.14	2.45 ^{A}	1.67 ^{A}	0.88 ^{B}
4-D@10 ppm	0.73 ^{EF}	0.60 ^{CD}	0.40 ^{C}	0.31 ^{B}	0.14 ^{C}	0.13	1.01 ^{D}	0.75 ^{EF}	0.53 ^{C}
NAA@25ppm	0.92 ^{DE}	0.74 ^{C}	0.38 ^{C}	0.32 ^{B}	0.16 ^{BC}	0.12	1.24 ^{CD}	0.90 ^{DE}	0.50 ^{C}
IAA@100 ppm	1.06 ^{D}	0.74 ^{C}	0.38 ^{C}	0.32 ^{B}	0.17 ^{BC}	0.12	1.38 ^{C}	0.91 ^{DE}	0.49 ^{C}
BA @1000 ppm	1.11 ^{CD}	0.76 ^{C}	0.41 ^{C}	0.41 ^{B}	0.19 ^{BC}	0.14	1.52 ^{C}	0.95 ^{D}	0.56 ^{C}
GA_3@50 ppm	1.61 ^{A}	1.16 ^{AB}	0.84 ^{A}	0.86 ^{A}	0.35 ^{A}	0.14	2.47 ^{A}	1.49 ^{AB}	0.97 ^{AB}
NAA @25 ppm + GA_3 50 ppm	1.43 ^{AB}	1.07 ^{B}	0.82 ^{A}	0.76 ^{A}	0.26 ^{AB}	0.15	2.18 ^{AB}	1.34 ^{BC}	1.23 ^{A}
IAA @100 ppm + GA_3 50 ppm	1.30 ^{BC}	0.98 ^{B}	0.65 ^{B}	0.74 ^{A}	0.26 ^{AB}	0.15	2.03 ^{B}	1.24 ^{C}	1.13 ^{AB}
Mean	1.16	0.88	0.56	0.54	0.23	0.13	1.70	1.10	0.76
SE(d)	0.11	0.08	0.06	0.08	0.05	0.03	0.15	0.09	0.15
LSD @ 5%	0.23	0.18	0.12	0.17	0.10	NS	0.32	0.20	0.31

Values followed by the same alphabets do not differ significantly

Table 2. Effect of growth regulators on leaf area, carotenoids and stomatal numbers in cashew

Treatment	Leaf area (cm^2)			Carotenoids			Stomatal number		
	Flushing	Flowering	Fruiting	Flushing	Flowering	Fruiting	Flushing	Flowering	Fruiting
Control	66.95 ^{F}	62.76 ^{F}	62.03 ^{F}	0.19 ^{G}	0.15 ^{H}	0.12 ^{D}	11.58 ^{E}	11.20 ^{F}	10.43 ^{D}
Ethrel@50 ppm	144.56 ^{A}	143.03 ^{A}	143.76 ^{A}	0.39 ^{B}	0.31 ^{C}	0.19 ^{A}	19.83 ^{B}	16.30 ^{A}	12.03 ^{BC}
2,4-D@10 ppm	71.33 ^{E}	67.10 ^{E}	67.30 ^{E}	0.27 ^{E}	0.23 ^{F}	0.14 ^{BC}	13.03 ^{DE}	12.23 ^{E}	11.47 ^{C}
NAA@25ppm	91.78 ^{D}	91.30 ^{D}	91.10 ^{D}	0.27 ^{E}	0.22 ^{G}	0.14 ^{BC}	18.50 ^{BC}	13.50 ^{D}	10.77 ^{D}
IAA@100 ppm	127.27 ^{C}	125.73 ^{C}	125.70 ^{C}	0.25 ^{F}	0.29 ^{D}	0.14 ^{BC}	13.90 ^{D}	13.90 ^{CD}	12.00 ^{BC}
BA @1000 ppm	130.31 ^{B}	128.70 ^{B}	128.73 ^{B}	0.32 ^{D}	0.26 ^{E}	0.13 ^{C}	17.67 ^{C}	14.10 ^{C}	11.60 ^{BC}
GA_3@50 ppm	145.08 ^{A}	142.60 ^{A}	142.97 ^{A}	0.41 ^{A}	0.35 ^{B}	0.15 ^{B}	21.87 ^{A}	14.17 ^{C}	12.00 ^{BC}
NAA@25 ppm+GA_3 50 ppm	129.14 ^{BC}	127.97 ^{B}	127.60 ^{B}	0.35 ^{C}	0.38 ^{A}	0.14 ^{BC}	18.83 ^{BC}	14.13 ^{C}	12.17 ^{B}
IAA@100 ppm+GA_3 50 ppm	128.78 ^{BC}	125.70 ^{C}	125.70 ^{C}	0.40 ^{AB}	0.33 ^{B}	0.15 ^{BC}	18.90 ^{BC}	15.53 ^{B}	14.30 ^{A}
Mean	115.02	112.77	112.77	0.32	0.28	0.14	17.12	13.90	11.86
SE(d)	1.05	0.60	0.60	0.009	0.007	0.008	0.91	0.23	0.31
LSD @ 5%	2.23	1.26	1.26	0.019	0.02	0.02	1.93	0.48	0.65

Values followed by the same alphabets do not differ significantly

It can also vary due to environmental factors such as light, air humidity, water availability and atmospheric CO_2 concentration. Spraying of GA_3 @ 50 ppm at flushing stage recorded highest stomatal number (21.9) and unsprayed (control) trees recorded least stomatal number (11.6). At flowering stage, spraying of ethrel @ 50 ppm resulted in highest stomatal number (16.3) whereas unsprayed (control) trees recorded least stomatal number (11.2). At fruiting stage, spraying of IAA @ 100 ppm + GA_3 @ 50 ppm resulted in highest stomatal number (14.3) whereas unsprayed (control) trees recorded the least (10.4) stomatal number (Table 2). In the present context, increased leaf area associated with growth regulators spray could be the reason for increased stomatal count.

Leaf area

Effect of foliar application of growth regulators on leaf area of cashew is presented in Table 2. At flushing stage, spraying with GA_3 @ 50 ppm resulted in highest leaf area (145.1 cm^2) which was on par with ethrel @ 50 ppm (144.6 cm^2), whereas, unsprayed (control) trees recorded least leaf area (67.0 cm^2). Spraying with ethrel @ 50 ppm during flowering stage resulted in highest leaf area (143.03 cm^2) which was on par with GA_3 @ 50 ppm (142.6 cm^2). At fruiting stage, spraying with ethrel @ 50 ppm resulted in highest leaf area (143.76 cm^2) whereas the least leaf area of 62.0 cm^2 was recorded with unsprayed (control) trees. The GA_3 is associated with leaf expansion of cereal grains (Akazawa *et al.,* 1990; Matsuoka 2003; Swain and Singh, 2005; Khassawneh *et al.,* 2006; Srivastava and Srivastava, 2007). GA_3 is widely regarded as a growth promoting compound that positively regulates leaf expansion (Swain and Singh, 2005). Role of growth regulators in increasing leaf area can be ascribed to their influence on cell division and cell elongation.

Plant leaf area is an important determinant of light interception and consequently of transpiration, photosynthesis and plant productivity (Goudriaan and Van Laar, 1994; Wahdan *et al.,* 2011). This increase in leaf area with GA_3 might be related to the fact that GA_3 promote leaf area through the increase of cell division in higher plant (Hartmann *et al,* 2002; Hopkins and Huner, 2004; Harris *et al.,* 2004). Higher leaf area values recorded with Ethrel and GA_3 may also be due to increased concentration of photosynthesis in the shoot (Nunez *et al.,* 1998; Zofoli *et al.,* 2009 and Zahoor *et al.,* 2011) as reported in grape.

Nut yield

Application of plant growth regulators had a role in increasing or decreasing various yield attributing parameters and yield of different crops. In the present study, it was concluded that the application of ethrel @ 50 ppm and NAA @ 25 ppm were found to be beneficial for increasing the nut yield. Considering the effects of different growth

Table 3. Effect of growth regulators on nut yield of cashew (*Anacardium occidentale* L.) var. Bhaskara

Treatment	Nut yield (kg tree^{-1})
Control	5.2
Ethrel@50 ppm	14.3
2,4-D@10 ppm	7.4
NAA@25ppm	12.2
IAA@100 ppm	7.6
BA @1000 ppm	10.3
GA_3 @50 ppm	11.6
NAA @25 ppm + GA_3 50 ppm	12.9
IAA @100 ppm + GA_3 50 ppm	8.0
SEm±	0.4
LSD ($p<0.05$)	1.1

regulators studied, spraying of ethrel @ 50 ppm results in highest nut yield (kg) per tree over other treatments. Lowest nut yield was recorded in control treatments. Increased nut yield with application of growth regulators could be attributed to increased number of bisexual flowers, fruit set, fruit retention and total number of nuts per tree (Veeraraghavathatham and Palaniswamy, 1983).

Spraying of growth regulators in all the treatments have given higher nut yield compared to control. However, spraying of ethrel and GA_3 independently and also in combination gave better yield compared to other treatments. It may be because of ethrel and auxin could induce better flowering in cashew (Aliyu *et al*., 2011). Auxin is known to induce flowering *via* ethylene production and also independently (Li and Xu, 2014). Other reasons for more nut yield compared to control are growth regulators/hormone sprayed leaf area mobilizes all the photosynthates and nutrients which will be utilized for flower production and fruit growth (Li and Xu, 2014). And other reasons might be increased stomatal number increases inflow of carbon dioxide into the mesophyll tissue resulting more photosynthates, latter partitioned towards nut resulted in more nut yield (Aliyu *et al*., 2011).

Conclusion

Irrespective of the stage of the crop growth such as flushing, flowering and fruiting, growth regulators treated plants resulted in more chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, leaf area and stomatal number compared to control plants. However, among the different growth regulators, spraying of ethrel @ 50ppm and GA @ 50 ppm independently resulted in more production of chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, leaf area and stomatal number compared to control. Therefore, it can be concluded from the present study that growth regulators like either ethrel @ 50 ppm or GA_3 @ 50 ppm could be effectively employed to increase leaf area, chlorophyll content and nut yield in cashew.

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