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Response of growth regulators to physiological and biochemical parameters of mango (*Mangifera indica* L.) under high density planting system

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Abstract
The experiment was conducted on eight years old mango cv. 'Sindhuram' grown under high density planting system with an objective to study the response of growth regulators on physiological and biochemical parameters in mango. The trees were treated with five treatments *viz*., paclobutrazol (PBZ) @ 8 ml/10 l (T_1), KNO_3 @ 4% (T_2), ethephon @ 200 ppm (T_3), salicylic acid @ 2000 ppm (T_4) and control (T_5) applied through foliar spray one month after pruning and applied twice at 15 days interval. The results revealed that foliar application of KNO_3 @ 4% (T_2) showed highest stomatal frequency (691.44 stomata/mm^2), stomatal conductance (0.134 mol m^{-2} s^{-1}), C/N ratio at flowering (14.47) and two months after flowering (13.82) and chlorophyll content (1.95 mg/g). However, no significant difference was observed in leaf photosynthetic and transpiration rate after growth regulator application.

Keywords: Growth regulator, mango, foliar spray, stomatal conductance, C/N ratio

1. Introduction
The mango (*Mangifera indica* L.) is the leading, most well-known tropical fruit and is referred to as the "King of Fruits" for its delightful flavour, outstanding aroma, appealing colour, and other desirable qualities. The world's oldest fruit is in cultivation for more than 4,000 years. Mango is India's national fruit because of its long-standing close ties to the country's religious, cultural, aesthetic and economic values. It is the most well-known tropical fruit in the Anacardiaceae family and is indigenous to Indo-Burma in Southeast Asia.
With thousands of different varieties of mangoes growing all over the country, India has the most abundant mango genetic diversity. This cultivar is commercially grown in west coast of India comprising of Maharashtra, Goa, Karnataka and Gujarat states.
Mango production is limited by a lack of environmental factors to flowering, Flowering and fruiting is a complex phenomenon in mango. The conceptual model of mango flowering has been described by Davenport and Nunez-Elisea (1997) [2] to simplify the interaction of environmental and internal factors that control the commencement and induction of vegetative and reproductive shoots in mango trees in tropical and subtropical environments. The biochemical components of floral stimuli at the bud break stage have a significant impact on mango flowering. Additionally, the maturation of terminal shoots and the accumulation of carbohydrates in leaves as well as the shoot apex are certainly associated to the generation of the floral stimulus in mango trees. The enhancement of crop productivity in the current farming system heavily relies on chemically manipulating the physiological processes of the crop. Chemical regulators like paclobutrazol, KNO_3, ethephon, salicylic acid *etc*. are used to alter physiological and biochemical response of mango. The present study was therefore proposed to study the response of physiological and biochemical characters of mango cv. 'Sindhuram' as influenced by growth regulator application.

2. Materials and Methods
The present study was carried out in Muthalamada region of Palakkad district during 2019-2020. The experimental site is located at 10^0 61' N and 76^0 69' E and at an elevation of 103 meters above MSL. The temperature recorded during the period of study ranged from 26 ℃ to 36 ℃ with a rainfall ranging from 2.25 mm in to 304.0 mm. It was found that the soil type of the experimental field had sandy loam surface soils and sandy clay sub soils.

The experiment was laid out in Completely Randomized Design (CRD) with four replicates and five treatments *viz*., paclobutrazol (PBZ) @ 8 ml/10l (T_1), KNO_3 @ 4% (T_2), ethephon @ 200 ppm (T_3), salicylic acid @ 2000 ppm (T_4) and control (T_5) with three observational trees for each replication under high density planting system with a spacing of 6 m x 2 m. The above treatments were given as foliar spray one month after pruning and applied two times at 15 days interval. The observations of stomatal conductance, photosynthetic and transpiration rate were measured from third or fourth healthy leaf of mango cv. 'Sindhuram' by portable photosynthetic system (LICOR-6400) using natural light source between 8.00 to 11.00 am (Fig 1). Stomatal frequency was determined by counting the number of stomata present per unit area of leaf. It is done by applying a thin layer of suitable replica fluid (transparent nail polish) and observing the peeled replica under a light microscope (40 X magnification) (Fig 2). For leaf nutrient status (C/N ratio), taking matured leaves were taken at flowering and two months after flowering stage, and collected leaf samples were estimated for total carbohydrate and nitrogen. Total carbohydrate were estimated using anthrone reagent method (Sadasivam and Manickam, 1991) [8] as calculated as follows:

$$\text{Carbohydrate (\%)} = \frac{\text{OD (sample) x standard concentration x total volume}}{\text{OD (standard) x volume taken x weight of sample}}$$

Total nitrogen was determined by micro-kjeldahl method (Sadasivam and Manickam, 1991) [8] and calculated as follows:

$$\text{Nitrogen (\%)} = \frac{\text{Normality x titre value x 0.014 x 100}}{\text{Weight of sample}}$$

C/N ratio of leaf sample was obtained by dividing total carbohydrates to total nitrogen

Leaf chlorophyll content was determined using DMSO method and sample was read in UV-Vis spectrophotometer at 663 and 645 nm using pure DMSO as blank (Hiscox and Israelstam, 1979) [3]. Total chlorophyll was calculated as follows:

$$\text{Total chlorophyll (mg/g)} = \frac{(20.7 \text{ x } OD_{645}) + (8.02 \text{ X } OD_{663}) \text{ X dilution x volume}}{1000 \text{ x weight of sample}}$$

3. Result and Discussion

The physiological characters like stomatal frequency and stomatal conductance were significantly influenced by growth regulator application (Table 1). The highest stomatal frequency (691.44 stomata/mm^2) in mango cv. 'Sindhuram' was recorded with foliar application of KNO_3 @ 4% (T_2). Lakshmipathi *et al*. (2017) [5] reported that increased leaf area coupled with growth regulator spray may be the possible reason for increased stomatal frequency. Similarly, foliar application of KNO_3 @ 4% (T_2) showed the maximum stomatal conductance (0.134 mol m^{-2} s^{-1}). Increased leaf area may be associated with increased stomatal conductance (Ahmad *et al*., 2016) [1]. Larger leaf surface enables the tree to capture maximum light for physiological processes.

Table 1: Response of growth regulators on stomatal conductance and frequency on mango cv. 'Sindhuram'

Treatments	Stomatal frequency (No. of stomata/mm^2)	Stomatal conductance (mol m^{-2} s^{-1})
T_1	545.90	0.068
T_2	691.44	0.134
T_3	641.25	0.093
T_4	610.56	0.113
T_5	524.79	0.059
CD (0.05)	0.64	0.041

Application of growth regulators showed no significant influence on photosynthetic and transpiration rates in mango cv. 'Sindhuram' (Table 2). It is contradictory from the finding of Singh *et al*. (2001) [9], where they found out enhanced rate of photosynthetic rate and reduced transpiration level with the application of growth regulators in mango.

Table 2: Response of growth regulators on photosynthetic and transpiration rates on mango cv. 'Sindhuram'

Treatments	Photosynthetic rate (µ mol m^{-2} s^{-1})	Transpiration rate (m mol m^{-2} s^{-1})
T_1	0.17	2.03
T_2	0.21	2.13
T_3	0.26	2.24
T_4	0.16	2.21
T_5	0.22	1.78
CD (0.05)	NS	NS

The biochemical parameters like C/N ratio and total chlorophyll content were significantly influenced by growth regulator application (Table 3). The highest C/N ratio at the time of flowering (14.73) and two months after flowering (13.82) was recorded with foliar application of KNO_3 @ 4% (T_2) in mango cv. 'Sindhuram'. The C/N ratio plays a vital role in mango flowering and foliar spray of chemicals influence the C and N contents in the shoot (Malshe *et al*., 2020) [6]. The synthesis of floral stimulus in mango trees are associated with the accumulation of carbohydrate in the leaves and shoot apex (Kumar *et al*., 2013) [4]. Highest carbohydrate content and C/N ratio with the application of KNO_3 was observed by Sudha *et al*. (2012) [10]. This may be due to the elevation of nitrogen concentration over a threshold nitrogen level due to action of KNO_3, thereby synchronizing bud break and initiating floral induction which is mediated by ethylene (Protacio, 2000) [7].

The highest total chlorophyll content (1.95 mg/g) was recorded with the foliar application of KNO_3 @ 4% (T_2). Similar result was obtained by Sudha *et al*. (2012) [10]. Thus, application of growth regulators would be a possible reason for increased chlorophyll content in the present study.

Table 3: Response of growth regulators on C/N ratio and chlorophyll content on mango cv. 'Sindhuram'

Treatments	At flowering			Two months after flowering			Total chlorophyll (mg/g)
	Carbohydrate (%)	Nitrogen (%)	C/N ratio	Carbohydrate (%)	Nitrogen (%)	C/N ratio	
T_1	11.56	0.99	11.65	11.13	1.01	10.93	0.41
T_2	1308	0.88	14.73	12.66	0.91	13.82	1.95
T_3	12.61	0.94	13.25	12.21	0.97	12.46	1.64
T_4	12.23	0.98	12.37	11.87	1.01	11.71	1.56
T_5	10.97	0.99	11.45	10.57	1.02	10.30	0.82
CD (0.05)	0.719	0.046	0.475	1.06	0.04	0.47	0.037

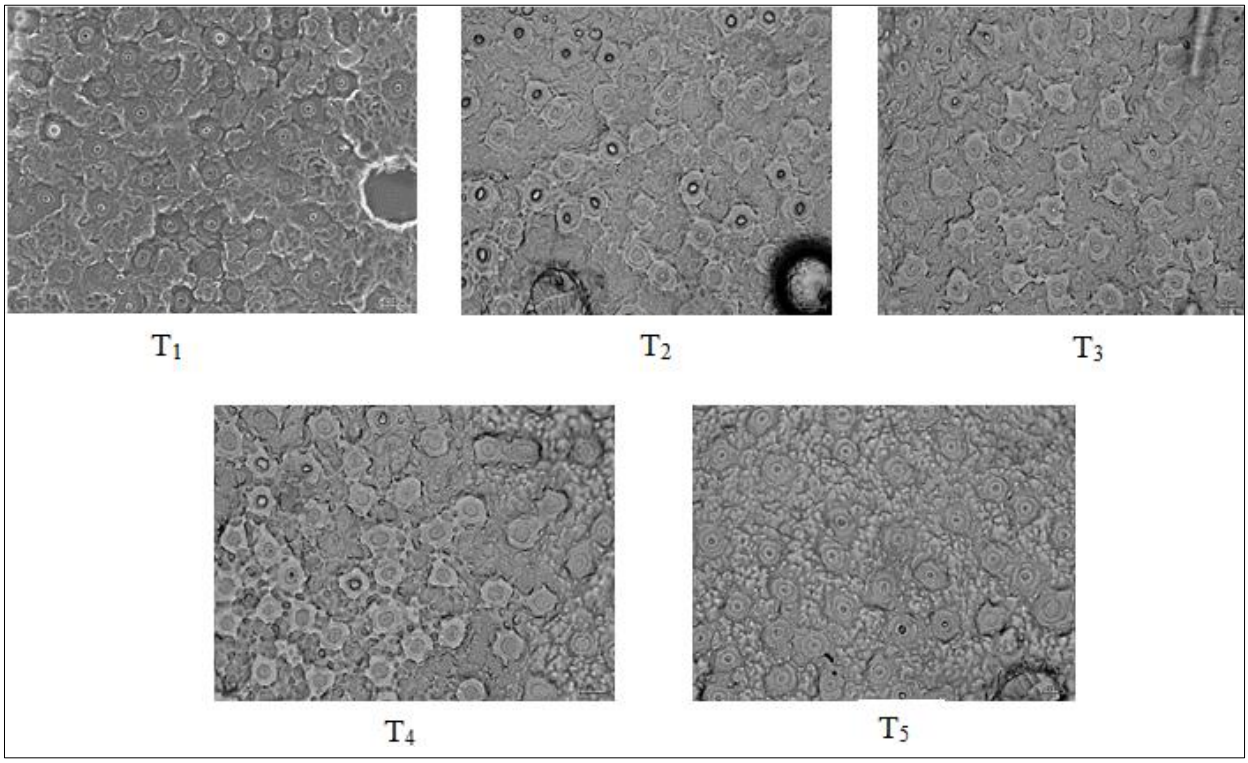

Fig 2: Response of growth regulators on stomatal frequency in mango cv. 'Alphonso'

4. Conclusion

Therefore, it was concluded that foliar application of KNO_3 @ 4% (T_2) at one month after pruning and applied twice at 15 days interval showed a higher stomatal frequency and stomatal conductance, C/N ratio at flowering and two months after flowering and total chlorophyll content in mango cv. 'Sindhuram'. However, photosynthetic and transpiration rates had no significant influence by growth regulator application.

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