

Effect of White, Red, Blue Light and Darkness on IAA, GA and Cytokinin Induced Stomatal Movement and Transpiration

Abstract: Plant growth regulators are the signaling molecules which control physiological as well as metabolic functions starting from seed germination to flowering and senescence. Along with this, light of different wavelengths also play an important role in the life span of plants starting from photosynthesis to transpiration. In this work we tried to find out the effect of plant growth regulators along with lights of various wavelengths on stomatal movement and transpiration rate. In this study we try to observe the influence of auxin, gibberellin, cytokinin in *Piper betel* L. under blue light, red light, white light and darkness in stomatal regulation. it was found that, red light inhibited transpiration rate and stomatal opening in auxin and gibberellin treated leaves although it accelerated cytokinin treated stomatal opening.

Key words: Growth regulators, senescence, transpiration, stomatal movement.

Introduction:

Behavior of stomatal movement is one of the many important aspects of plant growth and development that are governed by light and hormone. Stomatal pores are surrounded by pair of guard cells regulate CO₂ Uptake and water loss stomatal opening is driven by accumulation of

K⁺ salts and sugars in guard cells, which is mediated by electrogenic proton pumps in plasma membrane and / or by coordination of light signaling, light energy conversion membrane ion transport and metabolic activity in guard cells. Stomata regulate gas exchange between cell and atmosphere, optimize photosynthetic CO₂ fixation, and minimize transpirational water loss [1, 2, 3, 4, 5]. Activation of guard cell H⁺- ATPase by IAA may stimulate H⁺ extrusion and stomatal opening [3]. Stomatal opening Induced by IAA in epidermal strips of *Paphiopedilum tonsum* was preceded by a reduction of cytosolic pH [6]. High concentration of auxins such as PAA [7] and NAA [8], suppress stomatal opening. Several reports suggest that auxins can antagonize ABA induced stomatal closure. IAA alleviated the closing effect of ABA in epidermal peels is of

Commelina communis [9] and *Vicia faba*. [10,11] . The effects of foliar application of gibberellic acid (GA_3) are variable [12], although retardation of stomatal closure in water stressed leaves following GA_3 treatment has been observed [13] . This is consistent with a report that GA_3 could reverse triazole – induced stomatal closure in isolated epidermal strips of *Commelina benghalensis*. [14] . Stomatal responses to naturally occurring or synthetic cytokinins are variable although cytokinins can increase stomatal aperture [15, 16]. On the other hand light is a key regulator in developmental process. Light transverse electromagnetically along with other variable factors that influence growth and development of plants.

Plants can generate and perceive light signals. It was deduced that, plant can monitor the intensity quality and duration of light and modulate their development in order to optimize the acquisition of energy for photosynthesis. This process is collectively known as photo morphogenesis [17] showed that plant responses to light through photoreceptor for seed germination and also involved in green seedlings emergence through the soil surface.

In the intact leaves, blue light on a quantum basis is several to 20 times more effective than red light in opening stomata [18]. Blue light acts as a signal and red light as both signal and an energy sources. Blue light activates plasma membrane H^+ -ATPase [19]. Stomatal responses to exogenous auxin dependent plant species, age, environment and source of epidermis (adaxial or abaxial) [7] . Differential behavior on stomatal opening in response to naturally occurring and synthetic cytokinin occurs has been reported [15, 16]. It has been reported that triazole induced stomatal closure in isolated epidermal strips of *Commelina benghalensis* [14] . Light regulation of stomatal movement was showed [1]. Induction of stomatal opening induced by blue and red light was reported [5, 19]. Red light induced stomatal opening was reported by [20] . It has been reported that, opening response of stomata to red light requires a high light intensity and continuous illumination in most of the plant species with *Zea mays* being a notable exception [1] . Red Light induced stomatal opening result from combination of guard cell response and reduction in Cl and direct response of the guard cell chloroplast to red light was reported [21].

The goal of the present study is to examine the effect of blue light, red light, white and darkness in combination with auxin, gibberellin and cytokinin on stomatal movement.

Materials and methods:

Plant Materials: Fresh leaves of betel plant (*Piper betel* L.) of the family Arecaceae were collected from the adjoining area of Kalyani University, Kalyani, Nadia.

Sources of Lights: Zero watt bulbs of red, blue, white were purchased from the local market of Kalyani, Nadia.

Sources of Growth Regulators: 10^{-4} M IAA, 10^{-4} M GA₃, 10^{-4} M cytokinin were freshly prepared on the day of use from the stock solution of IAA, GA and cytokinin.

Experimentals:

Fresh petiolated betel leaves were taken and cut under water with a scalpel. 10^{-4} M IAA, 10^{-4} M GA₃, 10^{-4} M cytokinin was spread on the both sides of the leaves separately on each leaf. The

petiole of each leaf was tied with a glass rod to support the leaf and the leaves were placed under water in conical flask. Some oil was poured into the conical flask on the water surface to avoid physical evaporation. The experimental sets up were then placed under different light sources like red light, blue light, white light and darkness. Determination of transpiration rate was done by weighing method. Leaf areas were measured by graph paper method.

To examine the stomatal behavior, lower epidermis of the beetle leaves were peeled off and number of total stomata, number of opened and closed stomata were calculated under microscope. Percentage of opened stomata was calculated.

Results and Discussion:

Plants have frequently incorporated environmental signals into their normal developmental path ways. Present study of the paper incorporates some observations that have been examined by the

influence of auxin, gibberellin, cytokinin in *Piper betel* L. under blue light, red light, white light and darkness in stomatal regulation.

From the table 1 and 2 it may be concluded that among the three hormones Auxin, Gibberellin and cytokinin, cytokinin caused maximum stomatal opening and showed highest transpiration rate under blue, red and white lights. From table no.5, under darkness, stomatal opening was high without any hormone treatment. Moreover interpretation of light treatment showed that blue light induced maximum stomatal opening in auxin treated leaves (table no. 1), whereas from the table no.2 it may be concluded GA₃ showed increasing number of stomatal opening under darkness. Table No.3 showed increased no. of opened stomata and transpiration rate under white and red light in cytokinin. Without hormones treatment betel leaf showed highest no. opened stomata under darkness (Table no.4).

Red light inhibit stomatal opening in both auxin and GA₃ treated leaves (table no.1 and table no.2). This differential behavior of lights and hormones in *Piper betel* L. leaves in stomatal opening and transpiration rate may be cited as distinct mode of physiological behavior and this behavior may be responsible for regulating transpiration pattern and stomatal movement.

Finally, it was concluded that, cytokinin is more effective for stomatal opening than auxin and gibberellin under red, blue and white light whereas gibberellin does better stomatal opening in darkness. Again activity for stomatal opening is highest under the blue light. Finally C

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Reference:

- [1] Assmann SM, Lee DM, Markus P. 1992. Rapid Stomatal response to red light in *Zea mays*. *Photochem. Photobiol.* 56:685–89
- [2] Blatt MR, Thiel G. Kp 1994. Channels of Stomatal guard cells: bimodal control of the Kp inward-rectifier evoked by auxin. *Plant J.* 1994; 5:55–68.
- [3] Schroeder JI, Allen GJ, Hugouvieux V, Kwak JM, Waner D. 2001. Guard cell signal transduction. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* ; 52:627–658.
- [4] Willmer CM, Fricker MD. 1996. *Stomata*. London, UK: Chapman & Hall. 375 pp. 2nd ed.
- [5] Zeiger E. 1983. The biology of Stomatal guard cells. *Annu. Rev. Plant Physiol.* 34:441–75
- [6] Gehring CA, McConchie RM, Venis MA, Parish RW. 1998. Auxin-binding-protein antibodies and peptides influence Stomatal opening and alter cytoplasmic pH. *Planta*; 205:581-586.
- [7] Pemadasa MA. 1982. Effects of phenylacetic acid on abaxial and adaxial Stomatal movements and its interaction with abscisic acid. *New Phytol*; 92:21–30.
- [8] Snaith PJ, Mansfield TA. 1984. Studies of the inhibition of Stomatal opening by naphth-1-ylacetic acid and abscisic acid. *J. Exp. Bot*; 35:1410–1418.
- [9] Snaith PJ, 1982. Mansfield TA. Control of the CO₂ responses of stomata by indol-3ylacetic acid and abscisic acid. *J. Exp. Bot.* ; 33:360–365.
- [10] Ri'ca'nek M, Vicherkova' M. 1992. Stomatal responses to ABA and IAA in isolated epidermal strips of *Vicia faba* L. *Biol. Plant*; 34:259–265.
- [11] Dunleavy PJ, Ladley PD. 1995. Stomatal responses of *Vicia faba* L. to indole acetic acid and abscisic acid. *J. Exp. Bot.* ; 46:95–100.

- [12] Pospíšilová J. 2003. Participation of phytohormones in the Stomatal regulation of gas exchange during water stress. *Biol. Plant*; 46:491–506.
- [13] Aharoni N, Blumenfeld A, Richmond AE. 1977. Hormonal activity in detached lettuce leaves as affected by leaf water content. *Plant Physiol*; 59:1169–1173.
- [14] Santakumari M, Fletcher RA. 1987. Reversal of triazole-induced stomatal closure by gibberellic acid and cytokinins in *Commelinabenghalensis*. *Physiol. Plant*; 71:95–99.
- [15] Pharmawati M, Billington T, Gehring CA. 1998. Stomatal guard cell responses to kinetin and natriuretic peptides are cGMP-dependent. *Cell. Mol. Life Sci.* ; 54:272–276.
- [16] Incoll LD, Jewer PC. 1987. Cytokinins and stomata. In: Zeiger E, Farquhar GD, Cowan IR, eds. *Stomatal Function*. Stanford: Stanford University Press:281–292.

- [17] Attridge, T.H., 1990. Light & Plant responses. Edward Arnold, London, ISBN 0-713-2973-5.
- [18] Briggs WR. 2005. Phototropin overview. In *Light Sensing in Plants*, ed. M Wada, K Shimazaki, M Iino, pp. 139–46. Tokyo: The Bot. Soc. Jpn./Springer-Verlag
- [19] Briggs WR, Christie JM. 2002. Phototropins 1 and 2: versatile plant blue-light receptors. *Trends Plant Sci.* 7:204–10
- [20] Roelfsema MRG, Hedrich R. 2005. In the light of Stomatal opening: New insights into ‘the Watergate’. *N. Phytol.* 167:665–91
- [21] Vavasseur A, Raghavendra AS. 2005. Guard cell metabolism and CO₂ sensing. *N. Phytol.* 165:665–82

Table 1: Effect of GA, CYTOKIIN and AUXIN on Transpiration rate and stomatal behavior under white, blue, red light and dark treatment in beetle leaves

	GA		Cytokiin		AUXIN	
Treatment sets(light)	% of stomata open	trans.gm /sqcm	% of stomata open	trans.gm /sqcm	% of stomata open	trans.gm/ sqcm
White	45.34	0.011	64.48	0.0076	24.02	0.00406
Blue	50	0.01032	62.55	0.0096	42.2	0.0043
Red	29.34	0.0111	64.5	0.0084	19.65	0.01009
Dark	57.57	0.0036	62.18	0.0076	33.01	0.0045

Table 2; Statistical analysis of the data collect during the experiment:

ANOVA								
	AUXIN				GA and Cytokinin(each)			
	% of stomata open		Transpiration in gm/sq. cm		% of stomata open		Transpiration in gm/sq.cm.	
	F	Sig.	F	Sig.	F	Sig.	F	Sig.
Between Groups	41.70831	3.13E-05	303.7803	1.41E-08	64.36591	6.06E-06	50.89601	1.48E-05

Table 3: Effect of white, blue, red and dark light treatment on transpiration rate and stomatal behavior of beetle leaves.

Treatment sets(light)	% of stomata open	trans.gm/sqcm
White	41.26	0.0062
Blue	24.83	0.0133
Red	18.47	0.0103
Dark	71.38	0.0038

Table 4; Statistical analysis of the data collect during the experiment:

ANOVA	% of stomata open		Transpiration in gm/sq. cm	
	F	Sig.	F	Sig.
Between Groups	543.8663	1.39E-09	76.62596	3.11E-06