

STUDIES ON THE RELATIONSHIP OF PHYTOCHROME, AUXIN, PHENOL AND SUCROSE WITH ROOTING IN HYPOCOTYL CUTTING OF *VIGNA RADIATA* (L) WILCJECK.¹

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ABSTRACT

The hypocotyl cuttings of *Vigna radiata* L. with and without shoot apex and leaves cultured in SUC.+IAA under 10 min exposure of red light followed by 15 min of far red light gave profuse rooting (52.2 and 47.8 number of roots respectively), than the cuttings cultured in water, IAA (1 μ g/ml), and sucrose (0.5%) alone under different short term exposure to light. The initiation of roots increased in the cuttings with shoot apex and leaves under the exposure of far red light. The 10 min exposure of far red light followed by 10 or 15 min exposure of red light gave 22-25 number of roots in the cuttings with shoot apex and leaves, only the 5 min exposure of red light and far-red light gave the higher magnitude of rooting than other short term exposures. Both type of cuttings cultured in 1 and 5 μ g/ml concentration each of orcinol and hydroquinone in combination with sucrose (0.5%), IAA (1 μ g/ml) and suc+IAA respectively induced better rooting under the exposure of far red light followed by red light.

INTRODUCTION

Hypocotyl cuttings of mung bean (*Vigna radiata* (L.) Wilcjeck) are most sensitive to the factors inducing the development and differentiation of adventitious roots (Gill, 1979, 1982a) and thus have been extensively used as the most appropriate bioassay system to study the mechanism of rooting (Gill, 1982). Nanda and Anand (1970) demonstrated that morphophysiological status of rooting is determined by changes in the levels of endogenous regulatory substances and nutritional status. These in turn, are caused by changes in temperature and light conditions prevailing during the plant

growth. Proper balance between auxin and nutritional levels is necessary for differentiation and development of adventitious roots (Nanda and Jain, 1971; Gill, 1982). In the present paper, some experiments were undertaken to study whether the short term exposure to red and far red light conditions has any significant role on the regeneration of rootings in cuttings in relation to exogenously applied auxin, sucrose and phenols.

MATERIAL AND METHODS

Red and far red illuminations were obtained in the chamber by using cellophane filters and the light was also filtered through glass sheets. For red light, two

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red cellophane filters were used and for far red light a sheet of blue cellophane filter was placed between two red ones and the light was filtered through water and glass (Downs, 1955). Uniform seeds of mungbean Var. G31 were sown on the cotton pads in enamel trays (12"×15") in dark at 34±2°C. Two types of cuttings were prepared from the seedling which consisted of 3 cm of hypocotyl, about 6 cm of epicotyl, a pair of primary leaves and apical bud (Gill, 1982). The cuttings were separated into two lots, Lot I (cuttings without shoot apex and leaves) is divided into number of groups of ten cuttings. These cuttings were cultured in graded tubes (7.5×2.5 cm) containing water, 1AA (1µg/ml), sucrose (0.5%) and suc +1AA, respectively. Similarly, lot II (cuttings with shoot apex and leaves) was divided in the same way and cuttings were cultured in 1 or 5 µg/ml concentrations each of orcinol and hydroquinone, singly and in combination with sucrose, 1AA and suc +1AA, respectively. The hypocotyl

portion of cuttings was dipped in the culture solutions. Subsequently, cuttings were exposed to red and for red lights for 5,10,15 minute singly and in sequence (exposure of red followed by far red light and *vice versa*). Simultaneously, the cuttings were transferred and maintained in the same conditions under which the respective seedlings were raised. The test solutions were changed on alternate days to avoid microbial infection. Observations on the number of roots/cuttings were recorded after seven days after treatment. The experiment was repeated twice and average values are reported.

RESULTS AND DISCUSSION

The hypocotyl cuttings with excised shoot apex and leaves cultured in suc+1AA under 5 min exposure of red light enhanced better rooting (Table II) as compared to those exposed to far-red light, and effectiveness was more in cuttings with shoot apex and leaves (Table I). These results are in accordance

TABLE I

EFFECT OF IAA, SUCROSE AND EXPOSURE TO RED AND FAR RED LIGHT ON NUMBER OF ROOTS PER HYPOCOTYL CUTTINGS OF *V. RADIATA* (L) WILCJECK.

Culture medium/ treatments	Cuttings without apex and leaves				Cuttings with apex and leaves			
	Water	1AA (1 µg/ml)	Suc 0.5%	Suc+ 1AA	Water	1AA 1 µg/ml	Suc 0.5%	Suc+ 1AA
FR _{5min}	2.8	2.5	3.2	6.8	1.2	0	4.0	30.0
FR ₁₀	1.5	3.0	3.0	10.8	2.0	2.7	4.0	20.6
FR ₁₅	1.8	3.0	4.2	8.0	0	1.5	5.5	22.0
R ₅ →FR _{5min}	1.9	2.2	3.5	22.0	1.6	2.0	4.0	42.0
R ₁₀ →FR ₅	1.8	2.5	4.0	27.0	1.3	0	4.0	20.5
R ₁₅ →FR ₅	2.5	1.9	4.2	24.5	1.2	1.4	2.6	44.5
R ₅ →FR ₁₀	0	1.8	4.0	0	1.2	3.1	2.9	13.7
R ₁₀ →FR ₁₀	0	2.5	2.9	22.0	2.8	0	3.2	17.8
R ₁₅ →FR ₁₀	0	2.2	4.0	38.0	0	4.1	4.2	22.8
R ₅ →FR ₁₅	1.5	3.2	4.1	36.0	0	0	3.2	22.8
R ₁₀ →FR ₁₅	4.0	3.5	4.2	47.8	0	3.1	4.3	52.2
R ₁₅ →FR ₁₅	2.5	4.2	4.0	27.0	2.1	3.2	5.2	5.8

TABLE II

EFFECT OF IAA, SUCROSE AND EXPOSURE TO FAR RED AND RED LIGHTS ON NUMBER OF ROOTS PER HYPOCOTYL CUTTING OF *V. RADIATA* (L). WILCJECK.

Culture medium/ treatments	Cuttings without apex and leaves				Cuttings with apex and leaves			
	Water	IAA 1 μ g/ml	Suc 0.5%	Suc+ IAA	Water	IAA 1 μ g/ml	Suc 0.5%	Suc+ IAA
R _{5min}	2.1	2.0	4.2	18.0	0	0	4.2	7.3
R ₁₀	2.0	3.0	3.8	5.0	1.3	2.5	3.0	10.5
R ₁₅	1.5	1.2	3.5	7.5	3.1	2.5	6.1	5.2
FR ₅ →R _{5min}	1.8	0	3.2	16.8	1.0	3.0	4.2	14.2
FR ₁₀ →R ₅	0	3.9	7.0	17.8	0	0	3.0	21.2
FR ₁₅ →R ₅	1.9	1.8	3.4	14.0	1.2	2.1	3.0	22.0
FR ₅ →R ₁₀	2.0	0	3.2	11.8	1.0	4.0	3.2	23.0
FR ₁₀ →R ₁₀	0	5.1	4.1	22.0	2.0	2.1	3.8	13.0
FR ₁₅ →R ₁₀	1.0	0	4.0	11.8	1.0	2.9	5.0	15.0
FR ₅ →R ₁₅	1.3	2.0	4.6	32.0	1.8	2.1	5.0	22.0
FR ₁₀ →R ₁₅	0	1.8	6.0	25.0	0	0	3.3	15.0
FR ₁₅ →R ₁₅	0	0	4.0	15.7	0	0	4.0	30.2

with those of Hartman and Kester (1968) who reported that the effect of a particular bands of light is also dependent on the presence or absence of shoot apex and leaves and type of metabolites synthesized in particular quality of light. In the cuttings with shoot apex and leaves the effectiveness of red light exposure on rooting increased with increasing duration of exposure. On the other hand, effect of far red light and its interaction with red light exposure is governed by the absence of shoot apex and leaves and that too under specific time intervals of red light followed by far red light exposure (Table I). It is inferred that interaction of red and far red light is not only dependent on the presence of adequate levels of auxin and nutrition (A/N) but is dependent

upon the presence of shoot apex and leaves as these substitute the effect of optimal concentrations of auxin and nutrition for mediation of red and far red light exposures. This confirmed the observation of Nanda and Krishna (1968) and Varsha (1978) that phytochrome system works efficiently in regeneration of cuttings in presence of proper balance between auxin and nutrition (A/N) and it is the dose of red and far red light which determines the total potentiality of rooting of particular types of cuttings.

Exposure of cuttings to red and far red lights for varying duration in the presence of phenols (orcinol and hydroquinone) caused profuse rooting under far red light in cuttings having intact shoot apex and leaves contrary to their absence

under similar conditions. Although the differences were not visible on the cuttings exposed to red light regardless of the phenols and type of cuttings. The cuttings with shoot apex and leaves gave higher intensity of rooting (Nanda and Dhaliwal, 1974). In general, phenols in the presence of optimal concentration of auxin-nutrition caused significant increase in number of roots in cuttings with shoot apex and leaves, suggesting thereby, that red and far red lights are some way associated with synthesis and action of phenolics (Har-

bourne, 1974). This fact was further confirmed by the observation that even though cuttings were devoid of shoot apex and leaves, rooting increased several folds as compared to control in the presence of exogenously supplied orcinol and hydroquinone (Table III). On the other hand, intact shoot apex and leaves did not show increase in number of roots on exposure to red and far red lights and also when phenols were supplied in the medium. This confirmed that phenolic synthesis is triggered by red and far red lights

TABLE III

EFFECT OF IAA, SUCROSE, PHENOLS AND EXPOSURE TO RED AND FAR RED LIGHTS ON NUMBER OF ROOTS PER HYPOCOTYL CUTTINGS OF *V. RADIATA* (L) WILCJECK.

Culture media/treatments		Cuttings without apex and leaves				Cuttings with apex and leaves			
		Water	IAA	Suc	Suc+	Water	IAA	Suc	Suc+
					IAA				IAA
<i>Exposure to R_{15min}→FR_{5min}</i>									
Control		0	4.2	4.5	25.8	0	2.5	2.9	22.0
Orcinol	1 µg/ml	2.0	2.5	4.0	26.2	0	4.5	4.6	23.0
	5 µg	2.2	2.8	4.5	28.0	1.2	4.8	5.0	24.2
Hydroquinone	1 µg	2.1	2.5	4.2	27.0	1.0	4.2	4.5	25.0
	5 µg	2.4	2.6	4.6	29.0	1.5	4.5	4.8	27.0
<i>Exposure to R_{10min}→FR_{10min}</i>									
Control		2.0	1.8	3.9	20.0	4.0	3.5	4.2	47.8
Orcinol	1 µg/ml	0	2.2	4.5	36.0	0	3.2	4.5	53.0
	5 µg	1.1	2.8	4.8	39.0	0	3.5	5.0	56.0
Hydroquinone	1 µg	1.0	2.0	4.2	34.0	1.0	3.3	4.3	52.5
	5 µg	1.8	2.2	4.6	36.0	1.5	3.4	4.6	54.0
<i>Exposure to R_{5min}→FR_{15min}</i>									
Control		0	2.8	4.1	10.9	1.9	2.2	3.5	22.0
Orcinol	1 µg/ml	4.2	4.0	4.5	45.5	1.8	3.2	4.2	44.0
	5 µg	4.6	4.5	4.6	48.0	2.0	3.2	4.6	48.0
Hydroquinone	1 µg	4.0	3.9	4.0	42.1	2.0	2.9	4.0	43.0
	5 µg	4.3	4.2	4.3	48.4	2.2	3.4	4.5	45.0

TABLE IV

EFFECT OF IAA, SUCROSE, PHENOLS AND EXPOSURE TO FAR RED AND RED LIGHT ON NUMBER OF ROOTS PER HYPOCOTYL CUTTING OF *V. RADIATA* L.) WILCJECK.

Culture media/treatments		Cuttings without apex and leaves				Cuttings with apex and leaves			
		Water	IAA	Suc	Suc + IAA	Water	IAA	Suc	Suc + IAA
<i>Exposure to FR_{15min} → RR_{5min}</i>									
Control		0	5.2	3.0	36.0	11	0	3.2	16.8
Orcinol	1 µg/ml	1.5	2.2	3.2	22.2	0	4.0	7.5	19.0
	5 µg	1.8	2.6	3.8	25.0	1.5	4.2	9.0	20.0
Hydroquinone	1 µg/ml	1.2	2.0	3.1	22.6	1.0	3.9	7.2	18.0
	5 µg	1.9	2.5	3.6	24.4	1.5	4.0	7.6	22.0
<i>Exposure to FR_{10min} → R_{10min}</i>									
Control		0	6.0	4.1	30.0	0	5-1	4.1	22.0
Orcinol	1 µg/ml	1.0	4.0	3.6	23.8	1.0	5.5	4.5	23.0
	5 µg	1.1	4.5	3.9	26.4	1.2	5.9	5.0	25.0
Hydroquinone	1 µg/ml	1.5	4.6	3.9	25.1	1.2	5.5	4.7	22.0
	5 µg	2.2	4.8	4.0	28.2	1.5	6.0	4.9	24.0
<i>Exposure to FR_{5min} → R_{15min}</i>									
Control		0	4.8	2.8	52.2	1.3	2.0	4.6	32.0
Orcinol	1 µg/ml	1.0	1.8	4.6	32.4	1.5	2.2	5.0	34.0
	5 µg	2.1	2.2	4.9	37.1	2.0	2.6	5.8	37.0
Hydroquinone	1 µg/ml	2.0	2.1	5.0	31.8	2.0	2.5	5.1	32.8
	5 µg	2.2	2.5	5.2	36.4	2.1	2.9	5.5	36.0

treatments and it is the leaf which synthesizes the phenols which are translocated to the basal portion of the cutting. This assumption is further confirmed from the data in Table IV where far red light followed by red light exposure of the cuttings produced similar trend as observed in earlier case.

It is concluded that the formation of adventitious roots in hypocotyl cuttings is influenced by the quality of light, which exerts its effect through nutritional and regulatory factors (Gupta *et al.*, 1977).

The manifestation of rooting is triggered when suitable doses of red and far red lights are given to the cuttings in a medium in which they are cultured and the effectiveness of short term exposure to red and far red spectrum is dependent on the auxin and nutrition ratio.

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