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Light Quality (Spectral Distribution and Transmission Wavelength Maxima), Influences Regeneration Efficiency and Microshoot Quality in *Chrysanthemum*

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Abstract

Influence of light quality (intensity, spectral distribution and transmission wavelength maxima) on regeneration efficiency and microshoot quality is described. We present a prospective potential to modify regeneration efficiency and microshoot quality by varying the quality of incident light. Light quality was evaluated by means of three tinted (blue, yellow & red) and one transparent culture container (control) all receiving light in the range of 400-700 nm. Primordia, number of microshoot, shoot and internodal length were significantly higher in yellow containers. Whereas the number of nodes and leaf length was significantly higher in blue containers but with lowest green coloration. On the other hand, significantly higher green coloration of shoots was observed only in control containers. Induction in light/dark and regeneration on two BAP levels had no consequence, indicative of light quality as the inimitable attribute influencing regeneration efficiency and microshoot quality in *chrysanthemum Dendranthema grandiflora*.

Keywords: *Chrysanthemum*; *Dendranthema grandiflora*; Light quality; Microshoot quality; Regeneration efficiency; Spectral distribution; Transmission Wavelength Maxima.

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Introduction

Failure to regenerate adventitious shoots is a shortcoming dealt with by changing culture media, temperature, tissue source, genotype, and hormone concentration. Incident light quality in addendum can to a large extent influence morphogenesis and quality of microshoots in numerous crop species. Varying light quality to alter regeneration efficiency (number of microshoots and nodes) and microshoot quality (length of shoot, internode and leaf and green coloration of leaves) is not well examined, as a result it turned out to be the focal point of our study.

Red light (655 ± 20 nm) enhanced initiation of somatic embryogenesis in date palm compared with white or blue (420 ± 12 nm) light [1]. Callus regeneration of *Actinidia deliciosa* showed regeneration only in red light [2]. Maximum induction of new growth occurred in triticales irrespective of whether blue or red light was used [3]. Likewise, in *chrysanthemum* we were interested in ascertaining the influence of light quality, on regeneration efficiency and microshoot quality. We intended to identify explicit traits of light quality for modifying exclusive traits in microshoot quality and improving regeneration efficiency.

Materials and Methods

Three experiments were conducted, each specifically aimed to study the influence of induction (light and dark), BAP levels and light quality on microshoot quality and regeneration efficiency, whose results are compiled in **Tables 1-4.** Light quality was varied using three tinted polystyrene containers (Sigma phytatrays II) and a transparent control. The three tints were blue, yellow and red **(Figure 1),** with 400-500, 550-610 and 610- 690 nm spectral distribution with 475, 580 and 660 nm transmission wavelength maxima respectively. The transparent container acting as control

Table 1 Induction in light and dark, two levels of BAP and four light quality parameters were the variables tested. ANOVA indicates significant influence of light quality on the number of microshoots (NM), number of nodes (NN), shoot length (SL), internodal length (IL) leaf length (LL) and green coloration of leaf (GC). Induction and BAP, and the interactions between the variables do not have any significant influence.

Source of Variation	NM	NN	SL	IL	LL	GC
	F.pr	F.pr	F.pr	F.pr	F.pr	F.pr
Induction	0.342	0.460	0.275	0.644	1.000	0.012
BAP levels	0.680	0.564	0.781	1.000	0.384	0.357
Quality of light	<0.001	<.001	<0.001	<0.001	<0.001	<.001
Induction × BAP	0.342	0.460	0.176	1.000	0.384	0.042
Induction × light	0.694	0.605	0.983	0.585	0.939	0.207
BAP × light	0.944	0.317	0.939	0.929	0.939	0.463
Ind × BAP × Qu of l	0.911	0.384	0.867	0.929	0.755	0.207

Table 2 Influence of light and dark induction, different levels of BAP (0.25 mg/l and 0.5 mg/l) on the different parameters are presented. Standard deviations are mentioned in parenthesis. Means are based on 16 replications of 10 explants each.

Treatment	Number of primordia/explant (0.50)	Number of shoots/ explant (0.05)	Number of nodes/shoot (0.95)	Length of shoot (cm) (0.05)	Length I. node (mm) (0.82)	Length of leaf (mm) (0.50)	Green colour Of shoots
Induction in light	10	2	11	3.5	7	7	100%
Induction in dark	9	1.9	9	3.4	5	6	100%
Regene. media 1 0.25 mg/l	9	2	9	3.5	6	7	100%
Regene. media 2 0.5 mg/l	9	2	10	3.4	6	7	100%

Table 3 The influence of light quality on the timing and the number of primordia per explant are presented. The Standard deviation is 5.08. Means are based on 16 replications of 10 explants per replication.

Light	Intensity in µ Mol	Day-3	Day-7	Day-10	Day-13	Day-21 (Total)
Blue	4.4	0	0	4.8	10.9	17.8
Yellow	15.4	0	5.9	10.9	15.2	17.5
Red	2.6	0	0	0	4.8	9.3
Control	53.6	0	0	0	5	8.7

Table 4 Number and quality of shoots regenerated on leaf explants of chrysanthemum cultured under different light quality. Standard deviations are mentioned in parenthesis). Means are based on a total of 16 replications of 10 explants per replication tested in three independent experiments.

Light	Number of shoots/ explant (1.15)	Number of Nodes/ Shoot (6.13)	Length of harvest. shoot (cm) (1.77)	Length of Internode (mm) (3.77)	Length of Leaves (mm) (4.96)	Degree of Green Coloration (21.74)
Blue-(475 nm)	3.9	19	06	03	15	50%
Yellow-(580 nm)	4.0	06	07	12	05	75%
Red-(660 nm)	1.9	06	3.5	06	04	90%
Control	2.0	10	3.5	06	08	100%

permitted white light with a spectral range from 400-700 nm. Light intensity in the climate room was measured using a lux meter as 55 µMolm^{-2}s^{-1}, however in the containers was 4.4 µMol-m^{-2}s^{-1} (blue), 15.4 µMol-m^{-2}s^{-1} (yellow), 2.6 µMol-m^{-2}s^{-1} (red) and 53.5 µMol-m^{-2}s^{-1} (control). Four containers were used per tint with 10 explants per container.

Surface sterilized greenhouse grown leaves of cv. 1581 were cut 0.5 cm away on either side of the midrib, which was subsequently sliced resulting in two strips. Each strip was sliced perpendicular to its length ending in uniform square explants on their abaxial surface on induction medium. The containers were placed in a climate room maintained at 25°C with a 16 h photoperiod.

The induction medium was composed of MS inorganic, B5 organic, 2 mgL^{-1} BAP, 1 mgL^{-1} NAA, 7 gL^{-1} Tissue culture Agar, 30 gL^{-1} sucrose and 3 mm MES [4]. Explants were induced in dark or in four different qualities of light and transferred to media composed as above but lacking NAA and differing in BAP levels (0.25 mgL^{-1} and 0.5 mgL^{-1}) [5].

Periodically on 3, 7, 10, 13 and 21 days of incubation, the number of primordia were counted and transferred to fresh containers on 21st day. On the 42nd day the number of microshoots and nodes, length of harvestable shoot, internode, leaf and green coloration of the shoots (estimation by eye) were recorded and statistically analyzed.

Results and Discussion

ANOVA designated light quality as a variable with significant influence on microshoot quality and regeneration efficiency. Other variables, induction in light was faintly superior over dark and two levels of BAP (0.25 & 0.5 mgL^{-1}) had no consequence **(Table 1, Figures 2 and 3).** Interactions amid induction, BAP levels and light quality were insignificant hence pooled data illustrated main effects **(Table 2).**

Figure 1 Layout and design of the tinted polystyrene containers.

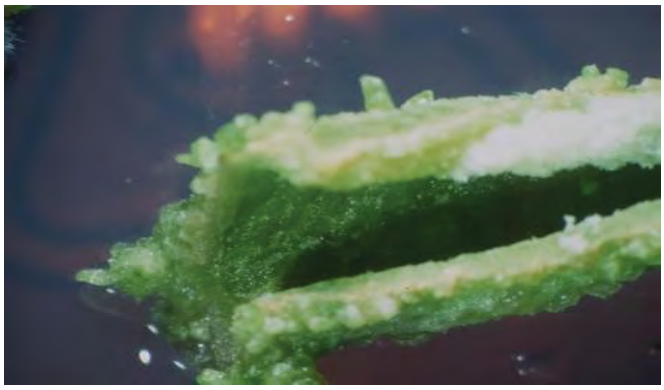

Figure 2 Primordia in a yellow container.

Figure 3 Leaf explant with microshoots from the three different TINTED containers and control. The variation in shoot length, internodal length, leaf length and green coloration is clearly visible from this photograph.

Primordia emerged exclusively from the cut edges of the explant **(Figure 2)** on day 7, 10 and 13 in yellow, blue and red/control containers respectively **(Table 3).** Number of primordia per explant in blue and yellow were ~18 **(Table 3)** while in red and control were ~ 9. Number of microshoots per explant in blue, yellow, red and control containers were 3.9, 4.0, 1.9 and 2.0 respectively **(Table 4).** Shoots from blue, yellow, red and control containers had 19, 06, 06 and 10 nodes respectively **(Table 4, Figure 3).** Prunus produced higher number of nodes at 475 nm than red (660 nm), far-red and white light [6] and also in *Azorina vidalii* [7]. Quality of light influenced number of primordia but not it's genetic engineering and auxiliary development demonstrating importance of light quality only during primordia production. Production of shoots in blue and yellow containers doubled the numbers present in red and control. Enhancing the number of primordia and number of harvestable shoots by shifting only the quality of light influenced regeneration efficiency. Higher number of nodes should be inferred as higher number of propagules per shoot, by which we can alternatively boost multiplication rate and regeneration efficiency.

The internodal length in blue, yellow, red and control containers were 3 mm, 12 mm, 6 mm and 6 mm **(Table 4 and Figure 3).** Between 400-550 nm the internodal length is shortest, but is longest between 550-610 nm and a value in-between is observed from 610-690 nm (red) and control. The microshoots length in yellow and blue containers were 100% and 71% longer (07 cm and 06 cm) than in red (3.5 cm) **(Table 4 and Figure 3).** Similarly, yellow light influenced shoot length in *chrysanthemum* [8] and rice [9]. In red containers (610-690 nm) the microshoot length was consistent with control, which is contrasting to rice [10] and *Argyanthemum* [11], wherein red light caused elongation. Subject to characteristics desired, one may possibly produce longer shoots with lesser nodes and leaves in *chrysanthemum* by culturing in yellow containers or vice versa by culturing in blue containers. Leaves from the blue, yellow, red and control containers were 15 mm, 05 mm, 04 mm and 08 mm long **(Table 4 and Figure 3).** Likewise, in *Arabidopsis* leaf area and petiole length were influenced by blue light irradiance [12]. Greenness of leaves (determined by eye) in control was 100%, while in blue, yellow and red containers were 50%, 75% and 90% green. None of the shoots in the tinted containers appeared greener than in control **(Table 4 and Figure 3).** White light with complete spectral range from 400 to 700 nm appears optimum for maximum chlorophyll synthesis. Diminished green coloration at 475 nm is a phenomenon also observed in rice [13]. This variation in greenness may be due to variation in levels of light harvesting *Cab* transcripts. Transcript levels of single light harvesting complex (*Lhc*) were determined in etiolated cress (*Lepidium sativum*) and maximum transcript was observed at 660 nm, followed by far red and 475 nm [14]. The etiolation observed at 475 nm and 580 nm may be due 3 reasons; firstly, due to lack of transcripts activated exclusively at 660 nm.

Secondly red and white lights are indispensable for protochlorophyllide reduction to chlorophyllide, which is subsequently esterfied to yield chlorophyll [15]. Thirdly wavelength of 638 nm is absorbed by chlorophyllide itself through its reduction to chlorophyllide [16]. Features observed

in the tinted containers can also be ascribed to the exclusive activation of phytochromes under that light quality. Accumulation of a particular phytochrome may augment or hinder the activity of other phytochromes. Consequently, a known phenotype for a specific quality of light may well be due to overproduction, complete absence or alterations in the ratio between distinct phytochromes.

Conclusion

There is no association between intensity and microshoot quality but there is with the spectral distribution and transmission wavelength maxima. As transmission wavelength maxima shifts from 660 nm to 475 nm the number of shoots, shoot length, leaf length increases but green coloration decreases. We propose spectral distribution and transmission wavelength maxima have a classic role in microshoot quality over light intensity. We advocate use of light with transmission wavelength maxima at 580 nm to turn out more shoots in shorter period to enhance regeneration efficiency. Those desiring to produce more nodes and broader leaves may perhaps try transmission wavelength maxima at 475 nm.

Conflict of Interest

The authors declare no conflict of interest.

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