

Multiple Shoots Regeneration of Strawberry under Various Colour Illuminations

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Abstract: Effects of colour illumination on multiple shoot regeneration from runner tip explants of strawberry were studied. Six colour (mixed, white, red, yellow, blue and green) illuminations were used in this study and among them mixed colour illumination showed the high percentage of shoot proliferation. Fresh and dry weights were also significantly higher under the mixed colour condition. Proliferated shoots showed 100% rooting in half strength of MS media. Plantlets were established successfully in soil.

Key words: Colour illuminations • multiple shoots • regeneration • strawberry

INTRODUCTION

Strawberry is a popular fruit eaten raw or used in making juice, desserts, jam, syrup and wine. It has been commercially cultivated in Canada, USA, Japan, Spain, Germany, Korea, Italy, Poland, Thailand and so many other countries in the world.

Plant regeneration mechanism in terms of physiological changes affected by photosynthetic apparatus and environmental factors has seldom been discussed. Light plays an important role in plant development, morphogenesis, photosynthesis and metabolism [1, 2] and it affects the accumulation of chlorophylls and development of thylakoid membranes, protein compounds and electron chain of photosystem I (PS I) and photosystem II (PS II) [3-5]. Organogenesis is a complex physiological and biochemical process associated with enzymatic, chlorophyll and endogenous growth regulators changes [6]. Plant growth regulators not only regulate cell dedifferentiation, multiplication and growth, but also control tissue organogenesis [7]. Establishment of an efficient regeneration system in strawberry would make a significant contribution in improving qualitative and quantitative characters of this fruit. Therefore, it is necessary to carry out research on strawberry shoot regeneration and its mechanism.

To obtain basic material for the establishment of large scale, stable and high yielding strawberry plantlets for cultivation requires mass production of uniform

propagules. Micropropagation is one of the effective means to achieve this goal. However, reports on micropropagation of strawberry are few [8-11]. To our knowledge, there have been no reports on *in vitro* multiple shoots regeneration using various colour illuminations in strawberry. In this paper we have compared growth and multiple shoot production using various colour illumination and growth regulators and have reported the best treatment for the mass production of strawberry plantlets.

MATERIALS AND METHODS

Runner tips were collected from 40-45 days old field grown strawberry mature plants. Explants were washed in running tap water for 20 min and then washed again thoroughly by adding a few drops of savlon and Tween-20 followed by 2-3 times washing with sterilized distilled water. They were then surface sterilized in 0.1% mercuric chloride solution 2-8 minutes followed by rinsing them 3-5 times with double distilled water inside the Laminar Air flow chamber. Sterile runner tips and terminal buds (3-4 mm) were dissected and cultured on MS supplemented with 1.0 mg/l BA + 0.5 mg/l Kin and MS0 (without plant growth regulators) adding 3% sucrose and 0.8% agar. The pH of the medium was adjusted to 5.8 with 0.1 NaOH before autoclaving at 1.06 kg/cm² and 121°C for 20 min. The cultures were incubated at 25 ± 2°C under 16 h photoperiod with various colour lights, viz. mix, white, red, yellow, blue and green.

Each experiment was completely randomized, with three replications and 25 explants per replication. Observations were recorded at seven weeks interval for percentage of response, number of microshoots per explant, length of longest microshoot, fresh and dry weight. The mean values were statistically compared using the Duncan multiple range test at $p < 0.05$.

RESULTS AND DISCUSSION

Effect of various colour illuminations on *in vitro* multiple shoot morphogenesis of strawberry were studied. All the characters were highly affected by colour illumination (Fig. 1). Among the various colour illuminations, the highest frequency (99%) of explants produced shoots was obtained in mixed colour

illumination followed by white and red colour illumination (Table 1). The highest number of microshoots per explants was obtained under mixed colour illumination followed by white and red colour illumination where as the green colour illumination gave the lowest result. Mixed colour illumination also showed higher longest micro shoot length after seven weeks of culture followed by red colour illumination. No significant difference was observed between mixed and white illumination for the characters fresh weight and dry weight of microshoots per explant. The mixed colour illumination might have special photosynthetic effect increasing the growth of the explants. The mixed colour was the combination of red and blue. All together 100% of illumination, red colour was covered by 80% illumination with the wave length 660 nm and blue colour covered 20% illumination with wave length 470 nm.

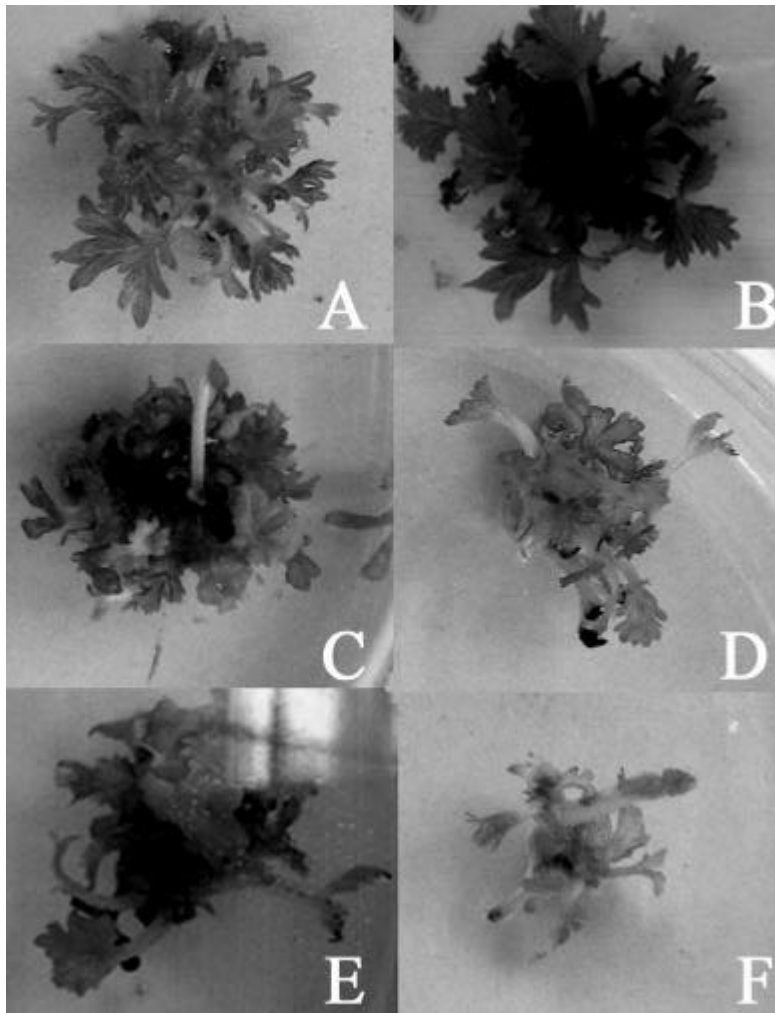


Fig. 1: Multiple shoot proliferation under mixed (A), white (B), red (C), yellow (D), blue (E) and green (F) colour illumination

Table 1: Effect of various colour illumination on multiple shoot proliferation. Media contained of MS+1.0 mg/l BA+0.5mg/l Kin. after seven weeks of culture

Colour illumination	% of responding explants	No. of micro shoots per explants	Longest micro shoot length (cm)	Fresh Weight (mg)	Dry weight (mg)
		$\bar{x}\pm SE$	$\bar{x}\pm SE$	$\bar{x}\pm SE$	$\bar{x}\pm SE$
Mix	99	42.75±3.49a	2.34±0.32a	843.3±53.31a	56.94±5.99a
White	98	40.33±3.10ab	2.05±0.30a	783.1±77.12a	53.97±6.06a
Red	95	38.61±5.90abc	1.32±0.11b	408.3±65.50b	30.31±3.68b
Yellow	93	33.75±4.47abc	1.24±0.17b	392.8±51.17b	29.31±3.70b
Blue	92	30.45±2.53bc	0.86±0.06b	346.7±46.17b	23.61±1.64b
Green	90	29.47±2.58c	0.72±0.09b	308.5±36.17b	20.89±1.77b
	CV	15.11%	25.52%	22.66%	23.46%
	LSD	9.868	0.6585	211.8	15.30

Many fluorescent lamps were used to illuminate plant tissue cultures to emit light deficient in the far-red wavelengths, thus care must be taken to provide the spectral balance suitable for growth of cultures [13, 14]. Morphogenesis of strawberry tissue cultures can be manipulated by light regimes and to improve efficiency of propagation.

The results obtained in this study showed that the mixed colour was the best for early and higher multiple shoot proliferation producing significantly higher fresh and dry weight for the tissue culture derived material.

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