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THE USE OF KINETIN FOR THE EFFICIENT *IN VITRO* INITIATION OF SOME CULTIVARS OF STRAWBERRY

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Abstract
Strawberry is one of the first horticultural species introduced in the plant tissue culture technology. Nowadays microprogation technology is one of the most important techniques to produce plants with high quality and resistance to biotic and abiotic stress factors. The researches were carried out in the Biotechnology and Physiology laboratory of University of Pitesti, with the following cultivars of strawberry: Premial, Elsanta and Senga Sengana. The purpose of this study was to examine the reaction of strawberry in vitro cultures on different kinetin concentration. Terminal buds were dissected and cultured on Lee and Fossard medium (1977). The explants were incubated in a growth chamber under 16/8 h light/dark cycle at 22 – 24 ^{o}C. LF medium supplemented with kinetin (1.25 mg l^{-1}) was found best for culture survival for all strawberry cultivars. Percentage survival of explants increased with increase in kinetin concentration from 0.5 mg l^{-1} to 1.25 mg l^{-1} for all strawberry cultivars.

Keywords: strawberry explants, in vitro culture, growing capacity, microprapagation technology

1. INTRODUCTION

Strawberry (*Fragaria x ananassa* Duch.) is a perennial fruit species of the Rosaceae family that belongs to the genus *Fragaria*. Plant tissue culture plays an essential role in plant biotechnology. *In vitro* micropropagation is an important tool for crop improvement in plant breeding (Biswas et al., 2009). A single explant from a wide categories of species (cultivated, endangered, threatened and rare species) can be multiplied into several thousand plants in relatively short time period and space under controlled conditions, irrespective of the season and weather on a year round basis (Idowu et al., 2009).

Tissue culture or microprogation technology is the *in vitro* aseptic culture of cells, tissues, organs or whole plant under controlled nutritional and environmental conditions used often to produce the clones of plants with superior quality, better disease resistance and stress tolerance capacities (Hussain et al., 2012).

Micropropagation of strawberry plants was introduced about thirty years ago. Immediately, the most important European nurseries producing several millions plants per year, were interested in this technique as it gave a definitive answer to the problems of soil fungi, causing a lot of damage to the strawberry fields and by another way, tissue culture plants seemed to produce more runners per mother plant in a short time (Mohan, et al., 2005). Since the initial report of in vitro strawberry propagation (Boxus, 1974), various culture conditions, basal media, and growth regulators have

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been investigated for adventitious bud and shoot regeneration from the leaves (Nehra et al., 1990; Rugini and Orlando, 1992; Passey et al., 2003), petioles (Rugini and Orlando, 1992; Passey et al., 2003), stems (Graham et al., 1995), stipules (Passey et al., 2003), roots, runners (Liu and Sanford, 1988) and sepals (Debnath, 2005) of strawberry.

2. MATERIALS AND METHODS

A study was conducted to evaluate the *in vitro* multiplication potential of 3 strawberry cultivars: Premial (A1), Elsanta (A2) and Senga Sengana (A3). Runner tips were collected from field grown stock plants. Disinfection of strawberry biologic material was achieved in ethylic alcohol 94^0 for 4 minutes and sodium hypochlorite 6% for 10 minutes. Terminal buds were dissected and cultured on Lee and Fossard basal medium (1977) supplemented with dextrose (40 g l^{-1}), agar-agar (7 g l^{-1}), IAA (indolyl-3-acetic acid) (0.27 mgl^{-1}), NaFeEDTA (sodium iron ethylenediaminetetraacetic acid) (32 mg l^{-1}) and 4 concentration of kinetin (table 1). The pH of all media was adjusted to 5.7. All media were autoclaved for 20 min at 121 ^oC. The explants were incubated in a growth chamber under 16/8 h light/dark cycle at 22 – 24 ^oC.

The experimental design was completely randomized with the (3 x 4), factorial and a total of 12 variants and three replications.

For the statistic interpretation of the results we used SPSS 16.0 for Windows program. Variance analysis was performed using Duncan test.

Table 1. Components of growing media in the initial stage of *in vitro* multiplication

Components (mg l^{-1})	Experimental variants			
	B1	B2	B3	B4
Macro elements*	LF	LF	LF	LF
Micro elements*	LF	LF	LF	LF
Vitamins*	LF	LF	LF	LF
Dextrose g l^{-1}	40	40	40	40
Agar g l^{-1}	7	7	7	7
K mg l^{-1}	0.5	0.75	1	1.25
AIA mg l^{-1}	0.27	0.27	0.27	0.27
NaFe EDTA mg l^{-1}	32	32	32	32

***LF: Lee and Fossard (1977)

3. RESULTS AND DISCUSSIONS

Percentage of growing explants in the initiation phase of *in vitro* culture was from 50 % for Senga Sengana cultivar to 98 % for Elsanta cultivar. LF medium supplemented with Kin (1 mg l^{-1} and 1.25 mg l^{-1}) (98 %) was found best for culture survival for Elsanta cultivar (figure 1). The lowest (50 %) culture survival was obtained on LF medium supplemented with 0.5 mg l^{-1} Kin for Senga Sengana cultivar. Growing kinetin concentration than 1 mg l^{-1} doesn't have significant influence of the survival percentage.

Premial cultivar had the lowest percentage of explants (76 %) on the nutritive medium supplemented with 0.5 mg l^{-1} Kin and the best percentage on B4 nutritive medium supplemented with 1.25 mg l^{-1} Kin (96 %).

Significant differences among B1, B2 and B3 media were observed for all three strawberry cultivars. The difference between B3 and B4 media was not significant ($p>0.05$).

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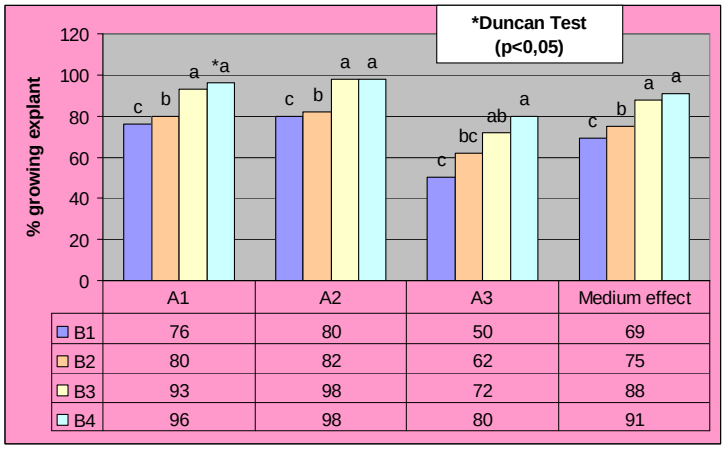
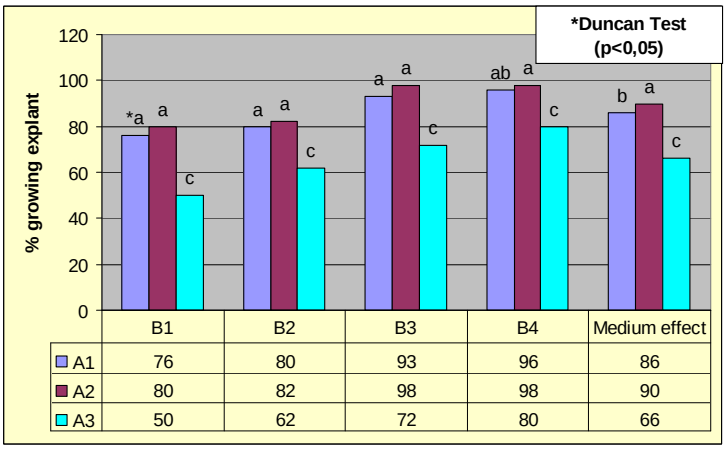


	A1	A2	A3	Medium effect
B1	76	80	50	69
B2	80	82	62	75
B3	93	98	72	88
B4	96	98	80	91

Figure 1. Influence of media on percentage survival of explants

The data presented in figure 2 showed that the maximum survival percentage on B1 nutritive medium supplemented with 0.5 mg l^{-1} Kin was obtained for cv. Elsanta, followed by cv. Premial and cv. Senga Sengana. The similar trend was observed on B2, B3 and B4 media.



	B1	B2	B3	B4	Medium effect
A1	76	80	93	96	86
A2	80	82	98	98	90
A3	50	62	72	80	66

Figure 2. Influence of genotype on percentage survival of explants

In figures 3, 4 and 5 observed the positive correlation between kinetin concentration and percentage survival of explant for cv. Premial, Elsanta and Senga Sengana. Value of Pearson correlation coefficient was 0.967 (positive significant correlation for 0.01 level) for cv. Premial, 0.918 (positive significant correlation for 0.01 level) for cv. Elsanta and 0.996 (positive significant correlation for 0.01 level) for cv. Senga Sengana.

Percentage survival of explants increased with increase in kinetin concentration from 0.5 mg l^{-1} to 1.25 mg l^{-1} for all strawberry cultivars.

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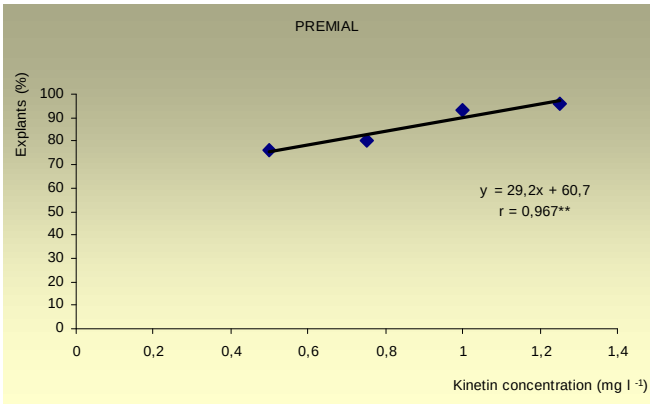

Figure 3. Correlation between kinetin concentration and survival percentage for cv. Premial (correlation is significant at the 0.01 level)**

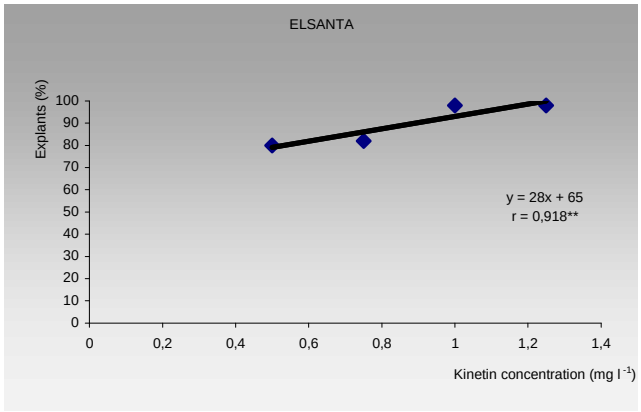

Figure 4. Correlation between kinetin concentration and survival percentage for cv. Elsanta (correlation is significant at the 0.01 level)**

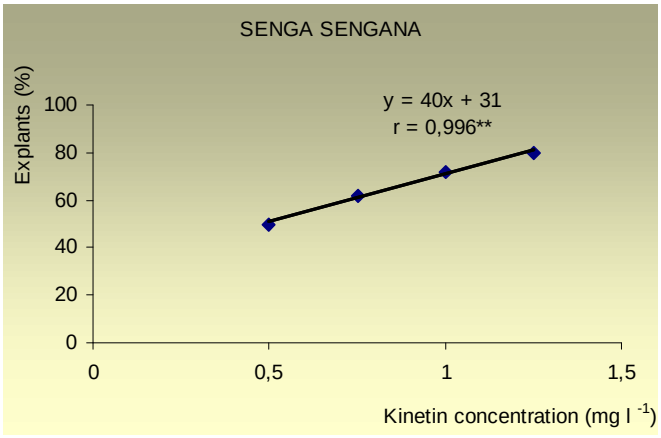

Figure 5. Correlation between kinetin concentration and survival percentage for cv. Senga Sengana (correlation is significant at the 0.01)**

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Aspect regarding the explants growing in the use of kinetin for the efficient *in vitro* initiation of some cultivars of strawberry is presented in figure 6.

Figure 6. Aspect regarding explants growing

Many authors have investigated influence of media and explant types on percentage survival of explants for *in vitro* multiplication of strawberry. Passey et al. (2008) obtained from leaf discs the high levels of survival percentage (100% for cv. Calypso an Tango) on MS media containing various combinations of the growth substances Benzyladenine (BA), 2,4-Dichlorophenoxyacetic acid (2,4-D), α-Naphthaleneacetic acid (NAA) and Thidiazuron (TDZ) and the minimum levels of survival percentage for Elsanta cv. (2%). Nehra et al. (1989) reported for Redcoat cv. 79% survival percentage of explants from leaf discs, while Sorvary et al (1993) reported 85.4% for Jonsok cv.
Negi M. (2008) reported that MS medium (Murashige and Skoog, 1962) supplemented with BAP (0,5 ml l^{-1}) and kinetin (0,5 ml l^{-1}) was found best for culture survival (88.95%) for cv. Chandler, while lowest (68,24%) culture survival was obtained on MS medium.
Munir et al. (2015) used for axillary/adventitious buds initiation in strawberry cultivars, a constant amount of glucose or sucrose (30 g l^{-1}) and for different growth regulators concentrations (Kinetin 0, 0.2, 0.4, 0.6, 0.8 mg l^{-1} and NAA 0, 0.2 mg l^{-1}). In their study, cultivar Osogrande initiated highest number of buds (20) at sucrose based MS media containing 0.8 mg l^{-1} Kinetin and 0.2 mg l^{-1} NAA.
Cappelletti et al. (2016) reported that the best regeneration efficiency for the leaves of strawberry cultivar Calypso were obtained culturing in a medium supplemented with thidiazuron (TDZ) 0.5 mg l^{-1} and 2,4-dichlorophenoxyacetic acid (2,4-D) 0.02 mg l^{-1} while for cultivar Sveva leaves the best regeneration efficiency was obtained culturing in a medium supplemented with N6-benzyladenine (BA) 3 mg l^{-1} and indole-3-butyric acid (IBA) 0.2 mg l^{-1}.

4. CONCLUSIONS

LF medium supplemented with kinetin (1.25 mg l^{-1}) was found best for culture survival for all strawberry cultivars.

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The maximum survival percentage of all strawberry cultivars was obtained on B4 nutritive medium supplemented with 1.25 mg l^{-1} kinetin, while the minimum survival percentage for all studied cultivars was observed on B1 nutritive medium.
Percentage survival of explants increased with increase in kinetin concentration from 0,5 mg l^{-1} to 1,25 mg l^{-1} for all strawberry cultivars.

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