ACTA SCIENTIARUM POLONORUM *Acta Sci. Pol., Hortorum Cultus 11(4) 2012, 129-142*

DEVELOPMENT OF *in vitro* METHODS FOR REGENERATION OF STRAWBERRY 'FESTIVAL' AND 'RUBYGEM' VARIETIES (*Fragaria* × *annanasa* Duch.)

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Abstract. The cultivated strawberry (*Fragaria* × *ananassa* Duch.), a member of the Rosaceae, is the most important soft fruit in the world. In vitro micropropagation is an important tool for clonal multiplication and is commonly applied for induction of somaclonal variation to create genetic variability. Optimized regeneration protocols and high efficiency are also important for gene transformation for improving new cultivars. For this purpose, in this study it was aimed to optimize a regeneration protocol for 'Festival' and 'Rubygem' strawberry varieties. Leaf disks and stipules from mature *in vitro* grown strawberry plants of 'Festival' and 'Rubygem' varieties (*Fragaria* × *annanasa* Duch.) were used as explant sources. Mc Cowns Woody plants including vitamins were used as a basal medium. Different concentrations of TDZ (Thidiazuron) (0, 1, 2, 3 and 4 mg l^{-1}) and IBA (Indole-3-butyric acid) (0, 0.2 and 0.4 mg l^{-1}) and the efficiency of 15 days dark treatment on the regeneration from leaf disks and stipules were also tested. Our results suggested that leaf stipules, a high concentration of TDZ, $AgNO_3$ and a dark treatment in culture medium successfully resulted in the induction of regeneration.

Key words: Strawberry, *in vitro*, organogenesis, regeneration, $AgNO_3$

INTRODUCTION

Strawberry (*Fragaria* × *ananassa* Duch.) is a natural hybrid of *Fragaria chiloensis* L. P. Mill. and *Fragaria virginiana* Duch. and belongs to the Rosaceae family [Ercisli 2004]. The berry is delicate in flavour and rich in vitamins particularly C, B1, B2 and minerals such as Ca, K, Cu, Fe [Chien-Ying et al. 2009; Kafkas et al. 2007]. Strawberries are also good sources of natural antioxidants [Wang et al. 1996; Heinonen et al. 1998; Koşar et al. 2004]. For these reasons in recent years strawberry is become favored food in the diets of millions of people in the worldwide. The cultivated strawberry is

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grown throughout the world and its production and growing areas increases year by year [Esitken et al. 2010].

In the view of the potential commercial value, it is highly desirable to develop methods of rapid, efficient and large scale multiplication of *Fragaria* × *ananassa* Duch. using tissue culture techniques. Micropropagation of strawberry plants was introduced about fourty years ago [Boxus 1974]. *In vitro* techniques are important tools for modern plant improvement programs introducing new traits into selected plants, to multiply elite selections and to develop suitable cultivars in the minimum time [Taji et al. 2002]. Used in conjunction with classical breeding methods, an efficient *in vitro* shoot proliferation and regeneration system could accelerate cultivar development programs. The ability to regenerate plants is crucial to the successful application of *in vitro* methods [Cao and Hammerschlag 2000]. This technique has been widely used especially in Europe and USA [Zimmerman 1981, Boxus 1989]. Nowadays, 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]. Adventitious shoot regeneration is a prerequisite for any plant improvement programme aimed at improving plants by *in vitro* techniques and genetic engineering. Plant biotechnology and genetic engineering technologies are particularly useful for fruit trees since these technologies have the potential to reduce the time needed for traditional breeding programme [Petri and Burgos 2005]. Addition to the cytokinins some other chemicals such as thidiazuron (TDZ) can be used *in vitro* propagation of many plant materials. TDZ, a substituted phenylurea (1-phenyl-3-(1,2,3-Thiadiazol-5-yl)) urea; TDZ) is one of the several substituted ureas that have been investigated recently for their cytokinin-like activity in tissue culture applications. TDZ is known to be more active than Zeatin for stimulating the growth when added to a tissue culture medium at a low concentration and auxin-like effects induces in vitro shoot regeneration in several species of recalcitrant woody plants [Huetteman and Preece 1993; Debnath 2005].

TDZ is used as a synthetic herbicide and a plant growth regulator to stimulate high rate of axillary shoot proliferation in many woody plant species [Malik and Saxena 1992]. It has been known to be more effective than most of the commonly used cytokinins [Huetteman and Preece 1993]. Thidiazuron releases the lateral bud dormancy and stimulates shoot formation in wide variety of plant species [Fiola et al. 1990; Malik and Saxena 1992].

According to Capelle et al. [1983], TDZ directly promotes growth due to its own biological activities in a fashion similar to that of an N-substituted cytokinin or it may induce the synthesis and accumulation of an endogenous cytokinin. In woody plant species, low levels of TDZ induce the axillary shoot proliferation but higher levels may inhibit it. Higher levels, on the other hand, promote callus and somatic embryo formation [Huetteman and Preece 1993]. The light and dark cycles are also known to affect tissue regeneration responses, although the effects seen can differ, depending on the genotype-PGR interactions [Liu and Sanford 1988; Nehra et al. 1989].

Silver nitrate has proved to be a very potent inhibitor of ethylene action and is widely started to use in plant tissue culture experiments. It is also having easy availabil-

ity, solubility in water, specifity and stability traits make it very useful for various applications in exploiting plant growth regulation and *in vitro* and *in vivo* morphogenesis The medias including silver ion responses seem to be involved in polyamines as plant growth regulators ethylene and calcium-mediated pathways and play a crucial role in regulating physiological process including morphogenesis. Silver ions in the form of nitrate such as $AgNO_3$ play a major role influcing somatic embryogenesis, shoot formation and efficient root formation which are the most important on gene transformation process [Kumar et al. 2009].

In this study, it was aimed to examine the interaction of various level of TDZ, IBA and $AgNO_3$ combinations at different light/dark cycles of for regeneration leaf stipules and leaf disks of 'Festival' and 'Rubygem' varieties.

MATERIAL AND METHODS

The study was conducted The Plant Tissue Culture Lab. in Yaltır Tarım (Yalex) company during 2010 and 2011. 'Festival' and 'Rubygem' varieties were subjected to *in vitro* cultures from runner leaf disk, leaf stipule regeneration from *in vitro* and explants. Explants were washed in running tap water and then washed again thoroughly by adding a few drops of Tween-20. Surface sterilization were done using 6% sodium hypoclorite for 8 min. and then rinsed several times in sterile double distilled water inside the Laminar Air Flow Chamber. Runner tip meristems were dissected under a stereomicroscope. Subcultures were done every 3–4 weeks intervals.

Strawberry meristems were maintained in vitro on basal medium (McCown's woody plant basal salts) and including vitamins [Lloyd and McCown 1980], 20 g l^{-1} sucrose and 7.5 g l^{-1} agar. All medias were adjusted to pH 5.8 before autoclaving. All cultures were propagated at 25^{o}C under 16 h light (20–40 mol sec^{-1} m^{-2}) /8 h dark cycle.

Regeneration medium consisting of basal medium including TDZ (0, 1.0, 2.0, 3.0, 4.0 g l^{-1}) and IBA (0, 0.2, 0.4 mg l^{-1}) combination. TDZ was included into the medium to using sterile filter after autoclaving. This study was examined two different light conditions: continuously 16 h light/ 8 h dark photoperiod and first 15 day dark only then 16 h light/ 8 h dark photoperiod condition .

Obtained results were evaluated and the medias which were having higher shoots were also compared using various levels of $AgNO_3$ (0, 1, 2 and 3 mg l^{-1}).

The number of shoots per explant, regeneration percentage (%), callus induction rate (%), callus diameter was determined. Calli growth were measured after 40 days of culture using the following scale: 0 (no callus formation), scale 1 (calli diameter < 2 mm), scale 4 (calli diameter 2–13 mm), scale 8 (calli diameter > 13 mm).

Statistical Analysis. Obtained dates were recorded after 40 days for number of shoots per explant forty explants were used per treatment and repeated three times All experiments were conducted in a completely randomized design. All recorded data were analyzed using JMP soft pack programme at 5% significant level ($p \leq 0.01$) and mean separation by LSD.

RESULTS AND DISCUSSION

The results of number of average shoots, shoot regeneration percentage (%) and percentages of calli (%) per explant derived from stipule explants of Festival variety under dark and photoperiod conditions were given in Table 1. As seen in Table 1, the highest shoot numbers was obtained from 0 mg l^{-1} IBA with 3 mg l^{-1} TDZ, 0 mg l^{-1} IBA with 4 mg l^{-1} TDZ and 0.4 mg l^{-1} IBA with 3 mg l^{-1} TDZ combinations at 15 d dark conditions for Festival variety. Whereas the lowest obtained from 0 mg l^{-1} IBA with 0 mg l^{-1} TDZ, 0.2 mg l^{-1} IBA with 0 mg l^{-1} TDZ and 0.4 mg l^{-1} IBA with 0 mg l^{-1} TDZ combinations. The results show that, the lowest shoot was obtained from without TDZ combinations. As for the 16 hours light 8 hours dark photoperiod conditions, the lowest shoots was obtained from without IBA and TDZ or the lowest IBA combinations.

Table 1. The average number of shoots, percentages of shoot regeneration (%) and calli formation (%) derived from stipule explants of 'Festival' variety under dark and photoperiod conditions

Tabela 1. Średnia liczba pędów, procent regeneracji pędów (%) i tworzenia się kalusa (%) z przylistków roślin odmiany 'Festival' w warunkach zaciemnienia i fotoperiodu

Application Podanie		Shoot numer Liczba pędów		Shoot regeneration Regeneracja pędów (%)		Calli formation Tworzenie się kalusa (%)	
IBA	TDZ	D	P	D	P	D	P
0	0	1.0f	0.6h	66.6b	26.6de	0.0e	0.0d
	1	5.6e	3.3ef	93.3ab	60bcde	80.0ab	46.7bcd
	2	7.3c	5.6cd	66.6b	66.6abcde	86.7a	66.7abc
	3	11.3a	6.6 b	80.0ab	80ab	53.3bcd	80.0abc
	4	11.3a	3.6ef	73.3ab	53.3bcde	86.7ab	60.0abc
0.2	0	0.0g	0.6h	0.0c	26.6e	20.0de	0.0d
	1	10.3b	1.3h	86.6ab	66.6abcd	86.7ab	53.3abc
	2	7.3c	2.3g	100a	60.0bcde	100.0a	80.0abc
	3	7.0cd	4.0e	80.0ab	56.6bcd	100.0a	86.7ab
	4	6.3de	3.3ef	100a	73.3abc	100.0a	86.7ab
0.4	0	0.3fg	0.6h	20.0c	26.6e	40.0cde	40.0cd
	1	7.0 cd	3.0fg	100a	40.0cde	100a	93.3a
	2	10.6ab	5.3d	86.6ab	73.3abc	86.7ab	73.3abc
	3	11.3a	9.6a	100 a	93.3a	100.0a	86.7ab
	4	10.3b	6.3bc	73.3ab	80.0abc	86.7ab	86.7ab
$LSD_{0.01}$		0.82	0.86	28.9	26.25	3335.29	39.88

D – dark – ciemność; P – photoperiod – fotoperiod.

Values in columns marked with the same letter do not differ at $p \leq 0.01$ – Wartości w kolumnach oznaczone tymi samymi literami nie różnią się istotnie przy $p \leq 0{,}01$.

The results of average number of shoots, shoot regeneration (%) and calli formation (%) per explants derived from leaf disks of Festival variety under dark and photoperiod conditions were given in Table 2. As seen in Table 2, the highest shoots was obtained

from 0.4 mg l^{-1} IBA with 3 mg l^{-1} TDZ combinations both 15 d dark conditions and photoperiod conditions. Whereas the lowest obtained from 0 mg l^{-1} IBA with 0 mg l^{-1} TDZ, 0.2 mg l^{-1} IBA with 0 mg l^{-1} TDZ and 0.4 mg l^{-1} IBA with 0 mg l^{-1} TDZ combinations. The results show that, the lowest shoots was obtained from without TDZ combinations. As for the 16 hours light 8 hours dark photoperiod conditions, the lowest shoots was obtained from without IBA and TDZ or the lowest IBA combinations.

Table 2. The average number of shoots, percentages of shoot regeneration (%) and calli formation (%) derived from leaf disk explants of 'Festival' variety under dark and photoperiod conditions

Tabela 2. Średnia liczba pędów, procent regeneracji pędów (%) i tworzenia się kalusa (%) z dysków liściowych roślin odmiany 'Festival' w warunkach zaciemnienia i fotoperiodu

Application Podanie		Shoot numer Liczba pędów		Shoot Regeneration Regeneracja pędów (%)		Calli formation Tworzenie się kalusa (%)	
IBA	TDZ	D	P	D	P	D	P
0	0	0.0g	0.0 g	0.0b	0.0e	0.0c	0.0c
	1	2.6f	3.3de	60.0 a	20.0d	86.7ab	46.7bc
	2	3.6ef	3.3de	60.0 a	80.0 c	80.0b	86.7ab
	3	5.3c	5.0ab	73.3 a	86.6bc	100.0a	80.0ab
	4	4.3cde	4.6abc	80.0a	93.3ab	73.3b	100.0a
0.2	0	0.0g	0.0g	0.0b	0.0e	0.0c	0.0c
	1	3.6ef	0.6fg	73.3a	26.6d	100.0a	66.7ab
	2	4.0de	4bcd	80.0a	73.3c	100.0a	86.7ab
	3	5.0cd	4.6abc	73.3a	93.3ab	86.7ab	93.3a
	4	3.3ef	3.6cde	73.3a	73.3c	100.0a	73.3ab
0.4	0	0.0g	0.0g	0.0b	0.0e	0.0c	0.0c
	1	4.3cde	1.3f	80.0a	73.3c	100.0a	100.0a
	2	6.6b	2.6e	66.6a	80.0c	100.0a	66.7ab
	3	8.3a	5.3a	86.6a	100.0a	100.0a	100.0a
	4	7.0b	4.6abc	80.0a	93.3ab	100.0a	100.0a
$LSD_{0.01}$		1.21	2.85	28.9	28.9	35.29	35.23

D – dark – ciemność; P – photoperiod – fotoperiod.

Values in columns marked with the same letter do not differ at $p \leq 0.01$ – Wartości w kolumnach oznaczone tymi samymi literami nie różnią się istotnie przy $p \leq 0{,}01$.

The average number of shoots, percentage of shoot regeneration (%) and percentages of calli (%) derived from stipule explants of 'Rubygem' variety under dark and photoperiod conditions were given in Table 3. As seen in Table 3, average shoot numbers, the shoot regeneration percentage (%) and cali formation (%) of various levels of TDZ and IBA combinations were varied and this variation was found to be statistically significant ($p < 0.05$).

Statistically differences were found to be between the photoperiod conditions (16 h dark/8 h dark; continuously 15 d dark) (tab. 3). Some observations were seen during the experimental stage. When the leaf and stipule explants were inoculated di-

rectly continuously photoperiod conditions, the medium became browning. Whereas the explants, were inoculated under 15 d dark conditions, browning process decreased. Shoot regeneration is thought to be a positively affected 15 d dark conditions but did not give same response in all IBA and TDZ level combinations . The variation was also observed between light and dark photoperiod regimes. The number of shoot per explant derived from under 15 days dark conditions was found to be higher in some combinations compare to the light photoperiod conditions.

Table 3. The average number of shoots, percentages of shoot regeneration (%) and calli formation (%) derived from stipule explants of 'Rubygem' variety under dark and photoperiod conditions

Tabela 3. Średnia liczba pędów, procent regeneracji pędów (%) i tworzenia się kalusa (%) z przylistków roślin odmiany 'Rubygem' w warunkach zaciemnienia i fotoperiodu

Application Podanie		Shoot numer Liczba pędów		Shoot Regeneration Regeneracja pędów (%)		Calli formation Tworzenie się kalusa (%)	
IBA	TDZ	D	P	D	P	D	P
0	0	0.0g	0.0h	0.0h	0.0g	6.6c	0.0d
	1	2.0bcd	5.0c	46.7cde	46.7abc	66.7b	20.0cd
	2	1.7cde	5.3bc	46.7cde	53.3ab	86.7ab	100a
	3	0.7fg	6.3b	20.0g	60.0a	86.7ab	86.7ab
	4	1.0ef	0.3gh	26.7fg	6.7fg	73.3ab	80.0ab
0.2	0	0.0g	1.0fgh	0.0h	6.7fg	13.3c	46.7bc
	1	3.3a	1.3efg	73.3a	20.0de	100.0a	80.0ab
	2	2.3bc	3.0d	60.0abc	46.6abc	13.3c	80.0ab
	3	1.7cde	2.3de	53bcd	40.0abcd	93.3ab	100a
	4	1.3def	8.3a	46.6cde	60.0a	100.0a	93.3a
0.4	0	0.0g	0.3gh	0.0h	6.7fg	60.0b	80.0ab
	1	2.7ab	0.7fgh	73.3a	13.3ef	86.7ab	86.7ab
	2	2.3bc	2.3de	66.6ab	33.3bcd	93.3ab	86.7ab
	3	1.3def	2.3de	40.0def	33.3bcd	93.3ab	100.0a
	4	1.3def	1.7 ef	33.3efg	26.7cde	100.0a	93.3a
$LSD_{0.01}$		0.78	0.86	9.08	15.12	29.78	35.1

D – dark – ciemność; P – photoperiod – fotoperiod.

Values in columns marked with the same letter do not differ at $p \leq 0.01$ – Wartości w kolumnach oznaczone tymi samymi literami nie różnią się istotnie przy $p \leq 0{,}01$.

As seen in Table 4, the average numbers of shoot per plant, shoot regeneration percentage (%) and percentage of shoot regeneration at various levels of TDZ and IBA combinations were varied and this variation was found to be statistically significant ($p < 0.05$). The lowest average shoots per explant was obtained from non used TDZ (0 mg/l) medias while, the highest obtained without IBA and 2 mg/L TDZ media under dark and 3 mg/L TDZ media under photoperiod conditions. Addition to this, without TDZ (0 mg/l) and IBA combinations were also gave low shoot numbers (0 mg/l and 1.0 mg/l).

Table 4. The average number of shoots, percentages of shoot regeneration (%) and calli (%) derived from leaf disk explants of 'Rubygem' variety under dark and photoperiod conditions

Tabela 4. Średnia liczba pędów, procent regeneracji pędów (%) i tworzenia się kalusa (%) z tarcz liściowych roślin odmiany 'Rubygem' w warunkach zaciemnienia i fotoperiodu

Application Podanie		Shoot numer Liczba pędów		Shoot Regeneration Regeneracja pędów (%)		Calli formation Tworzenie się kalusa (%)	
IBA	TDZ	D	P	D	P	D	P
0	0	0.6ef	0.33f	13.3e	6.6e	6.6c	0.0c
	1	4.3b	2.6de	46.6bc	40.0bcd	100a	60.0b
	2	6.0a	3.0d	73.3a	46.7abc	73.3a	86.7ab
	3	2.0d	5.6a	40.0bc	66.6a	100a	80.0ab
	4	3.6bc	5.3ab	53.3ab	53.3ab	93.3a	100.0a
0.2	0	0.0f	0.3f	0.0f	6.6e	40.0b	80.0ab
	1	3.0c	2.0e	53.3ab	40.0bcd	100.0a	100.0a
	2	4.0b	4.6bc	33.3bcd	46.6abc	100.0a	100.0a
	3	5.3a	2.3de	53.3ab	33.3bcd	100.0a	100.0a
	4	5.6a	4.6bc	53.3ab	46.6abc	100.0a	100.0a
0.4	0	0.0f	0.0f	0.0f	0.0e	0.0c	13.3c
	1	0.6ef	4.0c	13.3e	46.6abc	100.0a	100.0a
	2	1.6d	4.3c	20.0de	26.6cd	93.3a	100.0a
	3	1.3de	3.0d	26.6cd	26.6cd	93.3a	100.0a
	4	1.3de	2.6de	20.0de	20.0d	100.0a	80.0ab
$LSD_{0.01}$		0.78	1.11	12.14	12.96	24.13	25.8

D – dark – ciemność; P – photoperiod – fotoperiod.
Values in columns marked with the same letter do not differ at $p \leq 0.01$ – Wartości w kolumnach oznaczone tymi samymi literami nie różnią się istotnie przy $p \leq 0,01$.

Debnath [2006] was also reported similar results with us. The same author reported that, average shoot growth and calli formation were not formed without TDZ applications. Sutter et al. [1997], were also reported that high concentrations off Thidiazuron (60–80 μM) was provided 100% regeneration of leaf disks in 'Camarosa', 'Parker' and 'Pajaro' varieties and in all three varieties and shoots were regenerated, relatively. Another study was reported by Barceló et al. [1998] and the authors were examined shoot regeneration from sepal, leaf and petiole explants by incorporating TDZ (2–4 μM) and dark treatment for 14 d before incubating the explants under a 16-h photoperiod. A dark treatment similar to our results was achieved and the highest response obtained from leaf generation. The other authors reported that, TDZ induced shoots were transferred to 2–4 μM zeatin-containing medium for elongation, also [Debnath 2005]. In another study, Żebrowska and Hortyński [2002], reported that effects of various concentrations of 6-benzylaminopurine (BAP): 0; 1.6; 3.2; 6.4 mg l^{-1} in Murashige-Skoog (MS) medium in clone B-302 and Kama strawberry variety on their petioles and leaf blades. Leaf explants regenerated only at concentrations of 3.2 mg l^{-1} and 6.4 mg BAP l^{-1} in the medium. According the their results, the higher shoot formation was found to be in clone

B-302 than in Kama variety and obtained at 3.2 mg l^{-1} of BAP level (average of 6 and 8 shoots per explant). However, at the same concentration of BAP in MS medium no shoot formation was observed in cultivar Kama which regenerated only at concentration of 6.4 mg l^{-1} BAP. The same author implied that the number of plantlets per leaf blade was higher when compared with petiole explants.

In the previous studies, no calli or shoot growth was observed in the medium without TDZ [Debnath 2006]. One disadvantage of TDZ and its frequent inhibitory effect on shoot elongation, as has been reported in several woody species [Huetteman and Preece 1993]. Inhibition of elongation occurs because the level of endogenous cytokinins is increased, which inhibits the action of cytokinin oxidase [Hare et al. 1994].

Cytokinins and auxins are known to be promote cali formation in tissue culture. The lowest callus formation rate observed without IBA medium, but no callus formation observed without TDZ medium, such as 1, 6 and 11 numbers medium. According to the results, TDZ showed similar affects both cytokinin and auxins. The highest calli formation was determined consisting of 0.2–0.4 mg l^{-1} IBA medium. Callus formation is thought to be a positive effect of 15 day darkness. The average percentage of callus formation 15 days in dark conditions was determined 75.12% , however photoperiod conditions was determined 62.7%.

Table 5. The average number of shoots and percentages of shoot regeneration (%) derived from stipule and leaf explants of 'Festival' variety, efficiency of various $AgNO_3$ levels under dark and photoperiod conditions

Tabela 5. Średnia liczba pędów i procent regeneracji pędów (%) z przylistków i liści roślin odmiany 'Festival', efektywność różnych poziomów $AgNO_3$ w warunkach zaciemnienia i fotoperiodu

Application Podanie			Stipule – Przylistek				Leaf – liść			
			shoot number liczba pędów		shoot regeneration percentage procent regeneracji pędów		shoot number liczba pędów		shoot regeneration percentage procent regeneracji pędów	
IBA	TDZ	$AgNO_3$	D	P	D	P	D	P	D	P
0.4	3	0	6.3d	3.3d	100a	86.6ab	5.3c	2.6c	93.3a	73.3a
	3	1	10.0c	15a	100a	93.3a	6.6b	3.0c	53.3b	53.3b
	3	2	14.3a	11.3b	100a	86.6ab	10.6a	5.6a	86.6a	66.6ab
	3	3	11.3b	10.3c	93.3a	60.0b	6.3bc	4.3b	73.3ab	60.0ab
$LSD_{0.01}$			1.33	0.94	14.44	25.0	1.09	0.94	22.4	11.58

D – dark – ciemność; P – photoperiod – fotoperiod.

Values in columns marked with the same letter do not differ at $p \leq 0.01$ – Wartości w kolumnach oznaczone tymi samymi literami nie różnią się istotnie przy $p \leq 0{,}01$.

Table 6. The average number of shoots and percentages of shoot regeneration (%) derived from stipule explants of 'Rubygem' variety, efficiency of various $AgNO_3$ levels under dark and photoperiod conditions

Tabela 6. Średnia liczba pędów i procent regeneracji pędów (%) z przylistków roślin odmiany 'Rubygem', efektywność różnych poziomów $AgNO_3$ w warunkach zaciemnienia i fotoperiodu

Application – Podanie			Shoot number Liczba pędów	Shoot regeneration percentage Procent regeneracji pędów
IBA	TDZ	$AgNO_3$	D	D(%)
0.2	1	0	3.0d	46.6b
	1	1	5.3c	53.3ab
	1	2	8.3a	73.3a
	1	3	6.6b	53.3ab
$LSD_{0.01}$			0.94	12.85

D – dark – ciemność.

Values in columns marked with the same letter do not differ at $p \leq 0.01$ – Wartości w kolumnach oznaczone tymi samymi literami nie różnią się istotnie przy $p \leq 0{,}01$.

Calli formation were measured after forty days of culture initiation using the following scale:0 (no callus formation), scale 1 (calli diameter < 2 mm), scale 4 (calli diameter 2–13 mm), scale 8 (calli diameter > 13 mm). The cali formation was observed in all medium.

Silver nitrate is known to promote multiple shoot formation in different plants. Addition to this, it inhibits ethylene action to employe the supresssing the development of female flowers and induces male flowers [Takashi and Caffe 1984]. Beyer [1976], reported that silver nitrate has been imployed in tissue culture studies for inhibiting ethylene action because of its water solubility and lack of phytotoxicity at effective concentrations. Silver ions are employed in the form of silver thiosulphate in several tissue culture studies [Eapen and George 1997]. For this purpose, we applied various levels of silver nitrate using obtained best results among the medias. According to the results, various levels of silver nitrate was applied to each variety, separately. As mentioned above and seen in table 1 and 2, the best results were obtained from 0.4 mg l^{-1} IBA and 3 mg l^{-1} TDZ combination both leaf stipule and leaf explants of 'Festival' variety. The results of average number of shoots and percentage of shoot regeneration combination of various level of silver nitrate under dark and photoperiod conditions of stipule and leaf explants of Festival variety were given in Table 5. As seen in Table 5, the higher shoot numbers and percentages of shoot regeneration were obtained from stipule explants of 'Festival' variety and dark conditions compare to the leaf explants and photoperiod conditions in all levels. However, the highest shoot numbers (15) were obtained only in 1mg/l silver nitrate combination under photoperiod condition stipule explants of 'Festival' variety.

Table 7. The average number of shoots and percentages of shoot regeneration (%) derived from stipule explants of 'Rubygem' variety, efficiency of various $AgNO_3$ levels under photoperiod conditions

Tabela 7. Średnia liczba pędów i procent regeneracji pędów (%) z przylistków roślin odmiany 'Rubygem', efektywność różnych poziomów $AgNO_3$ w warunkach fotoperiodu

Application – Podanie			Shoot number Liczba pędów	Shoot regeneration percentage Procent regeneracji pędów
IBA	TDZ	$AgNO_3$	P	P(%)
0.2	4	0	8.3a	73.3a
	4	1	7.6a	46.6b
	4	2	6.6b	46.6b
	4	3	4.0c	26.6b
$LSD_{0.01}$			0.94	13.2

P – photoperiod – fotoperiod.

Values in columns marked with the same letter do not differ at $p \leq 0.01$ – Wartości w kolumnach oznaczone tymi samymi literami nie różnią się istotnie przy $p \leq 0,01$.

As for the 'Rubygem' variety, due to the highest results obtained from a few combinations based on the shoot numbers, percentage of shoot and calli formation four combinations were applied using various silver nitrate combinations and results were given in Table 6, 7, 8 and 9.

Table 8. The average number of shoots and percentages of shoot regeneration (%) derived from leaf explants of 'Rubygem' variety, efficiency of various $AgNO_3$ levels under dark conditions

Tabela 8. Średnia liczba pędów i procent regeneracji pędów (%) z liści roślin odmiany 'Rubygem', efektywność różnych poziomów $AgNO_3$ w warunkach zaciemnienia

Application – Podanie			Shoot number Liczba pędów	Shoot regeneration percentage Procent regeneracji pędów
IBA	TDZ	$AgNO_3$	D	D(%)
0	2	0	5.6a	40.0b
	2	1	6.6a	66.6a
	2	2	3.3b	26.6b
	2	3	2.3b	33.3b
$LSD_{0.01}$			1.08	11.9

D – dark – ciemność.

Values in columns marked with the same letter do not differ at $p \leq 0.01$ – Wartości w kolumnach oznaczone tymi samymi literami nie różnią się istotnie przy $p \leq 0,01$.

The results of average number of shoots and percentages of shoot regeneration (%) derived from stipule explants of 'Rubygem' variety efficiency of various $AgNO_3$ levels under dark conditions were given in Table 6. As seen in Table 6, the highest average

shoot numbers (8.3) and percentage of shoot regeneration (73.3%) were detected 0.2 mg l^{-1} IBA, 1 mg l^{-1} TDZ and 2 mg l^{-1} $AgNO_3$ combinations under dark conditions.

Table 9. The average number of shoots and percentages of shoot regeneration (%) derived from leaf explants of 'Rubygem' variety, efficiency of various $AgNO_3$ levels under photoperiod conditions

Tabela 9. Średnia liczba pędów, procent regeneracji pędów (%) i tworzenia się kalusa (%) z liści roślin odmiany 'Rubygem', efektywność różnych poziomów $AgNO_3$ w warunkach zaciemnienia i fotoperiodu

Application – Podanie			Leaf shoot Pędy liściowe	leaf shoot(%) % pędów liściowych/
IBA	TDZ	$AgNO_3$	P	P(%)
0	3	0	5.0a	60.0a
	3	1	3.3b	53.3a
	3	2	2.0c	26.6b
	3	3	1.6c	26.6b
$LSD_{0.01}$			0.83	11.6

P – photoperiod – fotoperiod.

Values in columns marked with the same letter do not differ at $p \leq 0.01$ – Wartości w kolumnach oznaczone tymi samymi literami nie różnią się istotnie przy $p \leq 0{,}01$.

The results of average number of shoots and percentages of shoot regeneration (%) derived from stipule explants of 'Rubygem' variety efficiency of various $AgNO_3$ levels under photoperiod conditions were given in Table 7. As seen in Table 7, the highest average shoot numbers (8.3) and percentage of shoot regeneration (73.3%) were detected 0.2 mg l^{-1} IBA, 4 mg l^{-1} TDZ and 0 mg l^{-1} $AgNO_3$ combinations under photoperiod conditions.

The results of average number of shoots and percentages of shoot regeneration (%) derived from leaf explants of 'Rubygem' variety efficiency of various $AgNO_3$ levels under dark conditions were given in Table 8. As seen in Table 8 the highest shoot numbers and percentages of shoot regeneration obtained from 0 mg l^{-1} IBA, 2 mg l^{-1} TDZ and without AgNO3 combinations (5.6 and 40.0%, respectively).

Table 9 gives the results of the average number of shoots and percentages of shoot regeneration (%) derived from leaf explants of 'Rubygem' variety efficiency of various $AgNO_3$ levels under photoperiod conditions. As seen in Table 9 the highest shoot numbers and percentage of shoot regeneration were obtained a combination of 0 mg l^{-1} IBA, 3 mg l^{-1} TDZ and without $AgNO_3$ combination (5.0 and 60.0%, respectively).

CONCLUSION

The application of genetic modification technology in strawberry is related to the efficiency of the regeneration protocol. As a result of this experiment, the differences

were detected regeneration response of 'Festival' and 'Rubygem' strawberry varieties based on their explant types, dark or photoperiod conditions. Stipules were found to be higher efficiency compare to the leaf disks, 15 d dark condition found to be more effective than direct photoperiod conditions in most combinations. In addition TDZ, IBA and $AgNO_3$ combinations showed positive effect on regeneration and their induction was varied between genotypes, explant types and photoperiod and dark conditions.

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ROZWÓJ METOD REGENERACJI *in vitro* ODMIAN TRUSKAWKI ‘FESTIVAL’ I ‘RUBYGEM’

Sterszczenie. Truskawka uprawna (*Fragaria* × *ananassa* Duch.), należąca do rodziny Rosaceae, jest najważniejszym owocem miękkim na świecie. Mikrorozmnażanie *in vitro* jest ważnym narzędziem do rozmnażania przez klonowanie i jest powszechnie stosowane do wywoływania odmiany somaklonalnej dla wytworzenia zmienności genetycznej. Zoptymalizowane protokoły regeneracji oraz wysoka wydajność również są ważne dla transformacji genów w celu ulepszenia nowych odmian. Dlatego celem niniejszej pracy była optymalizacja protokołu regeneracji dla odmian truskawek ‘Festival’ i ‘Rubygem’. Blaszki liściowe oraz przylistki dojrzałych wyhodowanych *in vitro* roślin truskawek odmian ‘Festival’ i ‘Rubygem’ (*Fragaria* × *annanasa* Duch.) wykorzystano jako źródła eksplantów. Rośliny Mc Cowns Woody zawierające witaminy wykorzystano jako pożywkę zasadową. Przetestowano również różne stężenia TDZ (Tidiazuron) (0, 1, 2, 3 i 4 mg l^{-1}) i IBA (kwas indolowo-3-masłowy) (0, 0,2 i 0,4 mg l^{-1}) oraz wpływ 15-dniowego zaciemnienia

na regenerację z blaszek liściowych i przylistków. Nasze wyniki sugerowały, że przylistki, wysokie stężenie TDZ, $AgNO_3$ oraz zaciemnianie w pożywce hodowlanej pozytywnie wpłynęły na wywołanie regeneracji.

Słowa kluczowe: truskawka, *in vitro*, organogeneza, regeneracja, $AgNO_3$

ACKNOWLEDGEMENT

This study financially supported the project the of Ç.U Scientific Research Projects Unit (Project no: ZF2010YL57) and the Scientific and Technical Research Council of Turkey (TUBITAK; Project no: 7100041). The authors thanked to Biotechnology Research and Experimental Center of Univesity of Çukurova, also.

Accepted for print – Zaakceptowano do druku: 25.06.2012