ACTA SCIENTIARUM POLONORUM
Acta Sci. Pol. Hortorum Cultus, 16(5) 2017, 145–158
www.acta.media.pl ISSN 1644-0692 DOI: 10.24326/asphc.2017.5.15

REVIEW ARTICLE

Accepted: 12.06.2017

In vitro REGENERATION OF *Fragraria* PLANTS

Marta Rokosa✉, Małgorzata Mikiciuk

West Pomeranian University of Technology in Szczecin Department of Plant Physiology and Biochemistry,
Juliusza Słowackiego 17, 70-953 Szczecin

ABSTRACT

Fragaria is one of genus in *Rosaceae* family. The most popular representative is strawberry (*Fragaria* × *ananassa* Duch.) and wild (woodland) strawberry (*Fragaria vesca* L.), which taste attributes are very attractive for a huge number of consumers around the world. The plants have many beneficial traits, such as low-caloric, high amount of antoxidants and vitamin C, laxative, diuretic, astringent, antidiarrheal and antiseptic properties. Cultivation of *Fragaria* plants is widespread worldwide with particular emphasis on moderate climate zone, also with use of a plant tissue culture method. This thesis showed and contrasted other studies about *Fragaria* plants propagation under *in vitro* conditions. In this method the most often used explants are leaf explants. Very rarely are used seeds. Mainly, the plants are propagated on basal medium of mineral composition by Murashige and Skoog in 8 hour dark and 16 hour light contidions. The most efficient cytokinin used to root induction is indole-3-butyric acid (IBA). The plant acclimatization had varying effectiveness – from a few to several dozen survival percent. During micropropagation of *Fragaria* plants, somaclonal variation occurs, which is dependent on age culture, frequency of passage and medium composition.

Key words: micropropagation, *Fragaria*, strawberry, shoot multiplication

Abbreviations: 2,4-D – 2,4-dichlorophenoxyacetic acid; ABA – abscisic acid; BA – 6-benzyladenine; BAP – 6-benzylaminopurine; BSAA – 3-benzoselenienylacetic acid; EtOH – ethanol (C_2H_5OH); GA3 – gibberellic acid; $HgCl_2$ – mercury (II) chloride; IAA – indoleacetic acid; IBA – indole-3-butyric acid; KIN – kinetine; KNOP – medium of mineral composition by Knop; MS – medium of mineral composition by Murashige and Skoog; ½ MS – half strength medium of mineral composition by Murashige and Skoog; NAA – 1-naphthaleneacetic acid; NaOCl – sodium hypochlorite; PGRs – plant growth regulators; TDZ – thidiazuron

INTRODUCTION

One of the main plant biotechnology tool is plant tissue culture, which uses plant totipotency – natural ability of plant cells to regeneration [Haberlandt 1902]. Plant tissue culture were first described by Steward et al. [1958]. Both plant cell, tissue and organs cultures [Gantait et al. 2011] can be used to clonal propagation disease-free plant and to protection of the gene pool or genetic engineering. This tool is very helpful when propagation many species of plants that cause problems during conventional culti-

✉ marta.rokosa@zut.edu.pl

vation. One of these species is Fragaria, which is often infested by soil pathogens.

Species, which belongs to *Rosaceae* family take third place in terms of economic among species cultivated in moderate climate zone [Oosumi et al. 2006]. World production of strawberries is about 4 500 000 tons per year while leading European producers are Spain (about 290 000 ton/year), Germany (about 156 000 ton/year) and Poland (about 150 000 ton/year) [Sowik et al. 2015].

Fragaria plants (*Rosaceae* family) are a perennial plants, which taste attributes of fruits are considered very attractive for a huge number of consumers around the world [Debnath 2009]. There are more than 12 species in *Fragaria* genus. Numerous varieties of them are cultivated in 37 countries [Biswas et al. 2009]. The varieties significantly differ in size, color, taste and shape of fruits, fertility rate, time of maturity, disease resistance and chemical composition. *Fragaria* plants fruits are valuable because of their content of low-caloric carbohydrates. They are a good source of natural antioxidants such as carotenoids, vitamins, phenols, flavonoids, dietary glutathione and endogenous metabolites. Content of phenols (especially ellagic acid) in strawberries causes their health-promoting qualities [Skupień and Oszmiański 2004]. They have also antioxidative properties [Debnath 2009]. The strawberry fruits contain vitamin C in an amount exceeding oranges and lemons [Bhatt and Dhar 2000]. Woodland strawberry (*Fragaria vesca* L.), named also wild strawberry, has laxative, diuretic, astringent, antidiarrheal and antiseptic properties and is used as an antirheumatic agent in traditional medicine [Lamari et al. 2008]. Leaf extracts are used to treat gastrointestinal and skin diseases and fruit extract to treat colorectal mucositis [Yildirim and Turker 2014]. Although, woodland strawberry is not comercial cultivated species in *Fragaria* genus, its valuable traits as a research model have been repeatedly emphasized [Alsheikh et al. 2002].

Varieties of strawberry (*Fragaria* × *ananassa* Duch.) are a result of crossing two species: *F. chiloensis* Duch. and *F. virginiana* Duch. [Gruchała et al. 2004], which were imported to Europe at the turn of the sixteenth and seventeenth century and [Nyman and Wallin 1992].

Strawberry (*Fragaria* × *ananassa* Duch.) is heterozygous oktoploid (2n = 8x = 56) [Owen i Miller 1996], differently from the woodland strawberry (*Fragaria vesca* L), which genome size is much less than *Arabidopsis thaliana* genome [Oosumi et al. 2006]. This is because the woodland strawberry is diploid (2n = 2x = 14) [El Mansouri et al. 1996].

Propagation of fragaria plants

Large problem in strawberries cultivation are fungal disease, mainly caused by soil pathogens [Sowik et al. 2015]. The most important of them are: gray mold (grey mould), Fusarium wilt and Verticillium wilt respectively caused by *Botrytis cinerea, Fusarium oxysporum* f. sp. fragariae and *Verticillium dahliae* Kleb. [Mathews et al. 1995, Żebrowska 2011]. With genetic engineering and use of appropriate regenerative methods, resistance to *Fusarium oxysporum* f. sp. fragariae genotypes [Toyoda 1991] has been obtained. Research about obtaining healthy mother plants and progeny and strawberry resistance to fungal and viral pathogens [Harris et al. 1997, Lines et al. 2006] has been also carried out. The plant breeders main objective is to obtain plants resistant to diseases causing a huge economic losses. The biotechnological methods that use horizontal gene transfer and clonal propagation are practical means to stable one (or more) dominant gene transfer to achieve desirable plant traits [Mathews et al. 1995].

Fragaria plants are propagated by seeds and runners. Propagation by seeds is not widely used, due to progeny, much later than plants propagated from runners, enter a period of fruiting. Propagation of *Fragaria* plants by stolons gives a limited number of progeny. However, it should be emphasised that large vulnerability of *Fragaria* plants to fungal disease reduces efficiency of methods listed above [Bhatt and Dhar 2000]. It is necessary to develop effective, fast and efficient large-scale *Fragaria* plants propagation methods. Strawberry micropropagation (*Fragaria* × *ananassa* Duch.) was firstly described in 1974 by Boxus [1974]. Many clonal propagation methods of strawberries have already been described, but in most

cases the shoot regeneration rate was low and the obtained results were inconsistent. Moreover, a successful genetic transformation protocol depends on efficient *in vitro* plant regeneration [Jones et al. 1988].

To date, many *Fragaria* species in plant tissue cultures and genetic transformation area were studied (tab. 1). The missing element in many studies is to provide the regeneration rate of the variety. It has been taken into account only in a few studies. For 'Elsanta', regeneration rate of 4% using a variety of explants and MS (Murashige and Skoog) media supplemented with combinations of BA (6-benzyloadenine), TDZ (thidiazuron), NAA (1-naphthaleneacetic acid) and 2,4 D (2,4-dichlorophenoxyacetic acid) has been obtained [Passey et al. 2003]. For 'Cambridge Favourite' and 'Hapil', shoot regeneration from callus using lamina and petiole explants [Jones et al. 1988] were not obtained. Also for 'Dana' and 'Santana' there was not regeneration from callus using lamina and petiole explants [Rugini i Orlando 1992].

It should be also emphasized, that the plants, cultivated initially *in vitro*, grow more effectively than plants cultivated only conventionally [Cameron and Hancock 1986, Żebrowska et al. 2015]. Moreover, they are characterized by a greater vigour [Karhu 2001, Karhu and Hakala 2002]. Besides of that, plants cultivated both on solid and liquid media are characterized by a higher content of anthocyanins and antioxidants than those grown with conventional methods [Debnath 2009]. Different results has been obtained by Mohamed et al. [2007], who reported that, more efficient regeneration from lamina was obtained from explants taken from two months old plants growing in a greenhouse than from explants taken from plants growing earlier *in vitro*. Similar results were obtained by Nehra et al. [1990].

Explant selection and sterilization

To date, in studies about *Fragaria* × *ananassa* Duch. plant regeneration, many different explants were used (tabs 1 and 2). The most common explants chosen for culture initiation are apical meristems runners and other meristems, lamina, shoot fragments, stipules and petioles [Gantait et al. 2011]. It has been proven, that strawberry regeneration rate from stipules is higher than from leaf explants [Żebrowska and Hortyński 2002]. In case of studies about *Fragaria vesca* the most common primordial explant were seeds [El Mansouri et al. 1996, Haymes and Davis 1998, Alsheikh et al. 2002, Morozova 2002, Zhao et al. 2004, Oosumi et al. 2006, Yildirim and Turker 2014, Zhang et al. 2014].

At first, it has been shown that the strawberry meristem culture is the most effective technique of viruses elimination, mass reproduction and seeds protection [Boxus et al. 1977]. However, an attention should be drawn to the fact that cutting meristems is time-consuming and requires high technical skills. It is also difficult to use cultures of meristems to genetic transformation. As an effective alternative, the use of leaf blades as explants for plant regeneration was recognized [Nehra et al. 1989]. In this method there is no intermediate stage of callus and it avoids the risk of the occurrence of the phenomenon of genetic instability [Nehra et al. 1994]. Leaf explants are particularly useful to shoot regeneration and genetic transformation of *Fragaria* plants due to the fact that in the most of cases they have the highest regeneration frequency [Landi and Mezzetti 2006]. For *Fragaria vesca*, shoot regeneration rate from petioles was merely about 10%, wherein lamina utilizing could achieve more than 80% regeneration rate on MS media with addition of IBA and BA [Alsheikh et al. 2002].

There are a lot of efficient methods of *Fragaria* plant sterilization before cultures initiation. One of them is washing in tap water for 30 min initially and next, sterilization in 10% (v/v) Domestos solution for 20 min and after that rinsing six times in sterile distilled water [Passey et al. 2003]. Domestos formulation may be replaced by 5,25% solution of sodium hypochlorite (NaOCl) [Kumar et al. 1999] or 12% solution of a mixed solutions (vol/vol) of 0,63% NaOCl and 0,1% (v/v) Tween 20 formulation – sterilization for 15 min and next, rinse in 70% ethanol solution for 30 second [Debnath 2009]. Tween 20 formulation was also used by other authors [Yildirim and Turker 2014]. Jones et al. [1988] sterilized plants for 25–35 min with 10% (v/v) Domestos solution and four times rinsing in a sterile distilled water, without previously soaking in a tap water. Toyoda et al. [1990] used also 70% solution of ethanol (EtOH)

and 2% solution of NaOCl and next four times rinsing in a sterile distilled water. The most optimal concentration of NaOCl in *Fragaria* plants sterilization seems to be 0,5 to 1,0%. This concentration resulted with higher survival percent in such varieties as: 'Chandler', 'Oso Grande', 'Toro' and 'Islamabad Local' [Munir et al. 2015]. In Rattanpal et al. [2011] studies for sterilization, plants was dipped in 0,1% solution of mercury (II) chloride ($HgCl_2$) for 4 min.

There are only a few studies about use of *Fragaria* seeds as primordial explants with description of their sterilization method. Yildirim and Turker [2014] and Oosumi et al. [2006] used a standard protocol of seeds rinsing for 5 min in 70% ethanol (C_2H_5OH) solution and next, rinsing in 4,5% (or 1%) NaOCl solution for 5 min and four times rinsing in a sterile distilled water. Also was used a procedure of initial soaking seeds in water for one hour to stimulate germination [Alsheikh et al. 2002]. Zhang et al. [2014] sterilized seeds of *Fragaria vesca* in 30% mixed solution of 6% NaOCl solution and two drops of Tween 20 and next four times rinsed in a sterile distilled water.

Table 1. *In vitro* regeneration of *Fragaria* plants by various authors

Species	Variety	Explant	Response	Medium		Conditions	Authors
				Basal medium	PGRs (mg·dm^{-3})		
Fragaria × *ananassa* Duch.	n/d	Leaf mesophyll protoplast	Mult Sht	8p	1 2,4-D + 0IBA	n/d	Nyman and Wallin [1988]
		Shoot tip		MS	0,02 NAA + 2 BA	2000–8000 lux	Jeong et al. [1996]
	Redgauntlet Bogota Brighton Ostara Rapella Cambridge Favourite Hapil	Petiole, flower bud, hoot tip			0,2 BAP + 0,02 IBA + 0,21 GA_3	22°C, 16/8 PP, pH 5,6	Jones et al. [1988]
	Senga Sengana	Runner tip meristem			1 BA + 1 IBA	n/d	Petrovic and Jacimovic-Plavsic [1990]
	Syriusz	Shoot fragment			0,5 BA + 0,1 IBA	n/d	Małodobry et al. [1997]
	Camarosa	Apical meristem			0,4 TDZ + 0,9 BAP	n/d	Haddadi et al. [2010]
	Elsanta	Runner tip			0,5 IBA + 0,5 BA	pH 5,6–5,7, 16/8 PP, 23°C/18°C (day/night), 55 µmol m^{-2}s^{-1} light intensity	Sowik et al. [2015]
	Tristar Totem	Meristem, leaf, petiole			1 IAA + 1 BA + 0,01 GA_3	pH 5,8, 16/8 PP, 15–20 µmol m^{-2} s^{-1} light intensity	Mathews et al. [1995]
	Chandler	Leaf, petiole	Sht Reg		2 BAP + 0,25 NAA + 0,5 KIN	n/d	Kaushal et al. [2004]
		Vegetative bud	Mult Sht		0,5 KIN + 1 BAP + 2 GA_3	n/d	Kaur et al. [2005]
		Runner tip	Sht Reg		4 BAP	n/d	Lal et al. [2003]
				Knop	4 IBA + 0,4 SAP + 0,4 GA_3		
	Ofra						
	Oso Grande						
				MS	0,5 BA + 0,1 IBA	n/d	Rattanpal et al. [2011]
		Nodal fragment			4 TDZ	16/8 PP, 26°C ±1°C	Husaini [2010]
			El		0,1 IBA + 0,05 KIN + 2 GA_3		

	n/d	Leaf	Sht Reg		3,2 BAP	n/d	Żebrowska and Hortyński [2002]
		Petiole			0,5 – 1,5 IBA	n/d	Husaini and Srivastava [2006]
		Lamina			1 TDZ + 0,5-1 IBA	n/d	
					1,5 IBA + 0,75 NAA + 0,5 KIN	n/d	Karim et al. [2015]
	Gorella	Petiole, flower bud			2 2,4-D + 0,1-1 BAP	n/d	Foucault and Letouze [1987]
	Redcoat	Lamina			2,25 BA + 1,752 IAA	n/d	Nehra et al. [1989]
	Hiku Jonsok				0,5 BAP + 0,5 IBA + 0,2 GA_3	25°C ±1°C (day), 23°C ±1°C (night), 16/8 PP, 40–50 pmol·m^{-2} ·s^{-1} light intensity	Sorvari et al. [1993]
	Chandler	Anther			2 IAA +1 BA	pH 5,8, 16/8 PP, 130 µmol m^{-2} s^{-1}	Owen and Miller [1996]
	Honeoye Redchief				2 IAA +1 BA	pH 5,8, 16/8 PP, 130 µmol m^{-2} s^{-1} light intensity	Owen and Miller [1996]
	Calypso Pegasus Emily Bolero Eros Elsanta Tango	Lamina, petiole, stipule, root fragment			1 TDZ + 0,2 2,4-D	25°C, 16/8 PP	Passey et al. [2003]
					1 TDZ + 0,2 NAA		
					2 BA + 0,5 TDZ + 0,2 2,4-D		
					2 BA + 0,2 2,4-D		
			Mult Sht		1 BA + 0,1 IBA + 0,2 GA_3		
	Tango	Lamina	Sht Reg		2,25 BAP + 0,18 NAA	n/d	Khan and Spoor [2004]
					2,25 BAP + 1 IBA		
	Pajaro				0,1 2,4-D + 1 BA	pH 5,7, 16/8 PP, 25°C ±2°C, 40 µmol $m^{-2}$$s^{-1}$ light intensity	Ricardo et al. [2003]
	Hecker La Sans Rivale	Shoot fragment			2,2 TDZ + 0,3 IBA	pH 5,6, 16/8 PP, 25°C, 40 µmol $m^{-2}$$s^{-1}$ light intensity	Zhao et al. [2004]
			Mult Sht		0,56 BA + 0,1 IBA		
		Seed	Germ		MS withous PGRs		
	Tonoyoka	Shoot tip	Sht Reg		0,2 BA + 0,01 IBA + 0,1 GA_3	pH 5,8, 16/8 PP, 25°C ±2°C,	Yonghua et al. [2005]
			Mult Sht		0,5 BA + 0,03 NAA		
	Sweet Charlie	Petiole	Em		0,5 ABA	n/d	Pallavi et al. [2011]
			Sht Reg		Half strenght MS		
		Runner tip			1 TDZ	n/d	Rekha et al. [2012]
			Mult Sht		0,5 BAP + 0,5 IBA + 1 GA_3		
Fragaria vesca L.	n/d	Petiole, flower bud	Sht Reg		B5 + 2 2,4-D + 0,1-1 BAP	n/d	Foucault and Letouze [1987]
Fragaria indica	n/d	Nodal fragments			0,9 BA + 0,02 NAA	pH 5,8, 16/8 PP, 40 µmol $m^{-2}$$s^{-1}$ light intensity	Bhatt and Dhar [2000]

n/d – no data; Sht Reg – Shoot Regeneration; Mult Sht – Multiple Shoot; Em – Somatic embryo; El – Elongation; Germ – Germination; PP – Photoperiod

Table 2. Callus induction of *Fragaria in vitro* plants by different authors

Species	Variety	Explant	Medium [MS + PGRs (mg·dm^{-3})]	Conditions	Authors
Fragaria × *ananassa* Duch.	Tango	Lamina	1 2,4-D + 1 BAP	n/d	Khan and Spoor [2004]
	Pajaro		0,2-0,3 2,4 D + 2-4 BA	pH 5,7, 16/8 PP, 25°C ±2°C, 40 µmol m^{-2}s^{-1} light intensity	Ricardo et al. [2003]
	n/d		2 NAA + 0,5 IBA	n/d	Karim et al. [2015]
	Redcoat		1 BA + 1 2,4-D	n/d	Nehra et al. [1990]
			2,25 BA + 0,186 NAA	16/8 PP	
	Redgauntlet Bogota Brighton Ostara Rapella Cambridge Favourite Hapil		0,2 BAP + 0,2 2,4-D	22°C, 16/8 PP, pH 5,6	Jones et al. [1988]
	Hoko-Wase	Young leaf	0,1 2,4-D + 1 BAP	n/d	Toyoda et al. [1991]
	n/d	Leaf, petiole	0,5 BAP + 0,75 NAA	n/d	Kaushal et al. [2004]
	Sweet Charlie	Petiole	1 TDZ + 1 2,4-D	n/d	Pallavi et al. [2011]
	Honeoye Redchief	Anther	2 IAA +1 BA	pH 5,8, 16/8 PP, 130 µmol m^{-2} s^{-1} light intensity	Owen and Miller [1996]
	Chandler				
	Addie Dana Gea Santana	Stipule	2,25 BAP + 0,5 IBA	n/d	Rugini and Orlando [1992]
Fragaria moschata Duch.	n/d	Leaf, petiole	1 NAA + 0,025 BA	n/d	Infante et al. [1998]

n/d – no data; PP – Photoperiod

In Owen and Miller [1996] studies, to sterilize floral buds of strawberry varieties, dipping it in 70% ethanol solution for 30 second and next rinsing in 5,25% NaOCl solution for 20 min and rinsing three times in a sterile distilled water was used.

Culture initiation

Basal medium which is chosen by most authors for culture initiation is a medium of mineral composition by Murashige and Skoog (MS) [1962]. Also Knop and N30K medium are used [López-Aranda et al. 1994]. In order to gel the medium, due to low effectiveness of other gel agent such as Gerlite, mainly agar is used [Svensson and Johansson 1992, Owen and Miller 1996]. There are promising research results of possibility to use pulp from sugar cane, which is waste of the extraction process, as an agar substitute in rooting medium [Mohan et al. 2005].

The pH of initiation media is from 5,7 to 5,8 (tabs 1, 2 and 3). Several authors used other medium pH: 5,5 [Oosumi et al. 2006], 5,6 [Jones et al. 1988, Yildirim and Turker 2014, Zhao et al. 2004] and 6,0 [Toyoda et al. 1990]. The standard sucrose concentration in initial medium (30 g·dm^{-3}) is effective enough in the regeneration of strawberry plants [Debnath 2005]. There were also carried out studies which indicated that sucrose in the medium might be replaced effectively by a table sugar (20 g·dm^{-3}) [Kaur et al. 2005].

Table 3. Rooting of *Fragaria in vitro* plants by different authors

Species	Variety	Medium [MS + PGRs (mg·dm^{-3})]	Conditions	Authors
Fragaria × *ananassa* Duch.	n/d	Half strenght MS	2000–8000 lux	Jeong et al. [1996]
		Half strenght MS + 1 IBA	n/d	Husaini and Srivastava [2006]
		MS without PGRs	n/d	Karim et al. [2015]
	Senga Sengana	0,5 IBA	n/d	Petrovic and Jacimovic-Plavsic [1990]
	Hoko-Wase	MS without PGRs	n/d	Toyoda et al. [1991]
	Chandler	1 IBA	n/d	Lal et al. [2003]
	Ofra			
	Oso Grande			
		Half strenght + 1 IBA	n/d	Rattanpal et al. [2011]
	Calypso Pegasus Emily Bolero Eros Elsanta Tango	3 IBA	25°C, 16/8 PP	Passey et al. [2003]
		MS without PGRs		
	Camarosa	0,2 NAA + 0,2 IBA	n/d	Haddadi et al. [2010]
		MS without PGRs		
	Sweet Charlie	0,5 KIN	n/d	Rekha et al. [2012]
		0,5 KIN + 0,5 IBA		
Fragaria indica	n/d	Half strenght MS + 0,186 NAA	pH 5,8, 16/8 PP, 40 µmol m^{-2}s^{-1} light intensity	Bhatt and Dhar [2000]

n/d – no data; PP – Photoperiod

Plant multiplication and regeneration

Many variants of medium used to multiplication of *Fragaria* plants were studied (tab. 1). At first, the medium used to multiplication and regeneration was supplemented with macronutrients in an amount included in a Knop's medium and microelements in the amount from the MS medium [Jemmali et al. 1994, 1995, 2002]. Currently, the basal medium used to be the MS medium. The most commonly used and most efficient growth regulators used in multiplication and regeneration are TDZ and 2,4-D. TDZ was used in shoot regeneration of *Fragaria* plants by many authors [Passey et al. 2003, Zhao et al. 2004, Husaini and Srivastava 2006, Husaini 2010], similarly as BAP [Foucault and Letouze 1987, Sorvari et al. 1993, Żebrowska and Hortyński 2002, Lal et al. 2003, Kaushal et al. 2004]. One of disadvantages of TDZ is possibility of inhibitory effect occurring on shoot elongation [Debnath 2009] which is associated with their cytokinin activity [Huetteman and Preece 1993] which occured also in strawberries [Jones et al. 2003]. Consequently, a combination of TDZ and IBA (indole-3-butyric acid) activity induced the higher regeneration frequency on the same genotypes, which confirms positive effects of this auxin in strawberry leaf tissue callogenesis [Yonghua et al. 2004, Husaini and Srivastava 2006, Landi and Mezzetti 2006, Yildirim and Turker 2014]. For 'Camarosa', optimal concentration of TDZ and IBA was respectively 1,5 mg·dm^{-3} and 1,0 mg·dm^{-3} [Adak et al. 2010]. Relatively effective concentration of TDZ was also 0,4–0,8 mg·dm^{-3} [Debnath 2005, 2006], but there are reports describing the use of significantly higher concentrations – 13,2–17,6 mg·dm^{-3} effect of which was a hundred percent regeneration from leaf explants [Sutter et al. 1997]. Using TDZ at concentra-

tion of 1,0 mg·dm^{-3} in combination with NAA or 2,4-D at concentration of 0,2 mg·dm^{-3} allowed also to obtain satisfactory results of shoot regeneration [Passey et al. 2003]. Impact of other auxins, such as 2,4-D and BSAA on shoot regeneration was more dependent on plant genotypes [Landi and Mezzetti 2006]. By Yonghua et al. [2004], 2,4-D is increasingly used to strawberry shoot induction. By other authors, the regeneration media, on which plants developed the largest number of shoots is MS medium with a 6-benzyloaminopurine (BA) and indole-3-butyric acid (IBA) [El Mansouri at al. 1996, Barcelo et al. 1998, Balokhina et al. 2000, Passey et al. 2003]. According to Gruchała et al [2004], the optimal concentration of IBA in shoot regeneration from leaf explants for 'Elsanta' and 'Induka' was 0,4 mg·dm^{-3}. In earlier studies it has been shown that the most efficient concentrations of BA in shoot regeneration for different strawberry genotypes was 0,9–1,8 mg·dm^{-3} [Simpson and Bell 1989]. In case of using BAP exclusively, it has been shown, that efficient was only concentration 3,2 and 6,4 mg·dm^{-3} [Żebrowska and Hortyński 2002]. It is worth to mention that, low salt content in medium and lack of plant growth regulators (PGRs) may contribute to inhibition of growth and development of strawberry plants [Monticelli et al. 2002].

For *Fragaria indica* the most appropriate shoot induction medium was MS with addition of 1,0 mg·dm^{-3} BA and 0,02 mg·dm^{-3} NAA (pH 5,8, temp. 25 ±2°C, white light with intensity of 40 µmol·m^{-2}·s^{-1}) [Bhatt and Dhar 2000].

Usage of liquid cultures, which was described by Debnath [2009], significantly improves the yield of strawberry micropropagation. It was noticed that, on MS with 0,02 mg·dm^{-3} TDZ, on 20–30% of plants vitrification has been occurred, which was caused by high potential of water in leaves. That process was reversed by using medium with 0,2 mg·dm^{-3} zeatine [Debnath 2009].

High concentrations of PGRs may lead to plant vitrification, which is an unfavorable physiological condition constituting an unquestionable barrier in plant micropropagation [Barbosa et al. 2013]. In this research, on 'Burkley' and 'Dover' varieties, BA added in concentration of 3,0 mg·dm^{-3} to medium caused vitrification on 100% of grown plants [Barbosa et al. 2013].

According to Nehra et al. [1989], low light intensity (12,5 µmol·m^{-2}·s^{-1}) has a stimulating effect on plant regeneration [Nehra et al. 1989]. Fridborg and Eriksson [1975] reports, that a light intensity plays a key role in plant regeneration through its effect on auxin activity. In studies about the influence of light intensity on strawberry shoot regeneration, the most efficiency was achieved with using light intensity in range from 20 to 40 µmol·m^{-2}·s^{-1} [Barcelo et al. 1998, Tian et al. 2003, Yildirim and Turker 2014]. Also the influence of different light colour on plant regeneration was examined and it showed that red and green lights are more beneficial for *Fragaria* plant growth than blue and yellow, which is associated with low UV intensity. According to Yonghua et al. [2004], the usage of fluorescent light rays may be beneficial for the regeneration of strawberry.

Callus induction

For callus initiation, lamina explants [Jones et al. 1988, Infante et al. 1998] and petioles [Jones et al. 1988, Infante et al. 1998, Tian et al. 2003] were used (tab. 2). Plants were cultivated on medium with low PGRs concentrations in dark conditions [Svensson and Johansson 1992, Tian et al. 2003]. Callus of *Fragaria moschata* Duch. has been formed most effectively on MS with 0,1 mg·dm^{-3} NAA and 0,025 mg·dm^{-3} BA. After 18 months of passages, higher concentrations of NAA and BA was more stimulative [Infante et al. 1998].

Rooting

For rooting *Fragaria* plants many variants of the medium were used (tab. 3). According to Mohamed et al. [1991], in case of plants arisen through crossing *Fragaria* × *ananasa* Duch. and *Fragaria ovalis* L., paclobutrazol activity on root development was more stimulative, abscisic acid (ABA) activity was weaker and GA and BA activities were the weakest. According to other authors, the most effective *Fragaria* plants rooting is on MS medium without PGRs [Singh and Pandey 2004, Debnath 2005, Haddadi et

al. 2010, Karim et al. 2015] or on ½ MS with IBA [Jeong et al. 1996, Husaini and Srivastava 2006].

In Jemmali et al. [1995] studies, for strawberry rooting, medium with microelements from Knop's medium, microelements from MS, vitamins and 0,9 mg·dm^{-3} IBA and 0,5 mg·dm^{-3} activated charcoal was used [Jemmali et al. 1995]. Increase of IBA concentration in medium caused inhibition of root growth and addiction of activated charcoal had a stimulating effect [López-Aranda et al. 1994, Adak et al. 2010]. More effective seems to be also addition of humic acids solution to medium, due to its higher stimulation effects on root growth than in case of adding only auxins [Rzepka-Plevnes et al. 2011].

From Borkowska [2000] studies resulted, that the rooting of strawberry *in vitro* plants should be done *ex vitro*.

Acclimatization

In *Fragaria indica* micropropagation protocol, which was created by Bhatt and Dhar [2000], it was proved, that one of the best mediums for plant acclimatization in plant tissue culture was a mixture of soilrite and sand, on which the survival rate of plant was 70%. 'Camarosa' variety survival rate at about 90% was obtained on mixture of perlite, vermiculite and coconut fiber (2 : 1 : 2 v/v/v) [Haddadi et al. 2010]. A relatively high plant survival rate was also obtained during 'Oso Grande' acclimatization on mixture of garden soil and manure in the ratio 1 : 1 (77.3%) [Rattanpal et al. 2011].

Genetic stability

In vitro plant regeneration is a valuable technique which is used to mass propagation and seeds protection. It is also used in genetic engineering. The limiting factor of using plant tissue culture technique is occurance of genetic instability among plants. One of the main factor which affects rate of genetic stability during plant culture is regeneration method [Nehra et al. 1994]. Plant regeneration through somatic embryogenesis or direct organogenesis from single cells is considered to be the method which minimizes problem of genetic instability [Vasil and Vasil 1982, Maheswaran and Wiliams 1987]. Regeneration from adventitious shoots with callus stage, through cell suspension or protoplasts culture is however associated with high genetic instability rate of plants cultivated *in vitro* [Larkin and Scowcroft 1981, Meins 1983]. It needs to be noticed that, genetic instability occurred in several other cases of shoot tips or meristems cultures [Nehra et al. 1994] and anther cultures [Nyman and Wallin 1992]. Very large impact on genetic stability has cultivated *in vitro* plant genotype [Nehra et al. 1994]. Somaclonal variation of strawberry (*Fragaria* × *ananassa*) may be induced from callus leaf explants through increased interval between subsequent passages [Popescu et al. 1997], gamma irradiation in doses of 5–50 Gy [Kaushal et al. 2004] or using high concentration of BAP [Biswas et al. 2009].

According to Sansavini et al. [1990] and Kaushal et al. [2004], strawberry clonal propagation on large scale, while plant multiplying is maximized by using high concentration of PGRs, may induce high rate of somaclonal variation [Sansavini et al. 1990, Kaushal et al. 2004], which is a huge problem in production of genotypic and phenotypic uniform plants. Among these changes – leaf colour, occurring of dwarf plants and many others stands out [Cameron and Hancock 1986, Sansavini et al. 1990, Irkaeva and Matveena 1997]. Modified traits may be epigenetic and therefore they can disappear with time [Koruza and Jelaska 1993]. This temporary changes may be caused by DNA methylation. Methylation patterns was discovered in soy cell suspension cultures and maize callus cultures [Kumar et al. 1999]. Epigenetic variability is often unstable and may disappear after transferring cultures from *in vitro* conditions to the soil as well as after several passages [Kaeppler et al. 2000].

Somaclonal variation occurs most often in plants regenerated through callus culture, which went in dedifferentiation phase. Somaclonal variation is caused by changes in chromosomes quantity (polyploidy or aneuploidy), chromosomal damage (like insertions, deletions, translocations, mutations) or chromatin methylation changes. Chromosomal damage may occur during crossing-over, late replication in heterchromatic regions, transpozons. point mutations or chromosomal rearrangements [Biswas et al. 2009].

Results of some studies suggested that, the important factors in somaclonal variation induction in *in vitro* systems are concentration of auxins in medium and number of passages [Popescu et al. 1997, Biswas et al. 2009].

According to Nehra et al. [1992], genetic stability of 'Redcoat' variety and somaclonal variation occurence in this plant is dependent on PGRs concentrations as well as age of culture and time of passages [Nehra et al. 1992]. Factor, which influences on genetic stability in this species, is also long-term storage of plant tissues in freezing temperatures [Morozova 2002]. The differences between the varieties grown conventionally, micropropagated and stored for 1 year in liquid nitrogen were not seen by Soria et al. [2007].

SUMMARY

According to current knowledge, the most commonly used type of explants to the initiation of *Fragaria* plant tissue cultures are leaf explants, while the most rarely – seeds (tab. 1). Most researchers for plant propagation use MS medium containing IBA and BA at a concentration of 0,1 to 1,0 mg·dm^{-3} (tab. 1). For rooting, MS medium without PGRs or MS supplemented with IBA at a concentration from 0,5 to 3,0 mg·dm^{-3} is used preferably (tab. 3). The most effective callus culture medium seems to be MS supplemented with 2,4-D and BAP (or BA) at a concentration of 0,1–1,0 mg·dm^{-3} (tab. 2).

The studies involving genetic transformation require to choose the appropriate regenerating medium for the plant after modification. In the case of *Fragaria* plants researchers frequently used MS medium with addition of a combination of auxin and cytokinin (often also gibberellin). Typically, it was a combination of IBA and BAP in a concentration of from 0,01 to 4,0 mg·dm^{-3} (tab. 1). Studies on *Fragaria* plants micropropagation are already conducted for over 40 years, but to this day there are not developed an optimal clonal propagation protocol.

These conclusions, drawn from the available literature, unfortunately are not unequivocal. Research continuation is essential in order to standardize the micropropagation protocol of plants of the genus *Fragaria*.

REFERENCES

Adak, N., Kaynak, L., Pekemezci, M., Gubbuk, H. (2010). The effect of various hormone types on *in vitro* propagation of strawberry. Acta Hortic., 829, 305–308.

Alsheikh, M.K., Suso, H.P., Robson, M., Battey, N.H., Wetten, A. (2002). Appropriate choice of antibiotic and *Agrobacterium* strain improves transformation of antibiotic-sensitive *Fragaria vesca* and *F. v. semperflorens*. Plant Cell Rep., 20, 1173–1180.

Balokhina, N.V., Kaliayeva, M.A., Buryanov, Ya.I. (2000). The elaboration of a shoot regeneration system for the genetic transformation of the wild strawberry (*Fragaria vesca* L.). Biotekhnologiya, 16(1), 46–51.

Barbosa, L.M.P., de Paiva Neta, V.B., Dias, L.L.C., Festucci-Buselli, R.A., Alexandre, R.S., Iarema, L., Finger, F.L., Otoni, W.C. (2013). Biochemical and morpho-anatomical analyses of strawberry vitroplants hyperhydric tissues affected by BA and gelling agents. Revista Ceres Viçosa, 60(2), 152–160.

Barcelo, M., El-Mansouri, I., Mercado, J.A., Quesada, M.A., Alfaro, F.P. (1998). Regeneration and transformation via *Agrobacterium tumefaciens* of the strawberry cultivar Chandler. Plant Cell Tiss. Org., 54, 29–36.

Bhatt, I.D., Dhar, U. (2000). Micropropagation of Indian wild strawberry. Plant Cell Tiss. Org., 60(2), 83–88.

Biswas, M.K., Dutt, M., Roy, U.K., Islam, R., Hossain, M. (2009). Development and evaluation of *in vitro* somaclonal variation in strawberry for improved horticultural traits. Sci. Hortic., 122(3), 409–416.

Borkowska, B. (2000). Morphological and physiological characteristics of micropropagated strawberry plants rooted *in vitro* or *ex vitro*. Sci. Hortic., 89, 195–206.

Boxus, P. (1974). The production of strawberry plants by in vitro micro-propagation. J. Hortic. Sci. 49, 209–210.

Boxus, P., Quoirin, M., Lame, J.M. (1977). Large scale propagation of strawberry plants from tissue culture. In: Applied and fundamental aspects of plant, tissue and organ culture, Reinert, J., Bajaj, Y.P.S. (eds). Springer-Verlag, New York, p. 130–143.

Cameron, J.S., Hancock, J.E. (1986). Enhanced vigor in vegetative progeny of micropropagated strawberry plants. HortScience, 21, 1225–1226.

Debnath, S.C. (2005). Strawberry sepal: another explants for thidazuron-induced adventitious shoot regeneration. In Vitro Cell Dev-Pl., 41, 671–676.

Debnath, S.C. (2006). Zeatin overcomes thidiazuron-induced inhibition of shoot elongation and promotes rooting in strawberry culture *in vitro*. J. Hortic. Sci. Biotech., 81(3), 349–354.

Debnath, S.C. (2009). Characteristics of strawnerry plants propagated by *in vitro* bioreactor culture and *ex vitro* propagation method. Eng. Life Sci., 9(3), 239–245.

El Mansouri, I., Mercado, A., Valpuesta, V., Lopez-Aranda, J.M., Pliego-Alfaro, F., Quesada, M.A. (1996). Shoot regeneration and *Acrobacterium* – mediated transformation of *Fragaria vesca* L. Plant Cell Rep., 15, 642–646.

Foucault, C., Letouze, R. (1987). In vitro: regeneration de plantes de Fraisier a partir de fragmentes de petiole et de bourgeons floraux. Biol. Plantarum., 29, 409–414.

Fridborg, G., Eriksson, T. (1975). Partial reversal by cytokinin and (2-chloroethyl)-trimethyl-ammoniun chloride of near-ultraviolet inhibited growth and morphogenesis in callus cultures. Physiol. Plantarum., 34, 162–166.

Gantait, S., Mandal, N., Nandy, S. (2011). Advances in Micropropagation of Slected Aromatic Plants: A Review on Vanilla and Strawberry. Am. J. Biochem. Mol. Biol., 1(1), 1–19.

Gruchała, A., Korbin, M., Żurawicz, E. (2004). Conditions of transformation and regeneration of "Induka" and "Elista" strawberry plants. Plant Cell Tiss. Org., 79, 153–160, 2004.

Haberlandt, G. (1902). Plant cell culture experiment with isollierten. S. B. Vienna Ways Sci., 111, 69–92.

Haddadi, F., Aziz, M.A, Saleh, G, Rashid, A.A., Kamaladini, H. (2010). Micropropagation of strawberry cv. Camarosa: Prolific shoot regeneration from *in vitro* shoot tips using thidiazuron with 6-benzylaminopurine. HortScience, 45(3), 453–456.

Harris, D.C., Simpson, D.W., Bell, J.A. (1997). Studies on the possible role of micropropagation in the dissemination of the strawberry crown rot pathogen *Phytophthora cactorum*. J. Hortic. Sci., 72, 125–133.

Haymes, K.M., Davis, T.M. (1998). *Agrobacterium*-mediated transformation of "Alpine" *Fragaria vesca*, and transmission of transgenes to R1 progeny. Plant Cell Rep., 17, 279–283.

Huetteman, C.A., Preece, J.E. (1993). Thidiazuron: a potent cytokinin for woody plant tissue culture. Plant Cell Tiss. Org., 33, 105–119.

Husaini, A.M. (2010). Pre- and post-agroinfection strategies for efficient leaf disk transformation and regeneration of transgenic strawberry plants. Plant Cell Rep., 29, 97–110.

Husaini, A.M., Srivastava, D.K. (2006). Plant regeneration and *Agrobacterium*-mediated gene transfer studies in strawberry tissues (*Fragaria* × *ananassa* Duch.). Asian J. Microbiol. Biotechnol. Environ. Sci., 8(3), 671–678.

Infante, R., Mazzara, M., Rosati, P. (1998). Growth estimation of *in vitro* cultured callus and plant regeneration from leaf disk and petiole callus of musk strawberry (*Fragaria moschata* Duch.) Subcultured for 18 months. J. Jpn. Soc. Hortic. Sci., 67(1), 39–43.

Jemmali, A., Boxus, Pn., Dekegel., Van Huele, G. (1994). Occurence of spontaneous shoot regeneration on leaf stipules in relation to hyperflowering response in micropropagated strawberry plantlets. In Vitro Cell Dev. Biol., 30(4), 192–195.

Jemmali, A., Boxus, Ph., Kevers, C., Gasoar, T. (1995). Carry-over of Morphological and biochemical characteristics associated with hyperfloweting of micropropagated strawberries. J. Plant. Physiol., 147, 435–440.

Jemmali, A., Elloumi, N., Kevers, C., Dommes, J. (2002). Morphological and hormonal characterization of strawberry vitroplants raised through axillary or stipular adventitious shooting. Plant Growth Regul., 38, 273–278.

Jeong, H.B., Ha, S.H., Kang, K.Y. (1996). *In vitro* multiplication of strawberry by vertical rotary culture of shoot tip. RDA J. Agr. Sci. Biotechnol., 38(1), 273–278.

Jones, O.P., Waller, B.J., Beech, M.G. (1988). The production of strawberry plants from callus cultures. Plant Cell Tiss. Org., 12, 235–241.

Jones, C.M., Mes, P., Myers, J.R. (2003). Characterization and inheritance of the anthocyanin fruit (Aft) tomato. J. Hered., 94, 449–456.

Kaeppler, S.M., Phillips, R.L. (1993). Tissue culture-induced DNA methylation variation in Maize. P. Natl. Acad. Sci. USA, 90(19), 8773–8776.

Karhu, S. (2001). Growth characteristics of micropropagated strawberries. Acta Hortic., 560, 539–542.

Karhu, S., Hakala, K. (2002). Micropropagated strawberries on the field. Acta Hortic., 567, 321–324.

Karim, R., Ahmed, F., Krishna Roy, U., Ara, T., Islam, R., Hossain, M. (2015). Varietal improvement of Strawberry (*Fragaria* × *ananassa* Duch.) through somaclonal variation using *in vitro* techniques. J. Agric. Sci. Technol., 17(4), 977–986.

Kaur, R., Gautam, H., Sharma, D.R. (2005). A low cost strategy for micropropagation of strawberry (*Fragaria* × *ananassa* Duch.) cv. Chandler. Acta Hortic., 696, 129–133.

Kaushal, K., Nath, A.K., Kaundal, P., Sharma, D.R. (2004). Studies on somaclonal variation in strawberry (*Fragaria* × *ananassa* Duch.) cultivars. Acta Hortic., 662, 269–275.

Khan, S., Spoor, W.A. (2004). Study of an *in vitro* callus culture and regeneration system from leaf disc explants in strawberry (*Fragaria x ananassa*) cv. Tango. Int. J. Biol. Biotechnol., 68, 397–401.

Koruza, B., Jeleska, S. (1993). Influence of meristem culture and virus elimination on phenotypical modifications of grapevine (*Vitis vinifera* L., cv. Refosk). Vitis, 32, 59–60.

Kumar, M.B., Barker, R.E., Reed, B.M. (1999). Morphological and molecular analysis of genetic stability in micropopagated *Fragaria* × *ananassa* cv. Pocahontas. In vitro Cell. Dev. Biol.–Plant, 35, 254–258.

Lal, M. Sharma, S., Hegde, M.V. (2003). Micropropgation of strawberry (*Fragaria* × *ananassa* Duch.). Indian J. Agr. Res., 37, 231–234.

Lamari, Z., Landsberger, S., Braisted, J., Neggache, H., Larbi, R. (2008). Trace element content of medicinal plants from Algeria. J. Radioanal. Nucl. Ch., 276(1), 95–99.

Landi, L., Mezzetti, B. (2006). TDZ, auxin and genotype effects on leaf organogenesis in *Fragaria*. Plant Cell Rep., 25, 281–288.

Larkin, P.J., Scowcroft, W.R. (1981). Somaclonal variation – a novel source of variability from cell cultures for plant improvement. Theor. Appl. Genet., 60, 197–214.

Lines, R., Kelly, G., Milinkovic, M., Rodoni, B. (2006). Runner certification and virus elimination in commercial strawberry cultivars in Australia. Acta Hortic., 708, 253–254.

Liu, Z.R., Sanford, J.C. (1988). Plant regeneration by organogenesis from strawberry leaf and runner tissue. HortScience, 23, 1057–1059.

López-Aranda, J.M., Pilego-Alfaro, E., López-Navidad, I., Barceló-Muñoz, E. (1994). Micropropagation of strawberry (*Fragaria* × *ananassa* Duch.). Effect of mineral salts, benzyladenine levels and number of subcultures on the *in vitro* and field behaviour of the obtained microplants and the fruiting capacity of their progeny. J. Hortic. Sci., 69(4), 625–637.

Maheswaran, G., Williams, E.G. (1987). Uniformity of plants regenerated by direct somatic embryogenesis from zygotic embryos of *Trifolium repens*. Ann. Bot., 59, 93–97.

Małodobry, M., Dziedzic, E., Lech, W. (1997). Shoot cultures of strawberry cv. Syriusz. Folia Hort., 9(1), 105–112.

Mathews, H., Wagoner, W., Kellogg, J., Bestwick, R. (1995). Genetic transformation of strawberry: stable integration of a gene to control biosynthesis of ethylene. In Vitro Cell Dev. Biol., 31, 36–43.

Meins, F. (1983). Heritable variation in plant cell culture. Annu. Rev. Plant Biol., 34, 327–346.

Mohamed, F., Swartz, H.J., Buta, J.G. (1991). The role of abscisic acid and plant growth regulators in tissue culture induced rejuvenation of strawberry *ex vitro*. Plant Cell Tiss. Org., 25, 75–84.

Mohamed, F.H., Beltagi, M.S., Ismail, M.A., Omar, G.F. (2007). High frequency, direct shoot regeneration from greenhouse-derived leaf disks of six strawberry cultivars. Pak. J. Biol. Sci., 10(1), 96–101.

Mohan, R., Chuiu, E.A., Biasi, L.A., Soccol, C.R. (2005). Alternative *in vitro* propagation: use of sugarcane bagasse as a low cost support material during rooting stage of strawberry cv. Dover. Braz. Arch. Biol. Techn., 48, 37–42.

Monticelli, S., Gentile, A., Damiano, C. (2002). Regeneration and Agrobacterium-mediated transformation in stipules of strawberry. Acta Hortic., 567, 105–107.

Morozova, T. (2002). Genetic stability of pure lines of *Fragaria vesca* L. in micropropagation and long-term storage *in vitro*. Acta Hortic., 567, 85–88.

Munir, M., Iqbal, S., Baloch, J.U.D., Khakwani, A.A. (2015). *In vitro* explant sterilization and bud initiation studies of four strawberry cultivars. J. Appl. Hortic., 17(3), 192–198.

Murashige, T., Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant., 15, 473–497.

Nehra, N.S., Stushnoff, C., Kartha, K.K. (1989). Direct shoot regeneration from leaf discs. J. Am. Soc. Hortic. Sci., 114, 1014–1018.

Nehra, N.S., Stushnoff, C., Kartha, K.K. (1990). Regeneration of plants from immature leaf-derived callus of strawberry (*Fragaria* × *ananassa*). Plant Sci., 66(1), 119–126.

Nehra, N.S., Kartha, K.K., Stushnoff, Giles, K.L. (1992). The influence of plant growth regulator concentrations and callus age on somaclonal variation in callus culture regenerants of strawberry. Plant Cell Tiss. Org., 29, 257–268.

Nehra, N.S., Kartha, K.K., Stushnoff., Giles, K.L. (1994). Effect of *in vitro* propagation methods on field performance of two strawberry cultivars. Euphytica, 76, 107–115.

Nyman, M., Wallin, A. (1988). Plant regeneration from strawberry (*Fragaria* × *ananassa*) mesophyll protoplasts. J. Plant. Physiol., 133, 375–377.

Nyman, M., Wallin, A. (1992). Improved culture technique for strawberry (*Fragaria* × *ananassa* Duch.) protoplasts and the determination of DNA content in protoplast derived plants. Plant Cell Tiss. Org., 30, 127–133.

Oosumi, T., Gruszewski, H.A., Blischak, L.A., Baxter, A.J., Wadl, P.A., Shuman, J.L., Veilleux, R.E., Shulaev, V. (2006). High-efficiency transformation of the diploid strawberry (*Fragaria vesca*) for functional genomics. Planta, 223, 1219–1230.

Owen, H.R., Miller, A.R. (1996). Haploid plant regeneration from anther cultures of three North American cultivars of strawberry (*Fragaria* × *ananassa* Duch.). Plant Cell Rep., 15, 905–909.

Pallavi C.M., Rekha, R., Neelambika, T.M. (2011). Indirect somatic embryogenesis from petiole segment in strawberry cv. Sweet Charlie. Indian J. Hortic., 68(1), 24–27.

Passey, A.J., Barrett, K.J., James, D.J. (2003). Adventitous shoot regeneration from seven commercial strawberry cultiuvars (*Fragaria* × *ananassa* Duch.) using a range of explants types. Plant Cell Rep., 21, 397–401.

Petrovic, D., Jacimovic-Plavsic, M. (1990). Propagation of the strawberry cultivar Senga Sengana by *in vitro* meristem culture. Nauka Praksi, 20(1), 11–18.

Popescu, A.N., Isac, V.S., Coman, M.S., Radulescu, M.S. (1997). Somaclonal variation in plants regenerated by organogenesis from callus culture of strawberry (*Fragaria* × *ananassa*). Acta Hortic., 439, 89–96.

Rattanpal, H.S., Gill, M.I.S., Sangwan, A.K. (2011). Micropropagation of strawberry through meristem culture. Acta Hortic., 890, 149–154.

Rekha, R., Mandave, P., Meti, N. 2012. Micropropagation of strawberry cultivar Sweet Charlie through axillary shoot proliferation. J. Appl. Hortic., 14(1), 71–73.

Ricardo, Y.G., Coll Y., Castagnaro, A., Diaz, Ricci, J.C. (2003). Transformation of strawberry cultivar using a modified regeneration medium. HortScience, 38(2), 277–280

Rugini, E., Orlando, R. (1992). High-efficiency shoot regeneration from calluses of strawberry (*Fragaria* × *ananassa* Duch.) stipules of *in vitro* shoot cultures. J. Hortic. Sci., 67, 577–582.

Rzepka-Plevnes, D., Kulpa, D., Gołębiowska, D., Porwolik, D. (2011). Effects of auxins and humic acids on *in vitro* rooting of strawberry (*Fragaria* × *ananassa* Duch.). J. Food Agric. Environ., 9(3–4), 592–595.

Sansavini, S., Rosati, E., Gaggioli, D., Toshi, M.E. (1990). Inheritance and stability of somaclonal variation in micropropagated strawberry. Acta Hortic. (Wageningen), 280, 375–384.

Simpson, D.W., Bell, J.A. (1989). The response of different genotypes of *Fragaria* × *ananassa* and their seedling progenies to *in vitro* micropropagation and the effects of varying the concentration of 6-benzylaminopurine in the proliferation medium. Plant Cell Tiss. Org., 17, 225–234.

Singh, A.K., Pandey, S.N. (2004). Genotypic variation among strawberry cultivars for shoot organogenesis. Acta Hortic., 662(1), 277–280.

Skupień, K., Oszmiański, J. (2004). Comparison of six cultivars of strawberries (*Fragaria* × *ananasa* Duch.) grow in northwest Poland. Eur. Food Res. Technol., 219, 66–70.

Soria, C., Gálvez-Farfán, J., Ariza, M.T., Medina, J.J., González-Benito, M.E. (2007). Implementing a strawberry cryogenic genebank: A comparison in agronomic traits of plants derived from cryopreserved apices, *in vitro* micropropagation, and conventional propagation. Adv. Hortic. Sci., 21(4), 207–210.

Sorvari, S., Ulvinen, S., Hietarante, T., Hiirsalmi, H. (1993). Preculture medium promotes direct shoot regeneration from micro-propagated strawberry leaf discs. HortScience, 28, 55–57.

Sowik, I., Markiewicz, M., Michalczuk, L. (2015). Stability of Vertivillium dahliae resistance in tissue culture – derived strawberry somaclones. Hortic. Sci. (Prague), 42(3), 141–148.

Steward, F.C., Mapes, M.O., Mears, K. (1958). Growth and organized development of cultures cells: II. Organisation in cultured grown from freely suspended cells. Am. J. Bot., 45, 705–708.

Sutter, E.G., Ahmadi, H., Labavitch, J.M. (1997). Direct regeneration of strawberry (*Fragaria* × *ananassa* Duch.) from leaf disks. Acta Hortic., 447, 243–245.

Svensson, M., Johansson, L.B. (1992). Anther culture of *Fragaria* × *ananassa*: Environmental factors and medium components affecting microspore divisions and callus production. J. Hortic. Sci., 69(3), 417–426.

Tian, M., Gu, G., Zhu, M. (2003). The involvement of hydrogen peroxide and antioxidant enzymes in the process of shoot organogenesis of strawberry callus. Plant Sci., 165(2003), 701–707.

Toyoda, H., Horikoshi, K., Inaba, K., Ouchi, S. (1990). Plant regeneration of Callus Tissues Induced from Leaf Explants of Strawberry. Plant Tissue Cult. Lett., 7(1), 38–41.

Toyoda, H., Horikoshi, K., Yamano, Y., Ouchi, S. (1991). Selection for *Fusarium* wilt disease resistance from regenerants derived from leaf callus strawberry. Plant Cell Rep., 10, 167–170.

Vasil, V., Vasil, K. (1982). The ontogeny of somatic embryos of *Pennisetum americanum* (L) K. Schum. in cultured immature embryos. Bot. Gazette, 143, 454–465.

Yildirim, A.B., Turker, A.U. (2014). Effects of regeneration enhancers on micropropagation of *Fragaria vesca* L. and phenolic content comparison of field-grown and in vitro-grown plant materials by liquid chromatography-electrospray tandem massspectrometry (LC–ESI-MS/MS). Sci Hortic., 169, 169–178.

Yonghua, Q., Shanglong, Z., Asghar, A., Lingxiao, Z., Qiaoping, Q., Kunsong, C., Changjie, X. (2005). Regeneration mechanism of Toyonoka strawberry under different color plastic films. Plant Sci., 168(6), 1425–1431.

Zhang, Q., Folta, K.M., Davis, T.M. (2014). Somatic embryogenesis, tetraploidy, and variant leaf morphology in transgenic diploid strawberry (*Fragaria vesca* subspecies vesca 'Hawaii 4'). BMC Plant Biol., 14, 14–23.

Zhao, Y., Liu, Q.Z., Davis, R.E. (2004). Transgene expression in strawberries driven by a heterologous phloem-specific promoter. Plant Cell Rep., 23, 224–230.

Żebrowska, J., Kaczmarska, E., Gawroński, J. (2015). Comparative studies on the agronomic value of *in vitro* and conventionally propagated strawberry (*Fragaria* × *ananassa* Duch.) plants. Acta Sci. Pol. Hortorum Cultus, 14(3), 25–35.

Żebrowska, J.I. (2011). Efficacy of resistance selection to *Verticillium* wilt in strawberry (*Fragaria* × *ananassa* Duch.) tissue culture. Acta Agrobot., 64, 3–12.

Żebrowska, J.I., Hortyński, J. (2002). Plant regeneration from leaf explants in strawberry (*Fragaria* × *ananassa* Duch.). Acta Hortic., 567, 313–315.