J. Trop. Agric. and Fd. Sc. 45(1)(2017): 77 – 88

In vitro regeneration of *Fragaria vesca* L.

(Pertumbuhan semula *in vitro Fragaria vesca* L.)

A.C.K. Ling[1] and A. Wetten[2]

[1]Biotechnology and Nanotechnology Research Centre, MARDI Headquarters, Persiaran MARDI-UPM, 43400 Serdang, Selangor, Malaysia
[2]School of Agriculture, Policy and Development, University of Reading, Whiteknights, Reading, RG6 6AR, United Kingdom

Abstract
Fragaria vesca L., a diploid relative of the cultivated octoploid strawberry *Fragaria* x *ananassa*, consists of seasonal *Fragaria vesca* f. *vesca* (*F. vesca*) and everbearing *Fragaria vesca* f. *semperflorens* (*F. v. semperflorens*). Study on *F. vesca* L. showed that *F. v. semperflorens* had higher *in vitro* regeneration capabilities than *F. vesca* although they belong to the same genus and species in the hierarchical rank. *F. vesca* explants and *F. v. semperflorens* could not regenerate from either leaf or petiole segments on media without plant growth regulators. Plantlet regeneration from leaf explants was highest on media supplemented with 1 mg/litre 6-benzylaminopurine (BAP) in comparison with those cultured on media supplemented with 2, 3 and 4 mg/litre BAP. Plantlet regeneration from *F. v. semperflorens* petiole explants was best achieved at 0.1 mg/litre indole-3-butyric acid compared to 0, 0.2 and 0.4 mg/litre.

Keywords: BAP, IBA, wild strawberry, micropropagation

Introduction

Micropropagation is an alternative propagation method based on the principle of totipotency, the ability of a single living cell to produce a whole organism. It allows production of a large quantity of clonal materials in a relatively short period of time. Plants can be regenerated from various explants through organogenesis or somatic embryogenesis (Hansen and Wright 1999). Both events occur either directly from explants or indirectly through callus induced from explants (Charrière and Hahne 1998). *In vitro* culture of strawberry has been carried out using different initiation tissues such as leaf (Jones et al. 1988; Nehra et al. 1989; Alsheikh et al. 2002; Oosumi et al. 2006) and petiole (Jones et al. 1988; Alsheikh et al. 2002; Passey et al. 2003) sections. Due to the economic importance of cultivated strawberries, most of the tissue culture techniques for strawberry were developed based on *Fragaria* x *ananassa* (Jones et al. 1988; Nehra et al. 1990; Folta et al. 2006). Several studies involving tissue culture of *Fragaria vesca* f. *vesca* (*F. vesca*) have also been reported (El Mansouri et al. 1996; Alsheikh et al. 2002; Oosumi et al. 2006) as a result of its less complicated genetic background than the octoploid cultivated counterparts.

The European wild strawberry, *Fragaria vesca* L. is the most widely distributed species of the genus, spanning the northern hemisphere and South America (Darrow 1966; Hancock and Luby 1993; Heide and Sønsteby 2007). It is a low-growing perennial herb, belongs to the Rosaceae family. The species includes the wood strawberry, *F. vesca* and the Alpine

Article history
Received: 27.11.13
Accepted: 21.1.16

Authors' full names: Adrain Ling Chieng Kuang and Andrew Wetten
E-mail: adrain@mardi.gov.my

©Malaysian Agricultural Research and Development Institute 2017

strawberry, *F. vesca* f. *semperflorens* (*F. v. semperflorens*) (Darrow 1966). *Fragaria vesca* f. *vesca* is seasonal flowering and runnering while *F. v. semperflorens*, a mutant of *vesca*, has non-runnering and everbearing habbits (Darrow 1966; Battey et al. 1998).

Unlike the cultivated strawberry, *F.* x *ananassa*, *F. vesca* L. is a diploid plant (2n = 14, x = 7) (Brown and Wareing 1965). The lower ploidy number is a desirable trait for genetic studies. In addition, it has a relatively small haploid nuclear DNA content (genome size) at ~240 megabases (Shulaev et al. 2011) in comparison with the model plant, *Arabidopsis thaliana* at 211 Mbp, as estimated by Schmuths et al. (2004). It is 15 – 30 cm in height (Darrow 1966) which can be easily fit into a controlled environment growth cabinet. Its short generation time of about 4 months makes it a very attractive perennial model plant (Battey et al. 1998).

An efficient plant regeneration system is important for both micropropagation and *in vitro*-based genetic transformation of a plant. Although a broad spectrum of auxins have been reportedly used to induce shoots in *Fragaria* tissue culture, only indole-3-butyric acid (IBA) has been used for diploid strawberry while 6-benzylaminopurine (BA or BAP) is the most commonly used cytokinin in *Fragaria* regeneration (Folta and Dhingra 2006). Thidiazuron (TDZ) has also been used as the cytokinin in both diploid and octoploid *Fragaria* (Zhao et al. 2004; Landi and Mezzetti 2006). The most widely used genetic transformation method for strawberry is *Agrobacterium tumefaciencs*-mediated transformation system (Qin et al. 2008). Controlling the bacteria growth after *Agrobacterium*-mediated transformation is essential yet challenging as antibiotic can be phytotoxic especially at high concentration (Folta and Dhingra 2006). Carbenicillin was found to control the bacteria effectively while having the least impact on regeneration in *Agrobacterium*-mediated transformation of strawberry (Alsheikh et al. 2002). The work described here was designed to study *in vitro* regeneration efficiencies of different tissues of *F. vesca* and *F. v. semperflorens* based on modified versions of some of the reported regeneration systems.

Materials and methods

In vitro plant materials

In vitro plants were grown as described by Alsheikh et al. (2002) with minor modifications. *Fragaria vesca* L. seeds were surface sterilised under a laminar flow hood with 70% (v/v) ethanol (Fisher, UK) for 20 s, followed by 15 min in 10% (v/v) sodium hypochlorite (BDH, UK) containing 12% (w/v) sodium hypochlorite with addition of 0.1% (v/v) Tween®-20 (BDH, UK). A 38 mm stainless steel test sieve with 250 µm mesh size (Endecotts, UK) was used to assist in re-collecting and transferring of the seeds. Occasional shaking was applied during the sterilisation. The seeds were then washed with sterilised water at least 3 times or until no residual bubbles of Tween®-20 were visible, and blotted dry on sterile filter paper (Whatman, UK) before transferring on to Murashige and Skoog (MS) basal medium (Murashige and Skoog 1962). The sterilised seeds were germinated in 300 ml glass jars containing 60 ml of MS basal medium (Sigma, UK) supplemented with 30 g/l commercial grade sucrose and solidified with 0.2% (w/v) Phytagel (Sigma, UK) with a pre-autoclave pH of 5.7 – 5.8. Media were sterilised by autoclaving at 116 psi for 20 min. Plants were grown under 16 h photoperiod under fluorescent lights with a photosynthetic photon flux density of approximately 90 µmol/m^2/s at a temperature of 24 ^{o}C. Subculture was carried out using standard aseptic techniques every 8 – 10 weeks.

Nutrient media
Basal shoot induction medium (SM) for *F. vesca* L. *in vitro* regeneration experiments contained MS salts, B5 vitamins (Gamborg et al. 1968), 2% (w/v) sucrose and 0.2% (w/v) Phytagel (Sigma, UK), following the shoot induction medium composition described by Oosumi et al. (2006). The pH was adjusted to 5.7 – 5.8 prior to autoclaving.

Nutrient media supplements
BAP and IBA powders were dissolved firstly in a few drops of 1M NaOH solution and made up to 3 mg/ml and 1 mg/ml stock solutions, respectively, using reverse osmosis (RO) water. Carbenicillin stock solution was prepared by dissolving powder in RO water at 250 mg/ml. All prepared stock solutions were filter-sterilised with 0.2 µm cellulose acetate membrane filters (Nalgene, UK) before storing in a refrigerator. Plant growth regulators (PGRs) and antibiotic were added to post-autoclaved media.

Preparation of explants
Young leaves were excised from *in vitro* plant material. Both leaf lamina and petioles were used as explants, prepared according to Oosumi et al. (2006) and Alsheikh et al. (2002). For leaf lamina as explant, petiole and midrib were removed from a leaf and the leaf blades were cut diagonally across the secondary veins to produce leaf pieces 1.0 – 1.5 mm wide. The explant was cultured with its abaxial side up. Petioles were cut into 1.0 – 1.5 cm explants and placed horizontally when cultured on media. To prevent the explants from drying up, all excision was carried out on 2 – 3 layers of sterile water-damped filter paper (Whatman, UK) placed in the lid of a petri dish.

Shoot regeneration
A total of 3 experiments were carried out at different time to determine the regeneration capacity of both *F. vesca* and *F. v. semperflorens*. In all the experiments, each petri dish represented one replicate. In Experiment I, SM either supplemented with 3 mg/litre BAP, 0.2 mg/litre IBA and 500 mg/litre carbenicillin (SMT), or without supplements (SM0) as control was used. Explants were cultured on medium in disposable 90 mm petri dishes containing 25 ml medium per plate and sealed with Nescofilm (Nesco, Japan). *Fragaria vesca* f. *semperflorens* leaf explants on SMT consisted of 8 petri dishes, each containing 10 explants while *F. vesca* leaf explants on SMT had 4 petri dishes, each containing 10 explants. Both *F. v. semperflorens* and *F. vesca* petiole explants on SMT had 5 and 3 petri dishes respectively with 9 explants in each dish. *Fragaria vesca* f. *semperflorens* leaf explants on SM0 consisted of 5 petri dishes, each containing 9 explants while *F. vesca* leaf explants on SM0 had 6 petri dishes containing 10 explants in each dish. Both *F. v. semperflorens* and *F. vesca* petiole explants on SM0 had 4 and 5 petri dishes respectively with 6 explants per dish. The culture plates were placed inverted and incubated in a controlled environment growth room at 16 h photoperiod under fluorescent lights with a photosynthetic photon flux density of approximately 90 µmol/m^2/s at a temperature of 24 ^{o}C. Subcultures were carried out every 4 weeks after the initial culture. Observations were carried out 4, 9 and 11 weeks after culture establishment and shoot regeneration was recorded. Extended observation was carried out on *F. vesca* explants on SMT.

The same treatments, media and culture vessels as Experiment I were applied in Experiment II *Fragaria vesca* f. *semperflorens* leaf explants on SMT had 6 petri dishes while *F. vesca* leaf explants on SMT had 5 petri dishes, but all contained 9 explants in each petri dish. *Fragaria vesca* f. *semperflorens* leaf explants on SM0 consisted of 4 petri dishes, each containing 9 explants. *Fragaria vesca* f. *semperflorens* petiole explants on both SMT and SM0 had

3 petri dishes with 6 explants per dish. The same layout applied to *F. vesca*. The incubation conditions were the same as in Experiment I. Subcultures were carried out every 2 – 3 weeks. Observations were carried out 3, 6, 9, 11 and 13 weeks after culture. Shoot regeneration and leaflet formation were recorded.

In Experiment III, MS basal media with 2% (w/v) sucrose and solidified with 0.2% (w/v) Phytagel were used. Medium was adjusted to pH 5.7 – 5.8 before autoclaving. The media were supplemented with a combination of BAP (1, 2, 3 and 4 mg/litre) and IBA (0.1, 0.2 and 0.4 mg/litre) except for the control treatment with no added PGR. Explants were cultured in 130 ml glass jars containing 40 ml of medium. Each treatment involving leaf explants had 8 jars, each containing 3 explants except 4 and 5 jars for *F. vesca* and *F. v. semperflorens* control treatment respectively. Treatments involving petiole explants consisted of 8 jars per treatment with 2 explants in each jar. The control treatment for petiole explants had 2 jars, containing 2 explants each. The cultures were incubated in the growth room conditions as in Experiment I and subcultured every 3 – 4 weeks. Observations of plantlet regeneration were recorded 8 and 13 weeks after the culture establishment.

Results and discussion

Shoot and plantlet regeneration

Experiment I: Shoot regeneration

Callus formation was observed on *F. v. semperflorens* leaf and petiole explants cultured on SMT 4 weeks after culture. However, there was no shoot regeneration observed. Contamination occurred in 1 replicate from leaf explants on SMT and 2 replicates from petiole explants on SMT leaving the total replicates at 7 and 3 respectively. Neither callus induction nor shoot formation was observed in treatments involving SM0 throughout the experiment. After 9 weeks of culture, 21 – 26% of the explants produced shoots which later increased to 41 – 44% by week 11.
The results are summarised in *Table 1*. Explants derived from *F. vesca* did not produce any callus or shoots on either SM0 or SMT media 11 weeks after culture. The explants on SMT did exhibit callus and shoots 20 weeks after culture (*Table 2*).

Experiment II: Shoot regeneration

Fragaria vesca f. *semperflorens* leaf and petiole explants on SM0 did not produce any shoots. Between 9 and 11 weeks after the initial culture, the leaf explants on SMT that had produced shoots increased from 11% to 28% while petiole explants on SMT exhibited an increase from 22% to 39% (*Table 3*). No shoot regeneration was observed from *F. vesca* leaf and petiole explants on SM0 and petiole explants on SMT. Less than 10% of the leaf explants on SMT produced shoots between 9 and 11 weeks after culture (*Table 4*).

Regenerated shoots formed leaflets more than 9 weeks after the initial culture of the explants from both *F. v. semperflorens* (*Table 5*) and *F. vesca* (*Table 6*). After 13 weeks of culture, 17% of both *F. v. semperflorens* leaf and petiole explants, and 2% of *F. vesca* leaf explants on SMT produced shoots with leaflets (*Table 5* and *6*).

Table 1. Experiment I: shoot formation from *F. v. semperflorens* leaf and petiole explants

	Total no. replicates	Mean regeneration per explant		Replicates with regeneration (%)
		Week 9	Week 11	
SM0/leaf	5	0.00	0.00	0.00
SMT/leaf	7	0.21	0.44	71.40
SM0/petiole	4	0.00	0.00	0.00
SMT/petiole	3	0.26	0.41	66.70

Table 2. Experiment I: shoot formation from *F. vesca* leaf and petiole explants on SMT 20 weeks after culture

	Total no. replicates	Mean regeneration per explant	Replicates with regeneration (%)
		Week 20	
SMT/leaf	4	0.10	50.00
SMT/petiole	3	0.07	66.70

Table 3. Experiment II: shoot formation from *F. v. semperflorens* leaf and petiole explants

	Total no. replicates	Mean regeneration per explant			Replicates with regeneration (%)
		Week 9	Week 11	Week 13	
SM0/leaf	4	0.00	0.00	0.00	0.00
SMT/leaf	6	0.11	0.20	0.28	66.70
SM0/petiole	3	0.00	0.00	0.00	0.00
SMT/petiole	3	0.22	0.28	0.39	100.00

Table 4. Experiment II: shoot formation from *F. vesca* leaf and petiole explants

	Total no. replicates	Mean regeneration per explant		Replicates with regeneration (%)	
		Week 9	Week 11	Week 13	
SM0/leaf	4	0.00	0.00	0.00	0.00
SMT/leaf	5	0.07	0.07	0.09	60.00
SM0/petiole	3	0.00	0.00	0.00	0.00
SMT/petiole	3	0.00	0.00	0.00	0.00

Table 5. Experiment II: shoots with leaflets formed from *F. v. semperflorens* leaf and petiole explants

	Total no. replicates	Mean regeneration per explant		Replicates with regeneration (%)
		Week 11	Week 13	
SM0/leaf	4	0.00	0.00	0.00
SMT/leaf	6	0.09	0.17	33.30
SM0/petiole	3	0.00	0.00	0.00
SMT/petiole	3	0.11	0.17	33.30

Table 6. Experiment II: shoots with leaflets formed from *F. vesca* leaf and petiole explants

	Total no. replicates	Mean regeneration per explant		Replicates with regeneration (%)
		Week 11	Week 13	
SM0/leaf	4	0.00	0.00	0.00
SMT/leaf	5	0.00	0.02	20.00
SM0/petiole	3	0.00	0.00	0.00
SMT/petiole	3	0.00	0.00	0.00

Experiment III: Plantlet regeneration

Based on the results of Experiment II, the amount of shoots regenerated did not reflect the ultimate amount of plantlets with leaflets produced. Some of the regenerated shoots took a long time to elongate and become plantlets while some died while still at the stage of young shoot. Hence, plantlets with leaflets (*Plate 1*) were scored in Experiment III. Most regenerated leaf explants produced 1 – 3 plantlets per explant except 3 out of 96 replicates which produced 4 – 6 plantlets per explant. Therefore, the results were re-categorised as growth or no growth and analysed using a logistic regression model with p values adjusted for underdispersion (Menard 1995). Plantlet regeneration from *F. v. semperflorens* leaf explants was significantly better than from *F. vesca* leaf explants ($p = 0.008$) (*Table 7*) with 53% of *F. v. semperflorens* leaf explants producing plantlets, compared to 26% in *F. vesca* (*Table 8*). BAP had a significant effect on plantlet regeneration of the explants ($p = 0.023$) (*Table 7*) and seemed to provide the best regeneration at the concentration of 1 mg/litre (*Table 9*) for both *F. v. semperflorens* and *F. vesca*, regardless of the level of IBA. IBA did not appear to be a significant factor affecting the plantlet regeneration of the explants ($p = 0.332$) (*Table 7*). There is no evidence suggesting that there was any interaction between the *Fragaria* accessions and either of the PGR, and between the two PGRs (*Table 7*).

Fragaria vesca f. *vesca* petiole explants did not produce any plantlets in any of the BAP and IBA combinations. When analysed using a Poisson regression model, IBA showed significant effect on plantlet regeneration from *F. v. semperflorens* petioles ($p = 0.03221$) (*Table 10*). Plantlet regeneration appeared to be optimal at 0.1 mg/litre IBA. There is no evidence to suggest that BAP is a significant factor in plantlet regeneration from *F. v. semperflorens* petiole ($p = 0.08612$), nor of an interaction between IBA and BAP

(*p* = 0.70846) (*Table 10*). The count data are shown in *Table 11* and *Table 12*.

A combination of 2 or more PGRs of different classes is usually required in plant tissue culture (Gaspar et al. 1996). PGR appeared to be essential in shoot regeneration of *F. v. semperflorens* and *F. vesca* as no regeneration was observed on media without PGR supplementation in all three experiments. Sugar concentration in culture media can influence the morphogenic response in some explants (Jeannin et al. 1995; Ling et al. 2007). Somatic embryos can be induced in 12%, sucrose-rich medium without any addition of auxin in the sunflower immature zygotic embryo system (Charrière and Hahne 1998). The sucrose concentration of 3% (w/v) used in the experiments described here was not sufficient to induce any morphogenesis in *F. vesca* L. explants without the addition of exogenous auxin, IAA and cytokinin, BAP.

In general, *F. v. semperflorens* had higher *in vitro* regeneration than *F. vesca*, most likely due to a genotypic influence (*Tables 1, 2, 3, 4, 5, 6* and *8*). The same trend was reported by Alsheikh et al. (2002) when both accessions were studied. Several *F. vesca* accessions were tested by Oosumi et al. (2006) for transformation efficiencies and *F. vesca* Hawaii-4, accession PI 551572, was found to be best in terms of shoot regeneration. Hence, shoot regeneration in *F. vesca* is highly genotype dependent, as a difference at the level of subspecies, forma or even cultivar can influence the propensity for regeneration (Alsheikh et al. 2002; Oosumi et al. 2006).

Carbenicillin at 500 mg/litre was incorporated in the media in Experiment I and II as it was the choice of antibiotic at the same concentration to control post transformation growth of *Agrobacterium tumefaciens* strain LBA4404 in the study by Oosumi et al. (2006). The antibiotic at the level tested did not show a completely inhibitory effect on shoot regeneration on either forms of *F. vesca* L., which was in agreement with the findings by Alsheikh et al. (2002). They found that carbenicillin at 500 mg/litre inhibited shoot regeneration in *F. vesca* but the inhibition was the least when compared to cefotaxime and cefoxitin. Carbenicillin was also used in the development of transformation method for *F. vesca* by Pantazis et al. (2013). The petiole was shown to be an unsuitable explant for *F. vesca* as shoot regeneration was very low and slow (*Tables 2* and *4*) and *F. vesca* petiole explants failed to produce any plantlets in Experiment III for the entire PGR combination tested. Petiole sections of *F. v. semperflorens* have higher shoot regeneration than those of *F. vesca* (Alsheikh et al. 2002) and petiole explants are generally less regenerative than leaf explants (Oosumi et al. 2006).

Optimal shoot regeneration from leaf discs was previously reported to be on media supplemented with 3 mg/litre BAP and 0.25 mg/litre IBA for both *F. v. semperflorens* and *F. vesca* (Alsheikh et al. 2002) while the concentrations of BAP and IBA used by Oosumi et al. (2006) were 3 mg/litre and 0.2 mg/litre respectively. A higher BAP concentration of 4 mg/litre and IBA at 0.25 mg/litre was reported to induce shoot regeneration from *F. vesca* leaf explants optimally in another study (El Mansouri et al. 1996). However, a lower BAP concentration of 1 mg/litre seemed to provide better regeneration for leaf explants of both *F. v. semperflorens* and *F. vesca* when different BAP and IBA concentrations were tested in Experiment III (*Table 7* and *9*). IBA did not show any significant influence on plantlet regeneration at the range of concentrations studied (*Table 7*). In tissue cultures, internal hormone concentrations are influenced by the exogenous PGRs present in the media (Charrière and Hahne 1998). BAP uptake into explants depends on the sucrose concentration in the culture medium; higher

level of BAP uptake occurs on medium with lower sucrose concentration (Charrière et al. 1999). The accumulation of BAP within the tissue in turn promotes the induction of endogenous auxins and cytokinins (Charrière et al. 1999). The BAP level in the media was probably enough to trigger the induction of both auxins and cytokinins in the leaf explants of *F. v. semperflorens* and *F. vesca* that led to the morphogenesis observed.

Shoot regeneration from *F. v. semperflorens* petiole sections was reported to be the highest at 1 mg/litre BAP and 0.25 mg/l IBA as compared to 3 mg/litre and 0.25 mg/litre IBA for leaf discs (Alsheikh et al. 2002). IBA appeared to be a stronger factor in shoot regeneration from *F. v. semperflorens* petiole explants than BAP in this study (*Table 10*) and it was sufficient to promote plantlet regeneration at 0.1 mg/litre (*Table 11*), an excluded IBA level in the study by Alsheikh et al. (2002).

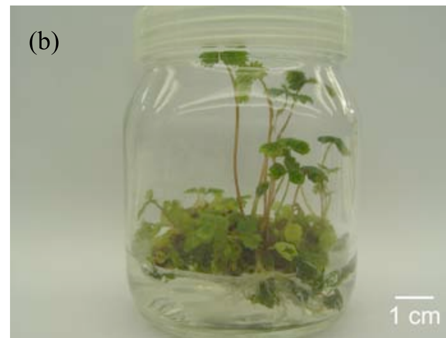

Plate 1. Plantlets formed from (a) F. vesca leaf explants (15 weeks after culture establishment), (b) F. v. semperflorens leaf explants (14 weeks after culture establishment), and (c) F. v. semperflorens petiole explants (14 weeks after culture establishment)

Table 7. Logistic regression output of plantlet regeneration from leaf explants under different PGR concentrations

	Deviance	Resid. Df	Resid. Dev.	F	*p*
		23	6.8471		
Accessions	1.86929	22	4.9778	1.53E + 01	0.008
BAP	2.495	19	2.4828	6.8081	0.023
IBA	0.32509	17	2.1577	1.3306	0.332
Accessions x BAP	0.58361	14	1.5741	1.5925	0.287
BAP x IBA	0.65804	8	0.9160	0.8978	0.550
Accessions x IBA	0.08326	6	0.8328	0.3408	0.724

Accessions: *Fragaria* accessions

Table 8. Percentage of responsive leaf explants from different *Fragaria* accessions

Accession	Responsive leaf explant (%)
F. v. semperflorens	53
F. vesca	26

Table 9. Percentage of responsive leaf explants at different BAP concentrations

BAP (mg/litre)	Responsive leaf explant (%)
1	63
2	35
3	40
4	21

Table 10. Poisson regression output of plantlet regeneration from *F. v. semperflorens* petiole explants under different PGR concentrations

	Df	Deviance	Resid. Df	Resid. Dev	*p*
			49	48.599	
IBA	3	8.7902	46	39.809	0.03221
BAP	3	6.5917	43	33.317	0.08612
IBA x BAP	6	3.7649	37	39.452	0.70846

Table 11. Number of plantlets regenerated from *F. v. semperflorens* petiole explants under different IBA concentrations

		BAP (mg/litre)				
		0	1	2	3	4
Number of plantlets	0	2	6	9	11	10
	1	0	5	2	1	1
	2	0	0	1	0	1
	3	0	1	0	0	0

n (control) = 2; n (treatment) = 16

Table 12. Number of plantlets regenerated from *F. v. semperflorens* petiole explants under different BAP concentrations

		IBA (mg/litre)			
		0	0.1	0.2	0.4
Number of plantlets	0	2	11	10	15
	1	0	2	6	1
	2	0	2	0	0
	3	0	1	0	0

n (control) = 2; n (treatment) = 12

Conclusion

In vitro regeneration of *Fragaria vesca* was both genotype and explant dependent. Although *F. vesca* and *F. v. semperflorens* are of the same genus and species, their *in vitro* regeneration abilities were very different. *Fragaria vesca* f. *vesca* explants took longer time to regenerate than *F. v. semperflorens*. Plantlet regeneration of *F. v. semperflorens* was better than *F. vesca* for both leaf and petiole explants. *Fragaria v. semperflorens* showed higher *in vitro* regeneration than *F. vesca*, and petiole explant was less regenerative than leaf explant. PGR has shown to be essential for *in vitro* regeneration of *F. vesca* L.

References

Alsheikh, M.K., Suso, H.P., Robson, M., Battey, N. H. and 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

Battey, N.H., Le Mière, P., Tehranifar, A., Cekic, C., Taylor, S., Shrives, K.J., Hadley, P., Greenland, A.J., Darby, J. and Wilkinson, K. J. (1998). Genetic and environmental control of flowering in strawberry. In: *Genetic and environmental manipulation of horticultural crops,* (Cockshull, K.E., Gray, D., Seymour, G.B. and Thomas, B., eds.), p. 111 – 131. Wallingford: CABI Publishing

Brown, T. and Wareing, P.F. (1965). The genetical control of the everbearing habit and three other characters in varieties of *Fragaria vesca*. *Euphytica* 14: 97 – 112

Charrière, F. and Hahne, G. (1998). Induction of embryogenesis versus caulogenesis on in vitro cultured sunflower (*Helianthus annuus* L.) immature zygotic embryos: Role of plant growth regulators. *Plant Sci.* 137: 63 – 71

Charrière, F., Sotta, B., Miginiac, É. and Hahne, G. (1999). Induction of adventitious shoots or somatic embryos on in vitro cultured zygotic embryos of *Helianthus annuus*: Variation of endogenous hormone levels. *Plant Physiol. Biochem* 37: 751 – 757

Darrow, G.M. (1966). *The Strawberry: History, Breeding and Physiology*. New York: Holt, Rinehart and Winston

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

Folta, K.M. and Dhingra, A. (2006). Transformation of strawberry: the basis for translational genomics in *Rosaceae*. *In Vitro Cell. Dev. Biol. Plant* 42: 482 – 490

Folta, K.M., Dhingra, A., Howard, L., Stewart, P.J. and Chandler, C.K. (2006). Characterization of LF9, an octoploid strawberry genotype selected for rapid regeneration and transformation. *Planta* 224: 1058 – 1067

Gamborg, O.L., Miller, R.A. and Ojima, K. (1968). Nutrient requirements of suspension cultures of soybean root cells. *Exp. Cell Res.* 50: 151 – 158

Gaspar, T., Kevers, C., Penel, C., Greppin, H., Reid, D.M. and Thorpe, T.A. (1996). Plant hormones and plant growth regulators in plant tissue culture. *In Vitro Cell. Dev. Biol. Plant* 32: 272 – 289

Hancock, J.F. and Luby, J.J. (1993). Genetic resources at our doorstep: the wild strawberries. *BioScience* 43: 141 – 147

Hansen, G. and Wright, M.S. (1999). Recent advances in the transformation of plants. *Trends Plant Sci.* 4: 226 – 231

Heide, O.M. and Sønsteby, A. (2007). Interactions of temperature and photoperiod in the control of flowering of latitudinal and altitudinal populations of wild strawberry (*Fragaria vesca*). *Physiol. Plant.* 130: 280 – 289

Jeannin, G., Bronner, R. and Hahne, G. (1995). Somatic embryogenesis and organogenesis induced on the immature zygotic embryo of sunflower (*Helianthus annuus* L.) cultivated *in vitro*: role of the sugar. *Plant Cell Rep.* 15: 200 – 204

Jones, O.P., Waller, B.J. and Beech, M.G. (1988). The production of strawberry plants from callus culture. *Plant Cell, Tissue Organ Cult.* 12: 235 – 241

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

Ling, A.C.K., Yap, C.P., Mohd. Shaib, J. and Vilasini, P. (2007). Induction and morphogenesis of *Phalaenopsis* callus. *J. Trop. Agric. and Fd. Sc.* 35: 147 – 152

Menard, S. (1995). Applied logistic regression analysis. *Sage University Paper Series on Quantitative Application in the Social Sciences,* 106 p. Thousand Oaks: Sage Publications Inc.

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

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

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

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

Pantazis, C.J., Fisk, S., Mills, K., Flinn, B.S., Shulaev, V., Veilleux, R.E. and Dan, Y. (2013). Development of an efficient transformation method by *Agrobacterium tumefaciens* and high throughput spray assay to identify transgenic plants for woodland strawberry (*Fragaria vesca*) using NPTII selection. *Plant Cell Rep.* 32: 329 – 337

Passey, A.J., Barrett, K.J. and James, D.J. (2003). Adventitious shoot regeneration from seven commercial strawberry cultivars (*Fragaria* x *ananassa* Duch.) using a range of explant types. *Plant Cell Rep.* 21: 397 – 401

Qin, Y., Teixeira da Silva, J.A., Zhang, L. and Zhang, S. (2008). Transgenic strawberry: State of the art for improved traits. *Biotech. Adv.* 26: 219 – 232

Schmuths, H., Meister, A., Horres, R. and Bachmann, K. (2004). Genome size variation among accessions of *Arabidopsis thaliana*. *Ann. Botany* 93: 317 – 321

Shulaev, V., Sargent, D.J., Crowhurst, R.N., Mockler, T.C., Folkerts, O., Delcher, A.L., Jaiswal, P., Mockaitis, K., Liston, A., Mane, S.P., Burns, P., Davis, T.M., Slovin, J.P., Bassil, N., Hellens, R.P., Evans, C., Harkins, T., Kodira, C., Desany, B., Crasta, O.R., Jensen, R.V., Allan, A.C., Michael, T.P., Setubal, J.C., Celton, J.M., Rees, D.J.G., Williams, K.P., Holt, S.H., Rojas, J.J.R., Chatterjee, M., Liu, B., Silva, H., Meisel, L., Adato, A., Filichkin, S.A., Troggio, M., Viola, R., Ashman, T.L., Wang, H., Dharmawardhana, P., Elser, J., Raja, R., Priest, H.D., Bryant, D.W.Jr., Fox, S.E., Givan, S.A., Wilhelm, L.J., Naithani, S., Christoffels, A., Salama, D.Y., Carter, J., Girona, E.L., Zdepski, A., Wang, W., Kerstetter, R.A., Schwab, W., Korban, S.S., Davik, J., Monfort, A., Denoyes-Rothan, B., Arus, P., Mittler, R., Flinn, B., Aharoni, A., Bennetzen, J.L., Salzberg, S.L., Dickerman, A.W., Velasco, R., Borodovsky, M., Veilleux, R.E. and Folta, K.M. (2011). The genome of woodland strawberry (*Fragaria vesca*). *Nature Genet.* 43: 109 – 116

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

Abstrak

Fragaria vesca L. ialah tumbuhan diploid yang merupakan saudara kepada tanaman strawberi oktoploid *Fragaria* x *ananassa*. Ia terdiri daripada *Fragaria vesca* f. *vesca* (*F. vesca*) yang bermusim dan *Fragaria vesca* f. *semperflorens* (*F. v. semperflorens*) yang tidak bermusim. Walaupun *F. vesca* dan *F. v. semperflorens* berada dalam hierarki genus dan spesies yang sama, tetapi kajian ke atas *F. vesca* menunjukkan *F. v. semperflorens* mempunyai pertumbuhan semula *in vitro* yang lebih tinggi daripada *F. vesca*. Eksplan *F. vesca* mengambil masa yang lebih lama untuk tumbuh semula berbanding dengan *F. v. semperflorens.* Tiada pertumbuhan semula ditunjukkan oleh *F. vesca* dan *F. v. semperflorens* sama ada daripada keratan daun ataupun keratan petiol apabila dikultur dengan medium tanpa pengatur pertumbuhan tanaman. Pertumbuhan semula plantlet daripada eksplan daun adalah paling tinggi pada medium yang mengandungi 1 mg/liter 6-benzylaminopurine (BAP) berbanding dengan medium yang mengandungi 2, 3 dan 4 mg/liter BAP. Eksplan petiol *F. v. semperflorens* mencapai pertumbuhan semula plantlet yang paling baik pada kepekatan asid indole-3-butyric 0.1 mg/liter daripada 0, 0.2 dan 0.4 mg/liter.