Middle East Journal of Agriculture Research
ISSN 2077-4605

Volume : 06 | Issue : 03 | July-Sept. | 2017
Pages:682-691

Influence of substrates on *in vitro* rooting and acclimatization of micropropagated strawberry (*Fragaria x ananassa* Duch.)

Wafaa, A. Fekry and H. M. Wahdan

Plant Production Department, Faculty of Technology and Development, Zagazig University, Egypt.

Received: 15 June 2017 / Accepted: 20 July 2017 / Publication date: 03 August 2017

ABSTRACT

This work was conducted at the Tissue Culture Lab., Center of Genetic Engineering and Biotechnology, Fac. Agric., Ain Shams University, Egypt, to optimize a successful technique for *in vitro* shoot rooting and acclimatization by using different substrates with strawberry (*Fragaria x ananassa* Duch.) cvs, "Festival" and "Marquez". As for *in vitro* rooting, various substrates were tested, Agar with 0.1 mg^{-1} IBA (control),peat moss, vermiculite or mixture of both (1:1 v/v). After four weeks of culture, rooting percentages ranged from 96 to 83.33% by both peat moss and vermiculite each alone, respectively. Marquez produces maximum number of roots (5.20 and 5.03) and shoot length (4.73 and 4.60cm) when plantlets were cultured on agar with 0.1 mg^{-1} IBA (control)and peat moss alone, respectively. Meanwhile , "Festival" had increased number of roots and shoot length on agar amended with 0.1mg IBA or the mixture of peat moss and vermiculite (1:1v/v). The longest root length was recorded by peat moss alone with the two cultivars. On the other hand, the lowest values of root features (number and length) as well as shoot length were recorded by using vermiculite as substrate with the two cultivars. Rooted plantlets of about 4.5 cm long were subsequently transferred to various substrates for acclimatization. The survival percentage reached 96 and 93%, respectively for the two cultivars "Marquez" and "Festival" when the transplants were acclimatized on a mixture of peat moss +vermiculite +sand (1:1:1v/v/v). The two studied cultivars had the same significant effect for increasing root parameters (number and length) when the transplant acclimatized on substrate consisted of peat moss +vermiculite+ sand (1:1:1v/v/v) and number of leaves when grown in peat moss alone. Also, Festival cv. gave the same maximum value of root length when acclimatized on vermiculite substrate alone or when mixed with peat moss and sand (1:1:1 v/v/v).

Key words: Strawbery, *in vitro* rooting, peat moss, vermiculite, Acclimation

Introduction

Micropropagation is a powerful biotechnology for plant multiplication. Plant regeneration through tissue culture has been widely utilized for rapid and mass multiplication of many species including strawberry (Amer, 2013).

In vitro rooting induction can be mediated by adding plant growth regulators to the culture medium (Rama, 2012 and Ghasemi *et al*., 2015). However, the survival percentage of these plants during acclimatization is reduced (Zhou *et al*., 2005) and this often becomes a practical problem. In this respect, Gonclaves *et al*. (1988) suggested that the lower survival rate during plant acclimatization may be due to non-functionality of the *in vitro* developed rooting system. Many commercial laboratories do not root microcutting *in vitro*, because it is labour –intensive and expensive. The process of rooting *in vitro* has been estimated to account for approximately 35 to 75% of the total cost of micropropagation (Debergh and Maene, 1981 and Hazarika, 2003). A variety of studies have shown that physiological functions of tissue cultured roots are different compared to the roots that develop on classical grown plants (Labrousse *et al*., 2012). Tissue cultured plants develop adventitious roots on micropropagated shoots, these roots must be strong and functional enough to support the plant in later growth and development (Richie, 1994). Usually, agar is used to solidify the medium of *in vitro* cultures. Different studies mentioned problems relating to the root quality when developed in agar medium (Keatmetha and Suksa-Ard, 2004; Labrousse *et al*., 20120). Anaerobic conditions in agar substrate due to the lack of development of root hairs in roots and this consequently made a difficulty to get a good functional

Corresponding Author: Wafaa, A. Fekry, Plant Production Department, Faculty of Technology and Development, Zagazig University, Egypt. E-mail: drwafaaadel@hotmail.com

root system *in vitro* and can dramatically reduce water and mineral nutrient uptake, thus representing a limiting key step to acclimatization in different substrates (Keatmetha and Suksa-Ard, 2004; Labrousse *et al*.,2012 and Barpete *et al*., 2014). Other substrates than agar like peat moss, vermiculite or mixture of them have physical properties which help to overcome the low O_2 pressure in agar medium. On the same time, decreased oxygen level in a medium had a relationship with poor rooting, decrease in root hair length as well as root respiration (Keatmetha and Suksa-Ard, 2004 and Labrousse *et al*., 2012).

It is very important to determine the factors affecting the ex vitro acclimatization of tissue cultured plants (Kumar and Rao, 2012). One of the important factors affecting on survival percentage of transplants during acclimatization is the type of potting substrate (Kaur *et al*., 2011; El-Zeiny *et al*., 2013 and Barpete, 2014).

Hardening is a time-consuming and labour- intensive process contributing to major portion of the production cost. In this respect, the goal of this research was to work out for selecting the favourable substrates increasing *in vitro* rooting efficiency and high survival rate as well as growth during acclimatization of Festival and Marquez strawberry cultivars.

Materials and Methods

The present study was carried out at the Tissue Culture Lab., Center of Genetic Engineering and Biotechnology, Fac. Agric., Ain Shams University, Egypt, during the period from 2013 to 2015 to study the effect of different substrates on *in vitro* rooting and during acclimatization on the rooting and survival percentage as well as growth of the two strawberry cultivars Festival and Marquez. Runner tips of two strawberry *(Faragaria x ananassa* Duch.*)* cultivars were used as plant materials.

Preparation and sterilization of plant material:

Runners (about 10-15 cm in length) were collected from greenhouse on June as a source of explants. Excised runner tips (5.0 cm in length) were washed under running tap water, then washed with sterilized distilled water. Explants were subjected to chemical sterilization under laminar air flow cabinet using 70% ethyl alcohol for 5 min. and rinsed by sterile double distilled water. Subsequently, the runner tips were sterilized again by 5 % sodium hypochlorite (NaOCl) solution with gently shaking for 20 min. followed by 3-5 times washing by sterile doubled distilled water.

Establishment stage and shoot proliferation:

The meristems were trimmed to 0.4 -0.5 mm and inoculated in jars containing 30.0 ml of MS medium (Murashig and Skoog 1962) supplemented with GA_3 at 0.1 mg l^{-1} and 0.1 mg l^{-1} BA as plant growth regulators and 30 gl^{-1} sucrose. The pH of the medium was adjusted to 5.7 and 7 gl^{-1} agar as well as 3 gl^{-1} activated charcoal (AC) were added before autoclaving at 1.06 kg/cm^{-2} ,121˚C for 20 min. The cultures were incubated at 25 ± 2 ˚C with 16h photoperiod under fluorescent lamps providing 25.2 µmol $m^{-2}S^{-1}$ of photosynthetic photon flux. Subcultures were done three times every 21 days interval. Regenerated multiple shoots about 10 mm in length were placed on a medium contained the same MS medium previously described and incubated under the same conditions. After about 3-4 weeks, elongated shoots were prepared for rooting.

In vitro rooting substrates:

Shoots with 2.0 cm length of the two tested cultivars Festival and Marquez were inoculated in different rooting substrates, i.e. agar (control), peat moss, vermiculite and mixture of peat moss and vermiculite (1:1 v/v), the total treatments were eight. As for agar substrate, shoots were cultured in jars containing 30.0 ml of MS medium supplemented with IBA at 0.1 mgl^{-1}, 30 g sucrose, 3 g l^{-1} activated charcoal and solidified with 7 g l^{-1} agar. The pH was adjusted to 5.7. As for the other substrates, jars were filled with thirty milliliters of peat moss, vermiculite or mixture of both (1:1 v/v) and moistened with 20 ml distilled water. All jars of the experiment were capped with aluminum foil before sterilization at 121˚C with pressure of 1.2 kg /cm^2 for 15 min. The shoots were cultured (five shoots/jar) under laminar air flow cabinet and kept in the incubation room at 25± 2 ˚C with 16h photoperiod.

After 4 weeks of culture the following parameters were evaluated: the percentage of rooting, number of roots, root and shoot length per plantlet.

Acclimatization substrates:

Rooted shoots of the two cultivars Festival and Marquez were removed from culture vessel and washed in distilled water in order to completely remove rooting substrate before being transferred to plastic pots (15 cm diameter) filled with peat moss, vermiculite, peat moss+ vermiculite (1:1v/v) and peat moss + vermiculite+ sand (1:1:1v/v/v). Pots were transferred to the greenhouse and covered with clear polyethylene sheets to create a high relative humidity. Moreover, spraying with water under the plastic sheets were carried out daily while the irrigation took place 2-3 times a week. After10 days of acclimatization, the plastic sheets were gradually opened. The acclimatization plants were fertilized (N/P/K 19/19/19) weekly with equal amount of complete fertilizer at $2gl^{-1}$ as foliar spray.

The following data were recorded in this stage: survival percentage (Number of succeeded transplants/total number of transplants x 100), number of roots, root length and number of leaves per transplant.

Experimental design and statistical analysis:

The two experiments were arranged in a split plot design where cultivars were arranged in the main plots and substrates were distributed randomly in the sub plots. All treatments were conducted in three replicates, five jars in each treatment for each replicate and five shoots were planted in each jar for the two cultivars during the *in vitro* rooting experiment, whereas contained one plant per pot for each replicate in the acclimatization trial. All statistical analysis were performed by using the SPSS software according to Pallant (2001). Means were compared according to L.S.D. at 5% level test to determine the significance differences among the treatments.

Results and Discussion

In vitro rooting substrates:

In vitro rooting of regenerated strawberry shoots started to induction about 15 days after the initiation of culture when the basal ends were swelling.

Effects of cultivars:

Data in Table 1 show clearly that all studied growth characters expressed as number of roots, root and shoot length were significantly affected by the two cultivars, Festival and Marquez. The highest number of roots (4.74) and root length (4.17cm) were produced by Marquez cv. On the other hand, higher value of shoot length (4.56cm) was obtained by Festival cultivar. Such differences in growth between the studied cultivars may be attributed to the potential of genetic differences among such genotypes. In this regard, Singh and Shymal (2001) and Barpete *et al*. (2014) recorded similar results.

Table 1: Effect of strawberry cultivars on root characteristics and shoot length during rooting stage

Cultivar	No. of roots/plantlet	Root length (cm)	Shoot length (cm)
Festavil	4.59	3.30	4.56
Marquez	4.74	4.17	4.51
LSD at 5%	0.02	0.12	0.03

Effect of substrates:

The presented data in Fig.1 show that rooting percentage increased when the plantlets were cultured in peat moss alone (96%) and followed by equal rooting percentage (90%) with the mixture

between peat moss and vermiculite or agar, but decreased to 83.33% when the plantlets were rooted in vermiculite alone.

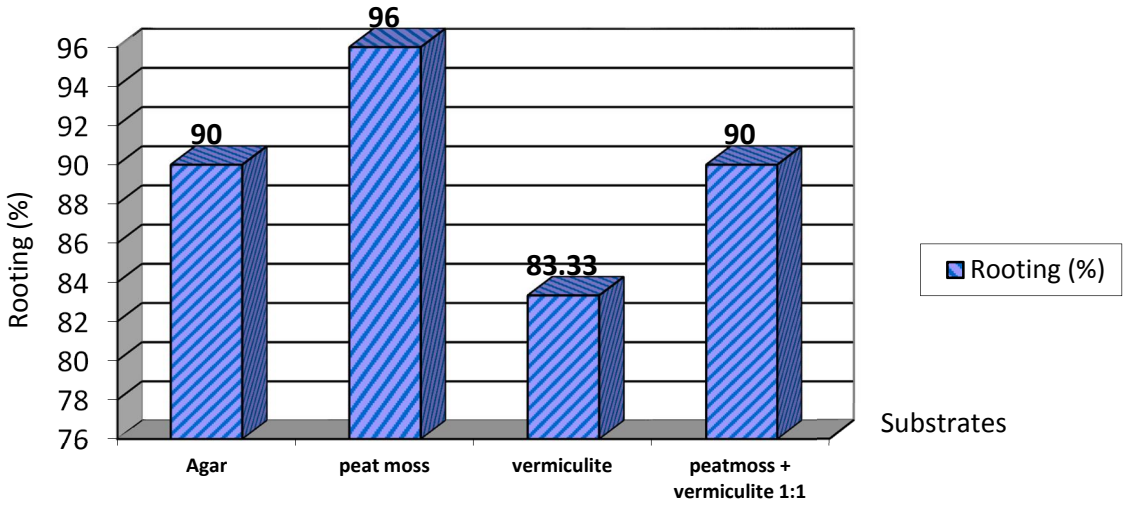


Fig. 1: Effect of rooting substrates on rooting percentage of strawberry plantlets during rooting stage.

As for the growth, obtained data in Table 2 show that the different used substrates had significant effect on root characters (number and length) and shoot length of plantlet. In this concern, agar amended with 0.1 mg^{-1} IBA (Control) was the best substrate for increasing number of roots (5.06 roots) and shoot length (4.73cm) of plantlet. In this respect, Li *et al*. (2009) revealed that auxins are the major plant hormones affect the rooting and the main role of auxin in tissue culture is stimulating cell division and development. On the same time, peat moss recorded the highest value of root length (4.47cm), but there is no differences between peat moss alone or when mixed with vermiculite on number of roots and shoot length. On the other hand, plantlets cultured in vermiculite alone recorded the lowest values of the studied characters, i.e. number of roots (3.91 roots), root length (3.38cm) and shoot length (4.18 cm).

Table 2: Effect of rooting substrates on root characteristics and shoot length during rooting stage

Substrate	No. of roots/plantlet	Root length (cm)	Shoot length (cm)
Agar	5.06	3.58	4.73
Peat moss	4.86	4.47	4.60
Vermiculite	3.91	3.38	4.18
Peat moss + vermiculite (1:1)	4.84	3.53	4.63
LSD at 5%	0.04	0.04	0.03

In this regard, Wahdan (1983) reported that greater numbers of rooted plantlets were recorded in Jiffy7 (in honey jars) kept on controlled environment room. Obtained results of peat moss are in agreement with those reported by Keatmetha and Suksa-Ard (2004) and Labrousse *et al*. (2012) who found that used peat moss as substrates *in vitro* cultured enhanced rooting and increase the number and length of roots. On the other side, obtained results of vermiculite alone on *in vitro* culture are not confirmed with those reported by Gabriel *et al*. (2002) and Keatmethas and Suksa-Ard (2004) who indicated that in order to optimize root induction *in vitro*, the replacement of the agar medium by another substrate such as vermiculite or a mixture of vermiculite and perlite is recommended. In this respect, previous studies demonstrated that different responses to rooting *in vitro* were the result of rooting substrates. Although there is a positive effect of agar for increasing number of roots, many authors have mentioned problems relating to the root quality when developing in agar medium before transplanting which can markedly affect the survival rate (Borkowska, 2001 and Keatmetha and Suksa-Ard, 2004). The lake of development of root hair in roots formed in agar medium generally made it difficult to get a good functional system *in vitro*. In addition, the roots were more fragile and did not present

ramification. Anaerobic root development without root hairs in agar medium considered the main problem and affect the vigor of plantlet after transplanting. In this concern, Galway *et al*. (1997), Bidel *et al*. (2000), Gabriel *et al*. (2002), Labrousse *et al*. (2012) and Aasim *et al*. (2013) indicated that absence of root hair and poor growth are attributed to hypoxia in agar medium and they hypothesized the presence of several limiting factors for root growth in agar medium, in addition to O_2 depletion, progressive dehydration and acidification may also have reduced the meristem growth. Moreover, actively tip-growing root hair cells are characterized by apolarized apex rich in golgi vesicles and mitochondria, suggesting important ATP needs for root hair growth where the high amounts of ATP in root hair imply a good O_2 in agar medium is lower than those found in conventional substrates. Consequently, to enhance root induction of shoot *in vitro*, more aeration of rooting substrate is recommended since it has higher dissolved oxygen rate and contains more water.

Effect of the interaction:

With regard to the effect of the interaction between the tested cultivars (Festival and Marquez) and *in vitro* rooting substrates (Agar with 0.1 mg^{-1} IBA , peat moss ,vermiculite and mixture between peat moss and vermiculite) data in Table 3 revealed that Marquez strawberry cultivar recorded the highest values of root number when rooted in agar with 0.1 mg^{-1} IBA (5.20) followed by the substrate peat moss alone (5.03) or in mixture with vermiculite (4.93).Festival cultivar had the same trend in this respect. The longest roots were obtained by Marquez cv (4.83 cm)followed by Festival cv (4.10 cm) when *in vitro* rooted in peat moss alone or when mixed with vermiculite. As for shoot length, both the two cultivars Marquez and Festival had the same trend mostly with the all used substrates in this respect. The best results obtained by agar with 0.1 mg^{-1} IBA or the mixture between peat moss and vermiculite or peat moss alone. On the other hand, rooted the two strawberry cultivars in the substrate vermiculite lonely gave the lowest values with all studied features.

Table 3: Effect of the interaction between strawberry cultivars (Festival and Marquez) and rooting substrates on root characteristics and shoot length during rooting stage

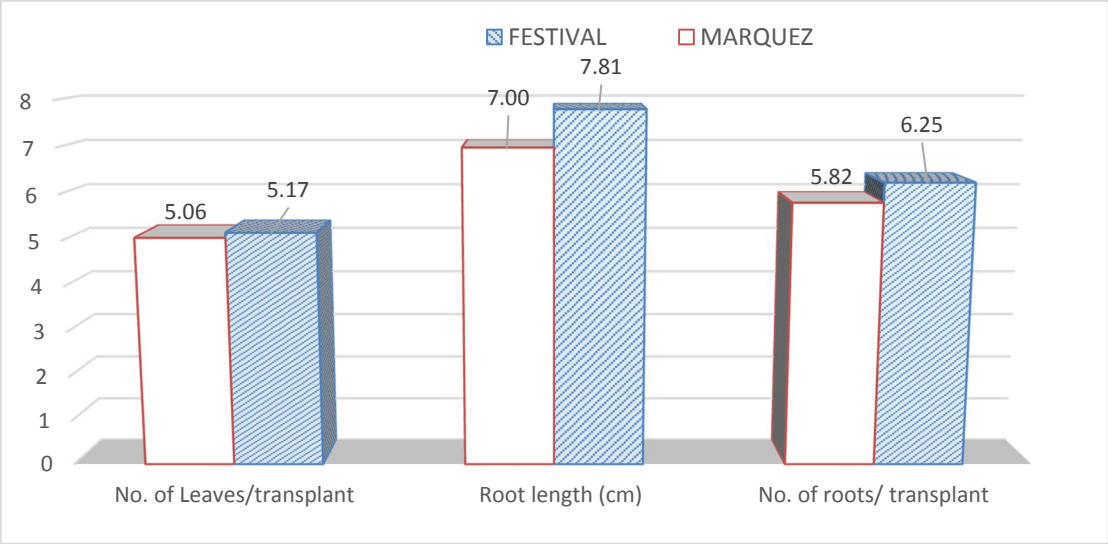
Cultivar	Substrate	No. of Roots /plantlet	Root length (cm)	Shoot length (cm)
Festival	Agar	4.93	3.06	4.73
	Peat moss	4.70	4.10	4.60
	Vermiculite	4.00	3.00	4.20
	Peatmoss + vermiculite (1:1)	4.75	3.06	4.73
Marquez	Agar	5.20	4.10	4.73
	Peat moss	5.03	4.83	4.60
	Vermiculite	3.83	3.76	4.17
	Peatmoss + vermiculite (1:1	4.93	4.00	4.53
LSD at 5%		0.06	0.06	0.05

It could be concluded from these treatments that the peat moss substrate used alone or mixed with vermiculite (1:1 v/v) were effective for increasing rooting percentage, number of roots, root and shoot length per plantlet *in vitro* rooting of the two strawberry cultivars Marquez and Festival as well as to overcome the problems relating to the root quality when developed in agar medium beside to save money and time.

Acclimatization substrates:

Effect of cultivars:

The two strawberry cultivars Festival and Marquez had significant effect on the studied growth parameters expressed as number and length of root as well as number of leaves per transplant during acclimatization stage as illustrated in Fig. 2. In this respect, Festival cv reflected the highest values in all studied growth characteristics. Such differences among the two cultivars may be attributed to the genetic differences between them as recorded by Mohamed, Nagwa (2003).



Characters	No. of leaves	Root length (cm)	No. of roots
LSD at 5%	0.18	0.17	0.12

Fig. 2: Effect of strawberry cultivars on transplants growth characteristics during acclimatization stage

Effect of substrates:

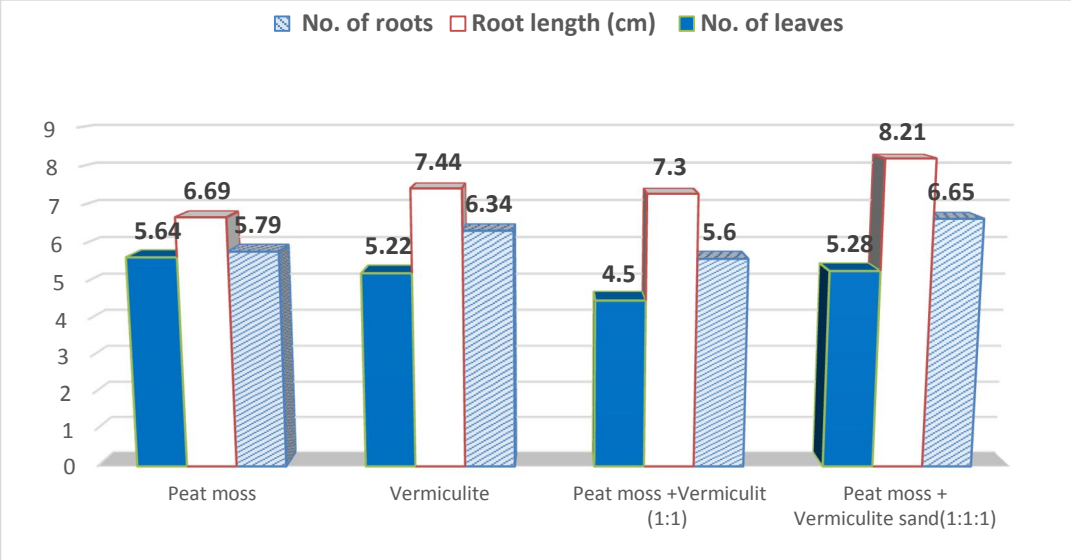
With regarded to the effect of various used substrates and their mixtures on growth of strawberry transplants, data in Fig.3 show that the plantlets acclimatized in the mixture of peat moss + vermiculite + sand (1:1:1 v/v/v) gave the maximum values of root growth (number and length) per transplant. However, the highest number of leaves was obtained using peat moss alone. In this respect, vermiculite alone show significant effect on number of roots (6.34)and root length (7.44 cm) compared with peat moss (5.79 and 6.69cm, respectively).

As for transplant survival percentage, data presented in Fig. 4 show that the survival rate recorded about 94% by the mixture of peat moss + vermiculite + sand (1:1:1v/v/v) and 73% by the vermiculite substrate alone. In this respect, Rajan (2007) reported that micropropagation was successful only when plants were transferred from culture condition to soil with high survival rate and better growth. The quality of transplant depends mainly on the substrate utilized. In this regard, Hartmann *et al*. (1997), Deoe Silva *et al*. (1998), Kampf *et al*. (1998) and Moreira *et al*. (2006) suggested that good growth, more adventitious roots and reduced mortality of plants during acclimatization depend on composition and quality of the substrate. The substrate must combine a light texture with adequate nutritive quality, good moisture-retaining capacity and good aeration. Regarding to vermiculite, it has less bulk density indicated less substrate compactness and more pore spaces which allowed better root aeration, nutrients and water uptake for subsequent growth enhancement and more water holding capacity and thus can hold nutrients in reserve to release them later (Hartmann *et al*., 1990 and Biter and Mohamed, 2009). As for the establishment success in peat, it may be due to better aeration, less water retention capacity, moderate pH and lack of any organic components (Hutchinson,1984)

Although vermiculite had increased number and length of roots the survival percentage was decreased (73%) compared with peat moss (86%) and this result may be ascribe to the increase of leaves number by used the substrate peat moss which due to stimulate the photosynthesis process and consequently the transplant growth and survival.

Obtained results for the effect of different substrates on growth during transplants acclimatization stage are in agreement with Badawi *et al*. (1990) and Amer (2013) on strawberry and Palee *et al*.(2012) and Barpete *et al*.(2014) on different plants with peat moss and Mohamed (1999) on strawberry, Mohamed *et al*. (2001), Bitar and Mohamed (2009) and El – Zeiny *et al*. (2013) by vermiculite. Obtained results of the mixture between peat moss and vermiculite are in a good line with those reported by Husaini *et al*.(2008), Moradi *et al*. (2011), Amer (2013) who found that the mixture of peat moss

+ vermiculite increased the survival percentage as well as number and length of roots. However, Amine and El-Atrach (2012) and El –Zeiny *et al.*(2013) came to the similar highest values of survival percentage (over 90%) ,number of roots and root length for transplants cultured in mixture of the substrates peat moss+ vermiculite+ sand during acclimatization stage.



Characters	No. of leaves	Root length (cm)	No .of roots
LSD at 5%	0.25	0.24	0.17

Fig. 3: Effect of various substrates and their mixtures on growth characteristics of strawberry transplants during acclimatization stage

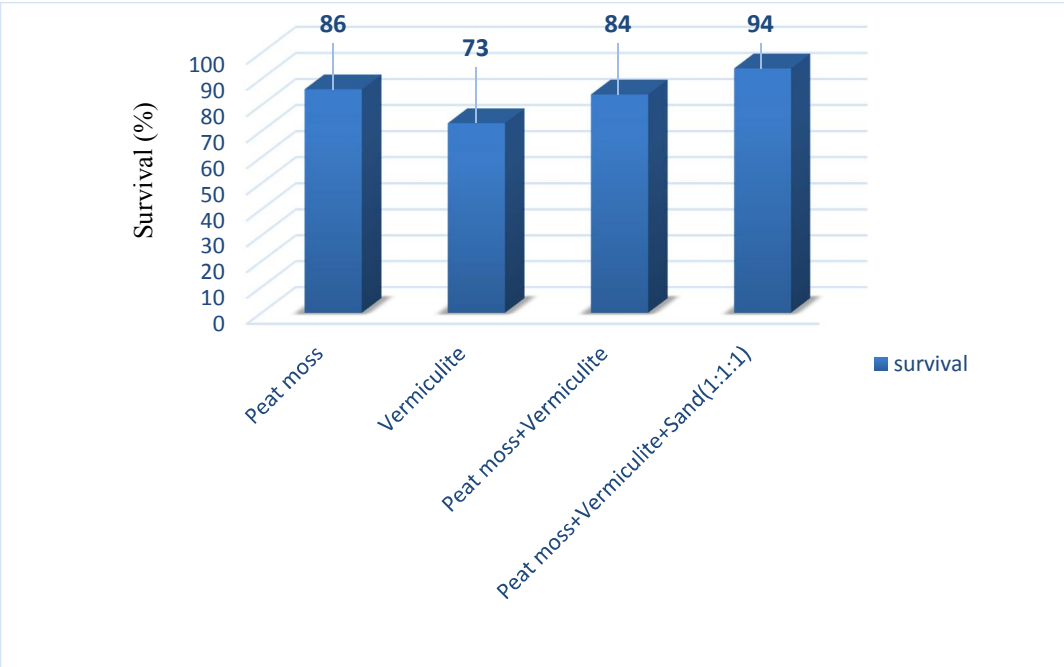


Fig. 4: Effect of various substrates and their mixtures on survival percentage of strawberry transplants during acclimatization stage

Effect of the interaction:

With regard to the interaction between Festival and Marquez strawberry cultivars and different substrates as well as their mixtures on growth characters at acclimatization stage, data recorded in Table 4 show that both cultivars Festival and Marquez achieved the highest values of roots number and length

by using the mixture of the substrates peat moss + vermiculite+ sand (1:1:1 v/v/v), while the highest number of leaves by peat moss alone.

Table 4: Effect of the interaction between strawberry cultivars (Festival and Marquez) and various substrates and their mixtures on growth characteristics of transplants during acclimatization stage

Cultivar	Substrate	No. of roots /transplant	Root length (cm)	No. of leaves /transplant
Festival	Peat moss	6.15	7.16	5.60
	Vermiculite	6.13	8.25	5.08
	Peatmoss + vermiculite (1:1)	5.58	7.58	4.70
	Peatmoss + vermiculite + sand (1:1:1)	6.55	8.25	5.31
Marquez	Peat moss	5.43	6.21	5.68
	Vermiculite	5.95	6.63	5.01
	Peatmoss + vermiculite (1:1)	5.15	7.01	4.31
	Peatmoss + vermiculite + sand(1:1:1)	6.75	8.16	5.25
LSD at 5%		0.24	0.47	0.50

Also, it is clear from such data that Festival cv. recorded the maximum values of root length per transplant when acclimatized in vermiculite alone or mixed with peat moss and sand (1:1:1v/v/v). Obtained results are confirmed by those reported by Mohamed (1999) and Mohamed *et al*., (2001) on the effective of vermiculite contained media than used alone. With respect to the survival percentage, data present in Fig.5 recorded the highest value (96%) with Marquez cultivar and 93% by Festival cv. when the transplant acclimatized in the mixture of peat moss + vermiculite+ sand (1:1:1v/v/v). On the other hand, the lowest value (70% and 76%), respectively for the two cvs, Festival and Marquez were showed by used vermiculite alone. In this respect, using vermiculite in combination with peat moss gave best result than used it alone. Obtained results are in agreement with those reported by Husaini *et al*., (2008) in this concern.

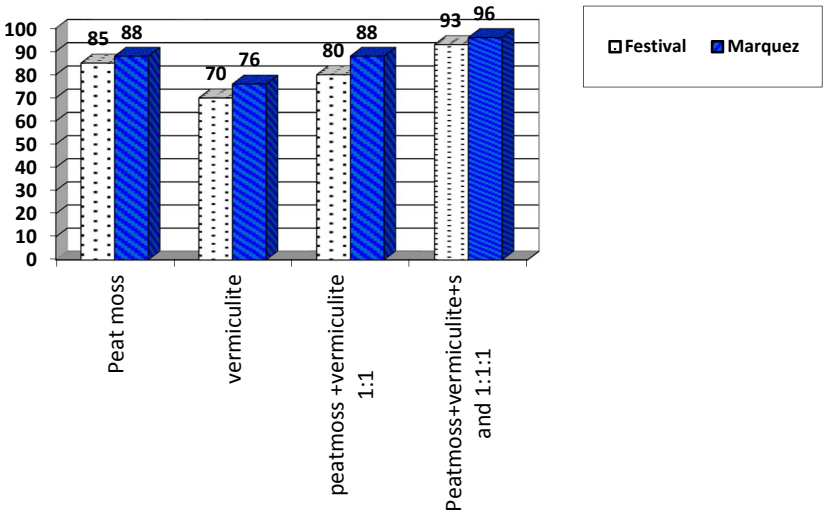


Fig. 5: Effect of the interaction between strawberry cultivars (Festival and Marquez) and various substrates and their mixtures on survival percentage of transplants during acclimatization stage.

Generally, it could be concluded that the two strawberry cultivars Festival and Marquez transplants had the best growth characteristics (number of leaves, root number and length) as well as the survival percentage over than 90% when grown during acclimatization in a mixture of substrates peat moss + vermiculite+ sand (1:1:1v/v/v)

Conclusion

Although agar could be used for micropropagation of the two strawberry cultivars, Festival and Marquez, the results showed that the enhancement of normal root development can be gained if rooting stage was performed on micro-porous substrates, i.e. peat moss or mixture of it with vermiculite which had better air permeability as well as appropriate retention and thus had deleterious effects on plant acclimatization in greenhouse. On the other hand, shoots rooted in these two substrates were easy to handle because roots are not disturbed and induce more quickly with simultaneous shoot growth. The improved rooting and acclimatization achieved by this work could optimize the commercial propagation of the two strawberry cultivars Festival and Marquez which may save time and labour cost.

References

Aasim, M., S. Day, F. Rezaei and M. Hajyzadeh, 2013. Multiple shoot regeneration of plumular apices of chickpea. Turkish J. of Agric. and Forestry, 37(1): 33-39.

Amer, Maha S.H.A., 2013. *In vitro* propagation of some strawberry cultivars. M.Sc. Thesis, Hort. Dept. Fac. Agric., Zagazig Univ., Egypt.

Amine, Mona A. and E. El-Atrach, 2012. The used of tissue culture technique for propagation of Bamboo (*Dendrocalamus giganteus Wallich*) Middle East J. Agric. Res., 1(1): 58-63.

Badawi, M.A., M. Alphonse, A.Z. Bondok and Y.A. Hosni, 1990. Propagation of some strawberry cultivars by means of tissue culture technique. Egy. J. of Hort., 17(1): 9-16.

Barpete, S., K.M. Khawar and S. Özcan, 2014. Differential competence for *in vitro* adventitous rooting of grass pea (*Lathyrus sativus* L.). Plant Cell, Tiss. Organ Cult., 119(1): 39-50.

Bidel, L.P.R., P. Renault, L. Pages and L.M. Riviere, 2000. Mapping meristem respiration of *Prunus persica* (L.) Batsch seedlings: potential respiration of the meristems, O2 diffusional constraints and combined effects on root growth. J. Eexp. Bot., 51(345): 755-768.

Bitar, A.D. and F.H. Mohamed, 2009. Effect of different substrate types on the growth of micropropagated banana transplant. Agric. Res. J. Suez Canal Univ., 9(1): 75-80.

Borkowska, B., 2001. Morphological and physiological characteristics of micropropagated strawberry plants rooted *in vitro* or *ex vitro*. Scientia Hort., Amsterdam, 39(3): 195-206.

Debergh, P.C. and L.J. Maene, 1981. A scheme for commercial propagation of ornamental plants by tissue culture . Sci. Hort., 14: 335-345.

Deoe Silva, S., S. Silva- Neto, R. Oliveira, A. De Matos, A. Prazeres and C. Oliveira, 1998. Development of pre-acclimatized banana plantlets obtained from tissue culture. Acta Hort., 490: 195-199.

El-Zeiny, O.A.H., U.A. El-Behairy, G. Zocchi and M. Rashwan, 2013. Commercial production of globe artichoke (*Cynara scolymus* L.) *In-vitro*. Egypt. J. Agric. Res., 91(3): 993-1007.

Gabriel, BL., L.F. Nelson and R.D. Gerson, 2002. Use of vermiculite as a substrate and effect of light on *in vitro* rooting of pears, cv. Bartlett and clone OH X F 97. Ciência e Agrotec, 26: 977-82.

Galway, M.E., J.W. Heckman and J.W. Schiefelbein, 1997. Growth and ultrastructure of Arabidopsis root hairs: the rhd3 mutation alters vacuole enlargement and tip growth. Planta, 201(2): 209-218.

Ghasemi, Y., S. Beaicknejad, F. Sohrevardi, M. Sharifani, E. Amiri and G.A. Nematzadeh, 2015. Adventitious shoot and root regeneration of wild strawberry (*F. viridis* Duch.) by means of tissue culture medium optimization. Biol. Forum –An Int. J., 7(2): 436-441.

Goncalves, J.C., G. Diogo and S. Amanico, 1998. *In vitro* propagation of chestnut *(Castanea sativa x C. crenata*) : effect of rooting treatments on plant survival ,peroxidase activity and anatomical changes during adventitious root formation. Scientia Hort.,72(3-4): 265-275.

Hartmann, H.T.D.E., F.T. Kester and J.R. Davies, 1990. Plant propagation: principles and practices. 5th Ed. Prentice-Hall. Inc. Englewood Cliffs N J.

Hartmann, H.T., D.E. Kester, F.T. Davies Jr. and R.L. Geneve, 1997. Plant propagation: principles and practices. 6th Ed.Regents/ Prentice Hall,N.J.

Hazarika, B.N., 2003. Acclimatization of tissue – cultured plants. Current Sci., 85(12): 1704-1712.

Husaini, A.M., S. Aquil, M. Bhat, T. Qadri, T.A. Kamaluddin and M.Z. Abdin, 2008. A high-efficiency direct somatic embryogenesis system for strawberry (*Fragaria× ananassa* Duch.) cultivar Chandler. J. Crop Sci. Biotech, 11(2): 107-110.

Hutchinson, J.F., 1984. Factors affecting shoot proliferation and root initiation in organ cultures of the apple 'Northern Spy'. Sci. Hort., 22(4): 347-358.

Kaity, A., A.M. Parisi, S.A. Ashmore and R.A. Drew, 2009. Root initiation and acclimatization of papaya plants. In A Romano, ed, Proc. IIIrd IS on Acclim. and Estabit. Of Micropropagated Plants, Vol 812. Acta Hort., pp: 387-394.

Kampf, A.N., E.O. Calvete and S. Bellé, 1998. Rooting media and plant acclimatization *ex vitro*. In: Combined Proceedings-International Plant Propagators Society. Univ. Washington. Int. Plant Propagation Soc., 48: 709-714.

Kaur, H. An. And M.D. Goyal, 2011. Optimization of potting mixture for hardening of *in vitro* raised plants of *Tylophora indica* to ensure high survival percentage. Int.J.Med.Arom. Plants, 1: 83-88.

Keatmetha, W., and P. Suksa-Ard, 2004. Effects of rooting substrates on *in vitro* rooting of *Anthurium andraeanum* L. cv. Avanti. Walailak J. of Sci. and Tech., 1(2): 49-55.

Kumar, K and I.U. Rao, 2012. Morphophysiological problems in acclimatization of micropropagated plants in –*ex vitro* conditions – a review J. Ornam. Hort. Plants, 2: 271-283.

Labrousse, P., D. Delmail, R. Decou, M. Carlué, S. Lhernould and P. Krausz, 2012. Nemesia root hair response to paper pulp substrate for micropropagation. The Scientific World J. pp: 1-7.

Li, S.W., L. Xue, S. Xu, H. Feng, and L. An, 2009. Mediators, Genes and Signaling in adventitious rooting. Bot. Rev., 75(2): 230-247.

Mohamed, F.H., M.M. Morsi, S. Seifi And T. Refaat, 2001. Effect of various substrate mixtures and container volumes on the quality of greenhouse propagated cucumber, melon and watermelon transplants. J.Agric. Sci. Mansoura Univ., 27(6): 4063-4081.

Mohamed, F.H., 1999. Effect of medium type and container volume on transplant quality, growth and yield of micropropagated strawberry. J.Agric. Sci. Mansoura Univ., 24(9): 5057-5070.

Mohamed, Nagwa A., 2003. Studies on som clonal variations regenerated by anther culture of strawberry cultivars (*Fragaria x ananassa* Duch). Ph.D. Thesis. Hort. Dept. (Veg.Crops), Fac. Agric., Ain Shames Univ., Egypt.

Moradi, K., M. Otroshy and M.R. Azimi, 2011. Micropropagation of strawberry by multiple shoots regeneration tissue cultures. J. Agric. Tech., 7(6): 1755-1763.

Moreira, M., J. Carvalho, M. G-Pasqual, F.C. Borges and A. Da. S. Bortolotto, 2006. Acclimatization of micropropagated pineapple plants cv. "pearl" substrate effect. Sci. and Agrotecnologia, 30(5): 875-879.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol Plant, 15: 473-497.

Palee, J., S. Dheeranupattana, A. Jatisatienr, S. Wangkarn, P. Mungkornasawakul, S. Pyne and T. Sastraruji, 2012. Influence of plantlet age and different soilless culture on acclimatization of *Stemona curtisii* Hook. f. Asian J. of Plant Sci., 11(6): 294-299.

Pallant, J., 2001. SPSS Survival Manual: A step by step Guide to Data Analysis using SPSS For Windows [Version 10], Allen an Vnwin.

Rajan, S., 2007. Propagation of horticultural crops (Vol. 6). New India Publishing.

Rama, B.P., M.K. Devi, H. Jayalaxmi, I. Sophia and P.S. Prajna, 2012. Effect of plant growth regulators on establishment and growth of strawberry (*Fragaria X ananassa* Duch.) var. Chandler *in vitro*. Agric. Sci. Res. J., 2(12): 623-632.

Richie, G.A., 1994. Commercial application of adventitious rooting to forestry.In: Davis TD.Haissig BE (eds) Biology of adventious root formation. Plenum press,New York: pp: 37-52.

Singh, S.K., and M.M. Shymal, 2001. Effect of media and physical factors on *in vitro* rooting in roses. Hortic J, 14: 91-97.

Wahdan, H.M.A., 1983. The balance between the fruiting and vegetative phases in the strawberry (*Fragaria x ananassa* Duch.). Ph. D. Thesis, Dundee Univ., Scotland, UK.

Zhou, Y.H., D.P. Guo, Z.J. Zhu and Q.Q. Qian, 2005. Effects of *in vitro* rooting environments and irradiance on growth and photosynthesis of strawberry plantlets during acclimatization. Plant Cell ,Tissue and Organ Cult., 81: 105-108.