THE EFFECTS OF CULTURE MEDIUM COMPOSITION ON *IN VITRO* ROOTING OF TWO INTERGENERIC HYBRIDS *Fragaria* x *Potentilla*

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Abstract. As an important stage in micropropagating ornamental strawberry, *in vitro* rooting of microshoots on media containing different concentrations of auxins was investigated in two intergeneric hybrids *Fragaria x Potentilla*, respectively "Pink Panda" and "Serenata". IBA at either 0.25 or 0.5 mg/l, and IAA at 0.5 mg/l concentration, were added to solidified Murashige and Skoog (1962) basal medium containing half strenght macroelements and half Lee-Fossard microelements. In all treatments, 0.1 mg/l of GA_3 was also added to the basal medium. IBA was found to be the most effective auxin in promoting rhizogenesis, with the concentration 0.25 mg/l giving the highest rooting rates for both varieties, respectively 100% for "Pink Panda", and 80% for "Serenata".

Keywords: *in vitro* culture, root induction, auxins, cytokinins, intergeneric hybrids *Fragaria x Potentilla*.

INTRODUCTION

Taking into consideration that large-scale micropropagation laboratories are providing millions of plants for the clonally-propagated crop and ornamental market, for many crops continued optimization of tissue-culture protocols is still required [5]. Because the conventional propagation of ornamental strawberry *Fragaria x Potentilla* does not allow the obtention of high number of stolons of guaranteed authenticity and biological value in a very short time, the *in vitro* micropropagation and succesfully rooting and acclimatization of shoots in a nursery environment is the first choice.

The phenomenon of adventitous rooting is still not fully understood, nor is it known why different cultivars have different rooting potentials. Certain physiological states and chemical treatments, such as juvenility, etiolation and application of growth regulators are known to favor rooting in many species [10].

To ensure rapid rooting of micropropagated shoots in strawberry, omission of cytokinin is recommended [4, 12]. However, it was reported that maximum *in vitro* rooting was induced on 1/4 MS medium supplemented with IBA 1.0 mg/l in *Fragaria x ananassa* cv. "Chandler" [13]. Also, satisfactory rooting can take place on full strength culture media, but is a very common practice to transfer shoots to be rooted from high strength media to less concentrated solution. This practice is used for herbaceous plants, as well as for woody ornamentals, fruit trees or forestry species [11, 14]. The favourable effect of a diluted mineral solution on rooting can be explained by the reduction of nitrogen concentration [8].

Knowing the fact that compromised rooting often results in excessive losses at this stage which are costly and inconvenient, and many plants remain unavailable as they are not able to be rooted and acclimatised reliably, we initiated a study aiming at the elaboration of an reliable protocol for the high *in vitro* rooting rate of the ornamental strawberry.

MATERIALS AND METHODS

The research work was carried on within Biotechnology Laboratory, at the Research Institute for Fruit Growing, Pitesti - Maracineni. Two varieties of ornamental strawberry (*Fragaria x Potentilla*), named "Pink Panda" and "Serenata", respectively, were established in the *in vitro* culture starting from meristems, and then subcultured successively on Murashige and Skoog [15] medium supplemented with various combinations of growth regulators (Table 1).

Table 1. The combinations and concentration of growth regulators added to MS medium in order to establish an efficient protocol for the micropropagation of *Fragaria x Potentilla* varieties.

Culture medium code	Basic medium	Growth regulators used and their concentration in the culture medium (mg/l)				
		BAP	IBA	IAA	GA_3	Kin
MM1	MS	0.5	0.1	-	0.1	-
MM2	MS	1.0	0.2	-	0.1	-
MM3	MS	0.5	-	0.5	0.1	-
MM4	MS	1.0	-	1.0	0.1	-
MM5	MS	2.0	-	1.0	-	-
MM6	MS	1.0	-	-	2.0	0.5

After four subcultures, shoots which regenerated from *Fragaria x Potentilla* explants were separated from the micropropagation basal media, when they were approximately 2-3 cm long, and placed on a medium suitable for root growth. Root growth was stimulated by supplementing the solidified Murashige and Skoog basal medium, containing half strength macroelements and half Lee-Fossard microelements, with the auxins IBA and IAA at different concentrations. In all treatments, 0.1 mg/l of GA_3 was also added to the basal medium (Table 2). As carbon

source in all culture media was used dextrose, at 40 g/l concentration.

The cultures have been incubated in a growth chamber at the temperature of 22-24°C, with a photoperiod of 16 hours light/8 hours darkness, and a light intensity of about 40 µmol m^{-2} s^{-1}.

In vitro rooting was followed by acclimatization to *ex vitro* conditions, plantlets being transfered in perlite in greenhouse conditions.

To avoid major statistical errors, at least 5 conical flasks (each with 30 ml of culture medium and closed with cotton-wool bungs and tinfoil) with 6 shoots per flask were used as repetitions in each of the experimental treatment investigated. In order to establish the efficiency of each treatment, the rooting rate, average root number and root length, were determined. Statistical analysis of the data obtained with "Pink Panda" and "Serenata" varieties respectively, on basal media containing different concentrations of auxins for *in vitro* rooting, were performed using Statistical Package for the Social Science (SPSS) statistical software (ver. 16.0) at $p < 0.05$.

Table 2. The combinations and concentration of growth regulators added to basic medium, tested in order to establish an efficient protocol for the *in vitro* rooting of *Fragaria x Potentilla* varieties.

Culture medium code	Basic medium	Growth regulators used and their concentration in the culture medium for *in vitro* rooting (mg/l)		
		IBA	IAA	GA_3
RM1	Macroelements MS 1/2 n, Microelements LF 1/2n, Vitamins MS n	0.25	0	0.1
RM2	Macroelements MS 1/2 n, Microelements LF 1/2n, Vitamins MS n	0.5	0	0.1
RM3	Macroelements MS 1/2 n, Microelements LF 1/2n, Vitamins MS n	0	0.5	0.1

RESULTS

After four weeks in culture, the percentage of microshoots rooted, number of roots and length of roots per culture was influenced by the different types and concentrations of auxins added in the rooting expression media and hormonal composition of the basal media used for explants micropropagation.

In "Pink Panda" variety, rooting induction of the shoots started about 10 days after the initiation of culture. A 100% rooting rate, was recorded in all treatments (RM1, RM2, RM3), but only for those shoots micropropagated in basal media supplemented with lower concentration of IBA and IAA (MM1-MS, MM2-MS, MM3-MS). The overall results indicates that rooting rate was higher for this variety, no significantly different rate being observed, compared with basal medium composition (Fig.1).

As compared to "Pink Panda", the "Serenata" variety of *Fragaria x Potentilla* rooting induction of

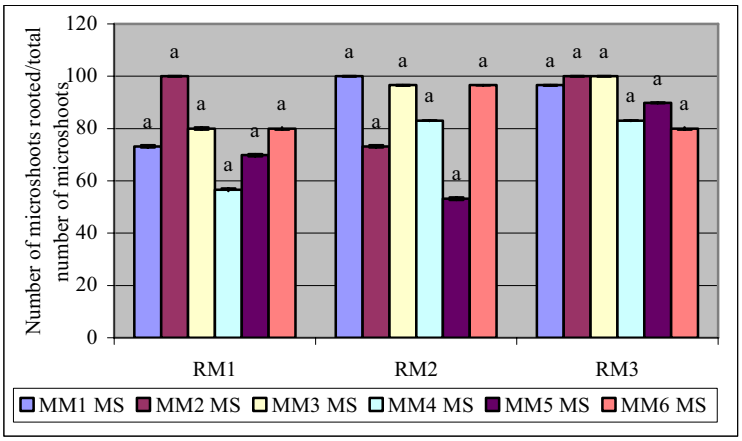

Figure 1. The rooting rate of "Pink Panda" variety (Statistical analysis were performed using ANOVA and Duncan's multiple range test; bars represent standard deviation; means followed by different letters are significantly different from each other, $p<0.05$).

the shoots started about 16 days after the initiation of culture and responded by a lower rate of rooting on all the three variants of culture media. Excepting the shoots obtained by treatment with 1.0 mg/l BAP, 0.2 mg/l IAA and 0.1 mg/l GA_3, no other combinations of growth regulators added to the LF basic medium resulted in significantly different rate of shoots rooting. The highest rooting rate (100%) calculated for "Serenata" variety (Fig. 2) was obtained on RM1 medium (Table 2). Shoots obtained on MM3-MS (Table 1) failed to induce rhizogenesis.

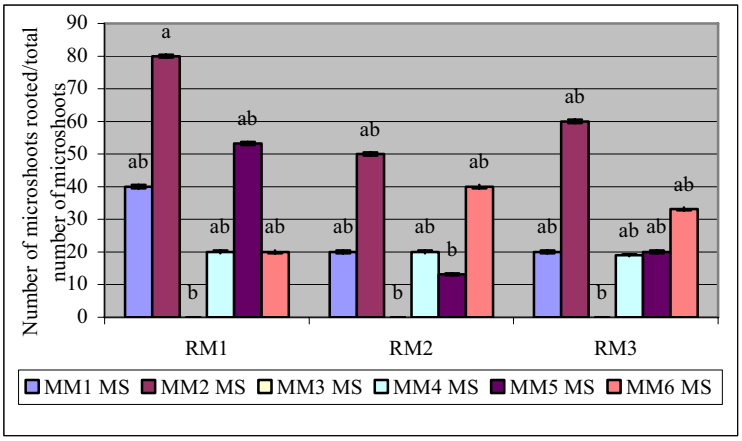

Figure 2. The rooting rate of "Serenata" variety (Statistical analysis were performed using ANOVA and Duncan's multiple range test; bars represent standard deviation; means followed by different letters are significantly different from each other, $p<0.05$).

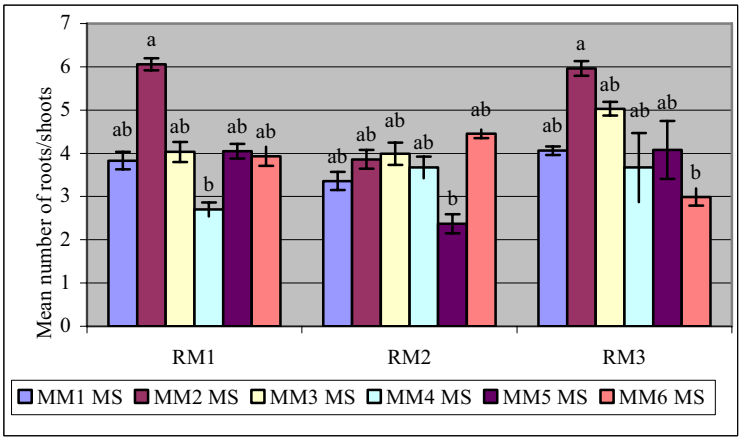

Figure 3. The mean number of roots per shoot in "Pink Panda" variety (Statistical analysis were performed using ANOVA and Duncan's multiple range test; bars represent standard deviation; means followed by different letters are significantly different from each other, $p<0.05$).

In "Pink Panda" variety, the mean root number per shoot varied between 2.37 and 6.06 (Fig. 3), and the average length of the roots between 5.46 and 18.49 mm (Fig. 5). Highest values were calculated for RM1 and RM3 (Table 2) rooting medium, but only for those shoots micropropagated in MM2-MS (Table 1). By increasing IBA concentration from 0.25 mg/l to 0.5 mg/l, a decrease in the mean root number and average roots length was observed, irrespective of the basal medium used for explant micropropagation (Fig. 3&5).

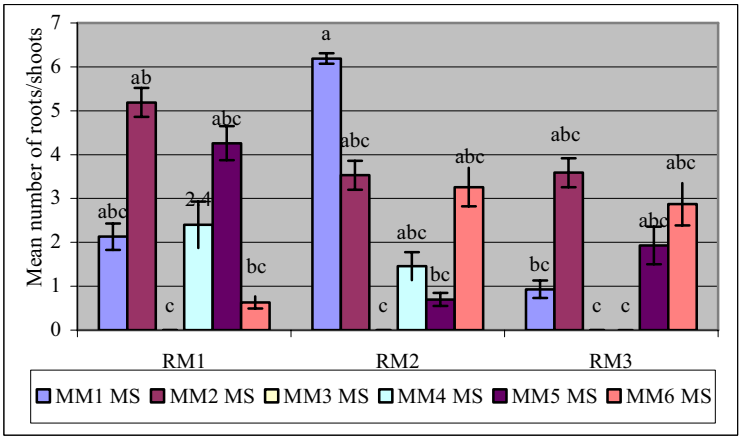

Figure 4. The mean number of roots per shoot in "Serenata" variety (Statistical analysis were performed using ANOVA and Duncan's multiple range test; bars represent standard deviation; means followed by different letters are significantly different from each other, $p<0.05$).

As compared to "Pink Panda", the "Serenata" variety of *Fragaria x Potentilla* responded by a lower average number of roots formed per shoot (Fig. 4). The statistical analysis revealed that the highest mean root number per shoot (6.19) and the highest average length of the roots (9.96) was induced in this variety by the combination 0.5 mg/l IBA and 0.1 mg/l GA_3 (Table 2), for those shoots cultured on MM1-MS variant of medium (Fig. 4 & 6). When increasing IBA concentration from 0.25 mg/l to 0.5 mg/l, or replacing

this auxin with IAA, a decrease in the mean root number and average roots length, respective an inability of rooting was observed for those microshoots obtained on MM4-MS basal medium (Fig. 4 & 6).

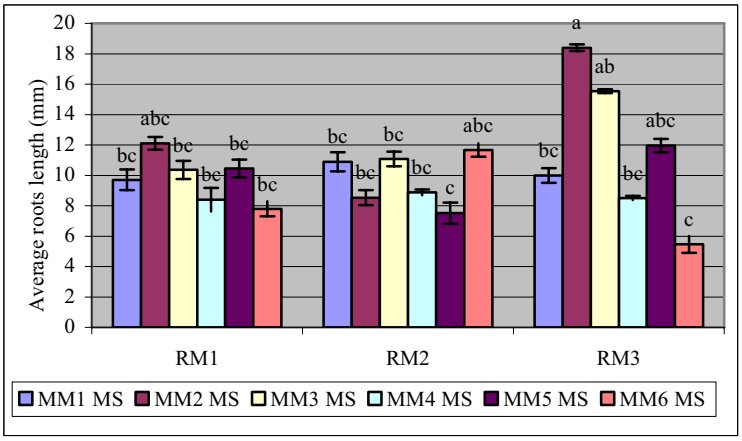

Figure 5. The average length of the roots in "Pink Panda" variety (Statistical analysis were performed using ANOVA and Duncan's multiple range test; bars represent standard deviation; means followed by different letters are significantly different from each other, $p<0.05$).

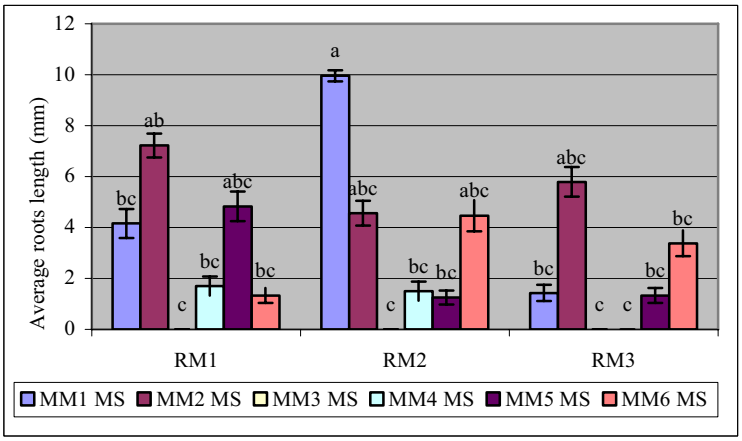

Figure 6. The average length of the roots in "Serenata" variety (Statistical analysis were performed using ANOVA and Duncan's multiple range test; bars represent standard deviation; means followed by different letters are significantly different from each other, $p<0.05$).

DISCUSSIONS

In vitro plantlets usually have a low photosynthetic ability and require sugar as carbon source for their growth [17]. Moreover, sufficient nutrient reserve are very important for a plantlet to overcome transition stress existing during the first week following transplanting [6], *in vitro* rooting media was adjusted with dextrose, at 40 g/l concentration.

The comparison of responses of "Pink Panda" and "Serenata" intergeneric hybrids, on different rooting media, have indicated that each genotype require different combinations and concentrations of growth regulators, for succesfully rooting of *in vitro* culture derived shoots.

In "Pink Panda" and "Serenata" intergeneric hybrids, low concentration of IBA (0.25 mg/l IBA) was enough for maximum number of root per explant. The IBA used for root development in MS medium, from meristem derived plantlets was also reported in different crops [1, 2, 16].

The precise concentration of growth regulators is critical in producing the desired response. A large amount of hormone can bring about an inhibition of rooting, rather than promotion. The "Pink Panda" variety responded by slightly lower rates of rooting when IBA concentration was higher (0.5 mg/l). Several authors have shown that auxin is only required during the initiation phase and becomes inhibitory for root out growth [7, 9].

As showed Bouza *et al.* [3] rooting capacity of explants was influenced by a preliminary accumulation of endogenous auxins and citokinines, absorbed from the multiplication medium. In "Pink Panda" variety, shoots regenerated on medium containing higher concentration of BAP (1.0 mg/l) and IBA (0.2 mg/l) responded by higher values for all three rooting caracteristics analysed, when rooting medium was supplemented with 0.5 mg/l IAA and 0.1 mg/l GA_3. In "Serenata" variety, lower concentration of BAP (0.5 mg/l) and IBA (0.1 mg/l) in the multiplication medium was associated with significantly higher average root number and root elongation, when IBA concentration in the rooting medium was higher.

In both intergeneric hybrids, higher concentration of auxins and citokinins in the multiplication media favoured rooting capacity of explants in those rooting media supplemented with lower concentration of auxins.

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