COMPORTAREA UNOR SOIURI DE CAIS ÎN FAZA DE MULTIPLICARE *IN VITRO*
BEHAVIOUR OF SOME APRICOT CULTIVARS DURING *IN VITRO* MULTIPLICATION PHASE

Mirela Călinescu [1], Maria Isac [1], Andi Ciobanu [2]
[1]Research Institute for Fruit Growing, Mărăcineni, Romania
[2]Craiova University, Faculty of Agriculture

Abstract

This paper presents relevant data regarding behaviour of 14 apricot cultivars during *in vitro* multiplication phase. The biological material was represented by meristems prelevated from one year old branches. In this phase 3 growing media were used: Murashige & Skoog (1962), Fossard (1972) and Lepoivre (1977), supplemented with: dextrose (40 g/l), GA_3 (0.1mg/l), BAP (1 mg/l), ANA (0.2 mg/l), and Na Fe EDTA (3.2 mg/l). The best results (in average 7.9 shoots per explants) were obtained when Fossard media was used, regardless the cultivar, the determinant role being held by the vitamin complex of this nutritive media. For all 14 cultivars the best multiplication rates was obtained when large size explants of 0.3 – 0.5 mm were used.

Keywords: explants, nutritive media, subculture, hormonal balance
Cuvinte cheie: explant, mediu nutritiv, subcultură, balanţă hormonală

1. Introduction

A factor on which depends the success of an *in vitro* culture is the nutritive media composition, specific for each and every species and cultivar, the requirements being different according to the explants type (Isac, 1983). Application of viruses release method of infected material must be done taking in account the optimal parameters which can conduct to a good efficiency, knowing that a series of factors are influencing the success of this process: explants size (Deogratias, 1987) number of subcultures and hormonal balance.

As is quoted in references (Boxus and Druart, 1989), in generally should to remember that the inocule must have a minimal dimension to express its cells totipotence under *in vitro* condition.

The *in vitro* multiplication capacity is defined by the multiplication rate which is given by the number of the adventives shoots obtained by explants. This rate is different according the species or inside the species according to the cultivar, this indicator ranging between 2 or 3 shoots and 30-40 shoots per explants (Isac M., 1996).

Knowing the multiplication rate, the number of subcultures can be established, according the plants number needed to be produced in a specified time period.

Present work objectives were focused on: establishment of the most efficient growing media for *in vitro* multiplication of the apricot; establishment of optimal dimension for the explants used at *in vitro* culture start; identification of some apricot cultivar very adequate for *in vitro* multiplication.

2. Material and methods

The biological material used to establish the *in vitro* cultures was represented by meristems excised from axilar buds, prelevated in November - February from one year old shoots belonging to 14 apricot cultivars: ‘Dacia’, ‘Harcot’, ‘Viorica’, ‘Carmela’, ‘Favorit’, ‘Rareş’, ‘Mamaia’, ‘Comandor’, ‘Olimp’, ‘Sulina’, ‘Litoral’, CR 2/63’, ‘NJA19’, ‘Excelsior’.

The used inocules had three dimensions: 0.1 mm (meristematic dom), 0.1 - 0.3 mm (1 - 2 foliar primordia), 0.3 – 0.5 mm (3 - 6 foliar primordia). The explants obtained at the end of differentiation phase were transferred on the multiplication media.

The growing media were prepared from macroelements, microelements, vitamins, and growing hormones and assembled in three media variants: Murashige & Skoog (1962), Lee Fossard (1972) and Lepoivre (1977) (table 1), supplemented with GA3 (0.1 mg/L), BAP (1.0 mg/L), NAA (0.2 mg/L), Na Fe EDTA (3.2 mg/L). After preparation, the growing media were sterilized by autoclavation at 115 - 120^0C and during 20 min. As an organic carbon source, dextrose 40 g /l and as gelifiant agent agar 10 g/l were used. The inocules were placed in glass phials under sterile laminated air flow. The growing conditions consisted in provide condition of 22 - 23^0 C temperature, 16h light photoperiod (2.000 - 2.500 lux light intensity) and 8h darkness.

3. Results and discussions

The experience was done during 4 subcultures, the multiplication rate was examinated after 3-4 weeks for each subculture. Analysis of the results obtained in the multiplication phase of the 14 apricots cultivars on three growing media (B.1, B.2, B.3), depending on dimensions of used explants (C.1, C.2, C.3), noticed that the high influence on multiplication rate is the one of explants dimensions.

When the used growing media is analyzed, it can be concluded that the highest average of the multiplication rate was obtained on B.3. (Fossard) media, with 7.93 shoots/explant, regardless the cultivar (Table 2). Analysis of the *in vitro* multiplication capacity of the studied apricot cultivars, according to the growing media, and dimension of the used explants reveal that the highest multiplication rate was registered when the explants had among 0.3-0.5 mm (C.3.), with an average of 8.5 shoots per explant (Table 3.).

Statistic interpretation of the cultivar influence on the multiplication rate evidence that for nutritive media average, the highest multiplication rate was registered with Viorica cultivar with 7.8 shoots per explants, 'Dacia' cultivar with 7.6 shoots per explant, 'Olimp', 'Sulina' and 'Excelsior' cultivars with a multiplication rate of 7.3 shoots per explant. The lowest multiplication rate was registered with Harcot cultivar, only 4.8 shoots per explants (Fig. 1). Analysis of the A x B factors (cultivar x nutritive media) shows that 'Viorica' cultivar registered the highest multiplication rate on B.3 (Fossard) nutritive media, with 9.3 shoots per explant. 'Dacia' and 'Sulina' cultivars are following with an lower rate of multiplication (9.0 shoots per explants).

A lowest multiplication rate on B.3 nutritive media of 5.3 shoots per explant was accomplished with Harcot cultivar.

The studied cultivars had an weak behavior on B.2 (Lepoivre) growing media, with a maximum of the multiplication rate of 8,0 and 7,7 shoots per explants obtained with Olimp and Sulina cultivars. On this nutritive media the lowest multiplication rates (4,3 shoots per explant) were obtained with Harcot cultivar (Fig. 1). On B.1 (Murashige & Skoog) nutritive media, first place was held by Viorica and Excelsior cultivars, with a multiplication rate of 6.7 shoots per explant, followed by 'Dacia' cultivar with 6.3 shoots per explant. On this media, the lowest multiplication rate was obtained with CR 2/63 cultivar with 4.0 shoots per explant

The experimental results regarding the influence of the growing media on explants multiplication rate shows that Fossard media provided the best *in vitro* growing conditions. On this media 7.9 shoots per explant being obtained, followed by Lepoivre growing media with 6.7 shoots per explant, compared with Murashige & Skoog media on which the multiplication rate was in average only 5.4 shoots per explant (Fig. 2).

The lowest multiplication rates were 4.0 shoots per explant in the case of CR 2/63 cultivar on B.1 - Murashige & Skoog growing media and 4.0 shoots per explant for 'Harcot' cultivar on B.2 – Lepoivre media.

Explants dimensions is a very important factor in the process of *in vitro multiplication* process. In this sense, as regard the average of the 14 apricot cultivars, the obtained results ranged from a minimum of 4.8 shoots per explant, in the case of the explants of 0.1 mm and up to 8.5 shoots per explants when explants of 0.3-0.5 mm were used.

For the studied cultivars, were evidenced the variants where the meristems used had 0.3-0.5 mm (3-6 foliar primordia) (Fig. 3).

Analysis of the interaction between nutritive media and average of the explants dimensions reveal that on B.3 (Fossard) media was obtained the best multiplication rate of 7.9 shoots per explant.

The highest value of the multiplication rate of 10.1:1 was achieved on B.3 (Fossard) multiplication media, for the explants with dimension of 0.3-0.5 mm (Fig. 4).

The results obtained regarding the influence of the explants dimensions on the multiplication rate showed that the best multiplication rate was in average 8.5 shoots per explants when at the initiation of the *in vitro* culture, meristems of 0.3-0.5 mm were used.

Assessment of the interaction between explants dimension, shows that the highest multiplication rates of 10.1:1, were obtained when meristems of 0.3-0.5 mm were used on B.3 (Fossard) nutritive media.

Explants dimensions has the same influence also on B.2 (Lepoivre) and B.1 (Murashige&Skoog) nutritive media.

In this sense, on B.2 (Lepoivre) nutritive media, the multiplication rate was 8.5 shoots per explants when explants of 0.3-0.5 mm were used and 4.9 shoots per explants when the meristems had 0.1 mm (Fig. 5).

4. Conclusions

On all 14 apricot cultivars higher multiplication rates were obtained in the variants where the used explants dimension was 0.3 – 0.5 mm (C.3.).

The 'Viorica' cultivar registered the highest average of the multiplication rate of 7.8 shoots per explant, followed at a small difference by Dacia cultivar with 7.6 shoots per explant.

Analyzing the factor **nutritive media** can be observed that the highest multiplication rate was obtained on B.3. (Fossard) media with 7.9 shoots/explant, regardless the cultivar, the determinant role being held by the vitaminic complex specific to this media.

The analyses of the cultivar x nutritive media (A x B interaction) reveal the fact that Viorica cultivar achieved the best multiplication rate of 9.3 shoots per explant on B.3. (Fossard) nutritive media.

The studied cultivars had a good behaviour and on the B.2. nutritive media (Lepoivre), with an average of multiplication rate of 7.9 shoots/explant.

References

1. Boxus Ph., Druart Ph., 1989. The production of fruit and vegetable plant by *in vitro* culture. Actual possibilities and perspectives. 49 annèe – n 396 – 4 – trimestre.

2. Deogratias J.M., 1985. *,,In vitro"* shoot tips micrografting from juvenile an adult *Prunus avium* L. and *Prunus persica* L. Batsch to produce virus free plants. Acta Horticulturae, 193.

3. Fossard, R.A., 1977. Tissue culture in horticulture – A perspective. Acta horticulturae, 455.

4. Isac Maria, 1983. Comportarea unor soiuri de prun în procesul de microînmulţire, Lucrările celui de al II-lea Simpozion Naţional de culturi de ţesuturi vegetale *in vitro* , vol. II.

5. Isac Maria, 1996. Contribuţii la îmbunătăţirea tehnologiei de microînmulţire *in vitro* a cireşului (*Prunus avium L.*) şi vişinului (*Prunus cerasus L.*), *Teză de doctorat*. Universitatea din Craiova.

6. Murashige T., Skoog F., 1962. A revised for rapid growth and bioassay with tobacco tissue cultures, Physiology Plant., 15: 473 – 497.

7. Quoirin M., Lepoivre P., 1977. Improved media for *in vitro* culture of *Prunus s*p. Acta Horticulturae 78: 437 – 442.

Tables and figures

Table 1. Composition of basic nutritive media

	Murashige&Skoog (1962) mg/l	Lepoivre (1977) mg/l	Fossard (1977) mg/l
Macroelements			
NH_4NO_3	1650	400	800
KNO_3	1900	1800	1011
$CaCl_2*2H_2O$	440	-	330
$MgSO_4*7H_2O$	370	360	370
KH_2PO_4	170	270	-
K_2SO_4	-	-	-
$Ca(NO_3)_2*4H_2O$	-	1200	-
NaH_2PO_4	-	-	138
Microelements			
$FeSO_4*7H_2O$	27.9	-	10.7
$MnSO_4*4H_2O$	22.3	0.75	8.45
$ZnSO_4*7H_2O$	8.6	8.6	5.75
H_3BO_3	6.2	12.0	3.09
$CuSO_4*5H_2O$	0.025	0.025	0.024
$Na_2MoO_4*2H_20$	0.25	0.25	0.024
$CoCl_2*6H_2O$	0.025	0.025	0.118
KI	0.83	0.08	0.415
Na_2EDTA	-	-	18.61
Na_2SO_4	-	-	144.99
Vitamins			
Inozitol	100	100	54.048
Tiamine HCl	0.1	0.4	0.674
Nicotinic acid	0.5	-	2.462
Piridoxine HCl	0.5	-	0.616

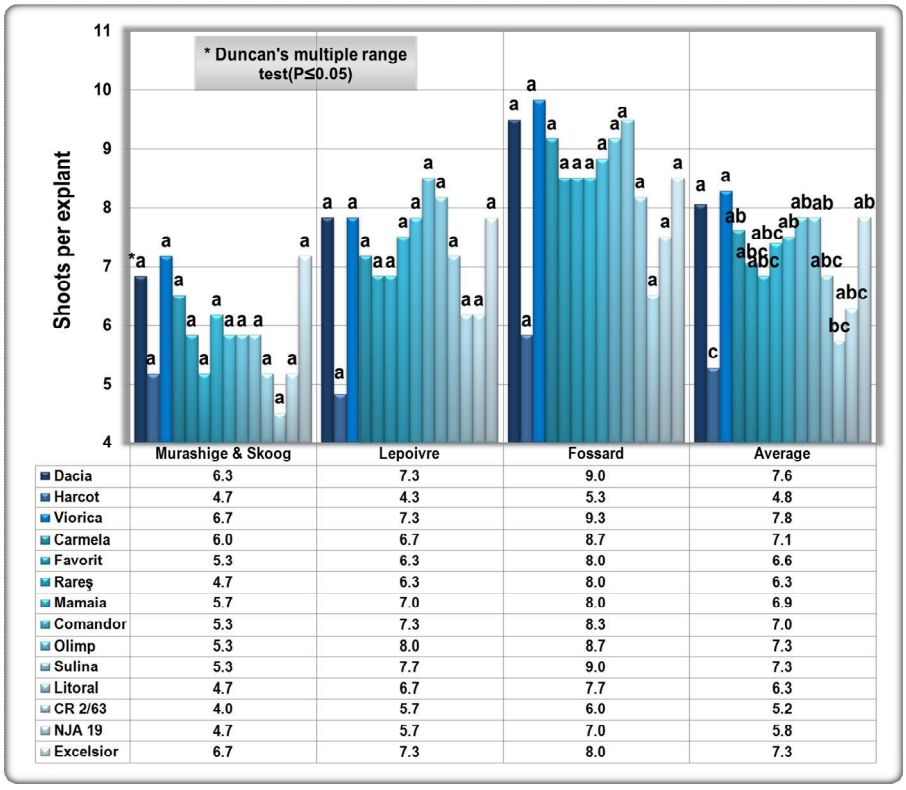
Glicine	2.0	-	-
Coline	-	-	0.104
Biotine	-	-	0.048
Calcium pantetonat	-	-	0.476
Riboflavină	-	-	0.376
Ascorbic acid	-	-	0.176

Table 2. *In vitro* multiplication capacity of apricot cultivars for each used growing media

Nutritive media	Cultivar	Average shoots/explant	Average
B.1 (Murashige&Skoog)	A.1 – Dacia	6.33	5.38
	A.2 – Harcot	4.66	
	A.3 – Viorica	6.66	
	A.4 – Carmela	6.00	
	A.5 – Favorit	5.33	
	A.6 – Rareş	4.66	
	A.7 – Mamaia	5.66	
	A.8 – Comandor	5.33	
	A.9 – Olimp	5.33	
	A.10 – Sulina	5.33	
	A.11– Litoral	4.66	
	A.12– CR 2/63;	4.00	
	A.13– NJA19	4.66	
	A.14– Excelsior	6.66	
B.2 (Lepoivre)	A.1 – Dacia	7.33	6.68
	A.2 – Harcot	4.33	
	A.3 – Viorica	7.33	
	A.4 – Carmela	6.66	
	A.5 – Favorit	6.33	
	A.6 – Rareş	6.33	
	A.7 – Mamaia	7.00	
	A.8 – Comandor	7.33	
	A.9 – Olimp	8.00	
	A.10 – Sulina	7.66	
	A.11– Litoral	6.66	
	A.12– CR 2/63;	5.66	
	A.13– NJA19	5.66	
	A.14– Excelsior	7.33	
B.3 (Fossard)	A.1 – Dacia	*9.00*	*7.93*
	A.2 – Harcot	5.33	
	A.3 – Viorica	*9.33*	
	A.4 – Carmela	8.66	
	A.5 – Favorit	8.00	
	A.6 – Rareş	8.00	
	A.7 – Mamaia	8.00	
	A.8 – Comandor	8.33	
	A.9 – Olimp	8.66	
	A.10 – Sulina	*9.00*	
	A.11– Litoral	7.66	
	A.12– CR 2/63	6.00	
	A.13– NJA19	7.00	
	A.14– Excelsior	8.00	

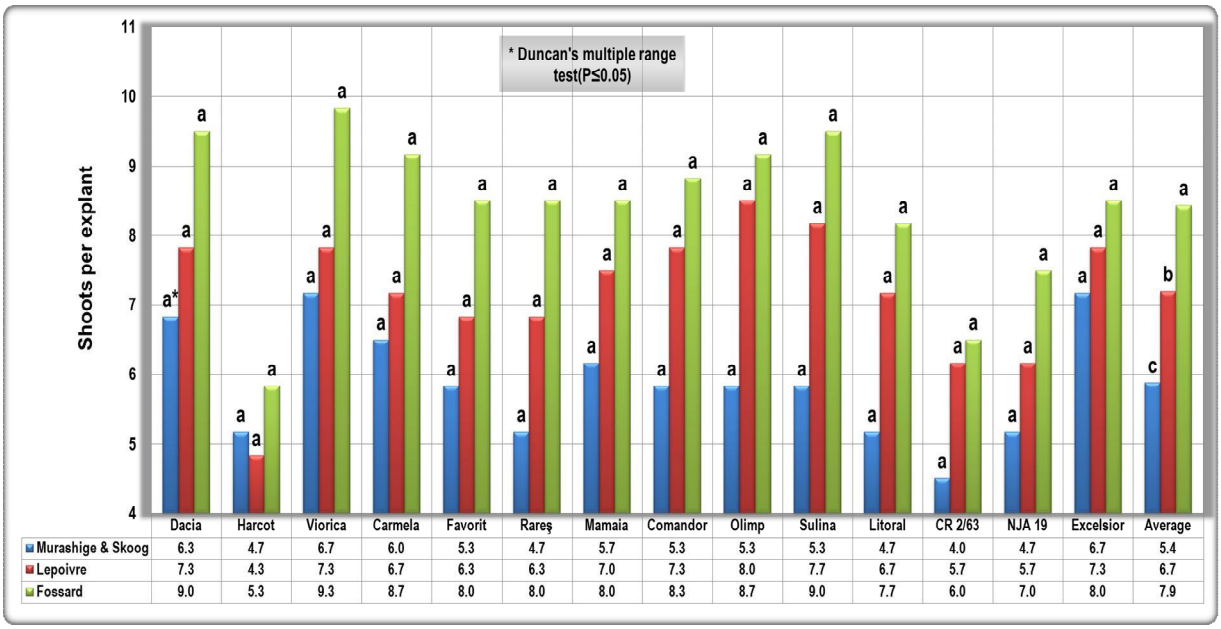
Table 3. *In vitro* multiplication capacity of apricot cultivars according to explant dimensions

Explant dimensions (mm)	Cultivar	Average shoots/explant	Average
C. 1. (0.1)	A.1 – Dacia	4.66	4.83
	A.2 – Harcot	3.00	
	A.3 – Viorica	6.00	
	A.4 – Carmela	5.66	
	A.5 – Favorit	5.00	
	A.6 – Rareş	4.66	
	A.7 – Mamaia	5.00	
	A.8 – Comandor	5.33	
	A.9 – Olimp	5.33	
	A.10 – Sulina	5.00	
	A.11– Litoral	4.33	
	A.12– CR 2/63;	4.00	
	A.13– NJA19	4.00	
	A.14– Excelsior	5.66	
C. 2. (0.1-0.3)	A.1 – Dacia	7.66	6.66
	A.2 – Harcot	4.66	
	A.3 – Viorica	7.66	
	A.4 – Carmela	7.33	
	A.5 – Favorit	6.33	
	A.6 – Rareş	6.33	
	A.7 – Mamaia	7.00	
	A.8 – Comandor	6.66	
	A.9 – Olimp	7.33	
	A.10 – Sulina	7.33	
	A.11– Litoral	6.33	
	A.12– CR 2/63	5.33	
	A.13– NJA19	6.00	
	A.14– Excelsior	7.33	
C. 3. (0.3-0.5)	A.1 – Dacia	*10.33*	*8.50*
	A.2 – Harcot	6.66	
	A.3 – Viorica	*9.66*	
	A.4 – Carmela	8.33	
	A.5 – Favorit	8.33	
	A.6 – Rareş	8.00	
	A.7 – Mamaia	8.66	
	A.8 – Comandor	9.00	
	A.9 – Olimp	9.33	
	A.10 – Sulina	*9.66*	
	A.11– Litoral	8.33	
	A.12– CR 2/63	6.33	
	A.13– NJA19	7.33	
	A.14– Excelsior	9.00	



	Murashige & Skoog	Lepoivre	Fossard	Average
Dacia	6.3	7.3	9.0	7.6
Harcot	4.7	4.3	5.3	4.8
Viorica	6.7	7.3	9.3	7.8
Carmela	6.0	6.7	8.7	7.1
Favorit	5.3	6.3	8.0	6.6
Rareş	4.7	6.3	8.0	6.3
Mamaia	5.7	7.0	8.0	6.9
Comandor	5.3	7.3	8.3	7.0
Olimp	5.3	8.0	8.7	7.3
Sulina	5.3	7.7	9.0	7.3
Litoral	4.7	6.7	7.7	6.3
CR 2/63	4.0	5.7	6.0	5.2
NJA 19	4.7	5.7	7.0	5.8
Excelsior	6.7	7.3	8.0	7.3

Fig. 1 *In vitro* multiplication rate variation (shoots per explant) according to the cultivar for different nutritive media



	Dacia	Harcot	Viorica	Carmela	Favorit	Rareş	Mamaia	Comandor	Olimp	Sulina	Litoral	CR 2/63	NJA 19	Excelsior	Average
Murashige & Skoog	6.3	4.7	6.7	6.0	5.3	4.7	5.7	5.3	5.3	5.3	4.7	4.0	4.7	6.7	5.4
Lepoivre	7.3	4.3	7.3	6.7	6.3	6.3	7.0	7.3	8.0	7.7	6.7	5.7	5.7	7.3	6.7
Fossard	9.0	5.3	9.3	8.7	8.0	8.0	8.0	8.3	8.7	9.0	7.7	6.0	7.0	8.0	7.9

Fig. 2 *In vitro* multiplication rate variation (shoots per explant) according to the nutritive media for different cultivar

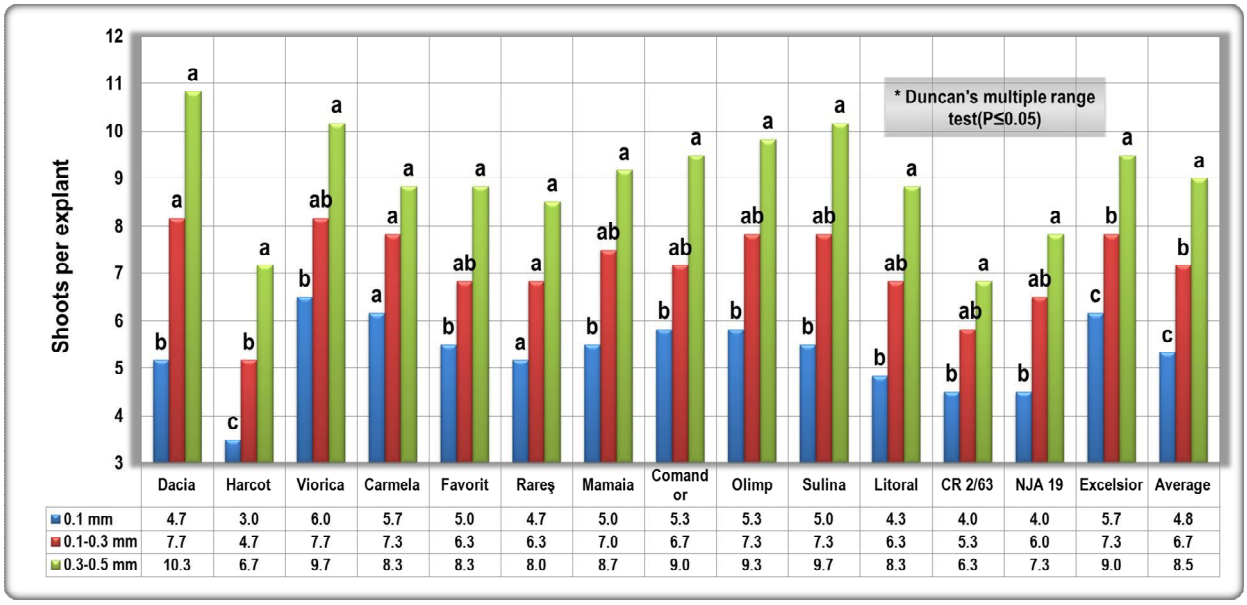


Fig. 3 *In vitro* multiplication rate variation (shoots per explant) according to the cultivar for different nutritive media

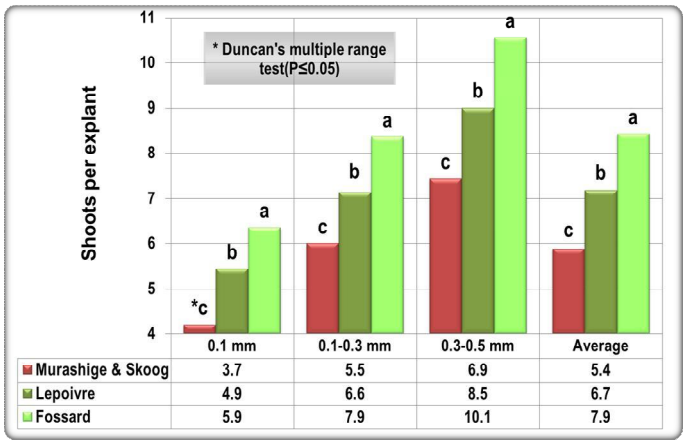


Fig. 4 *In vitro* multiplication rate variation (shoots per explant) according to the nutritive media for different explant dimensions

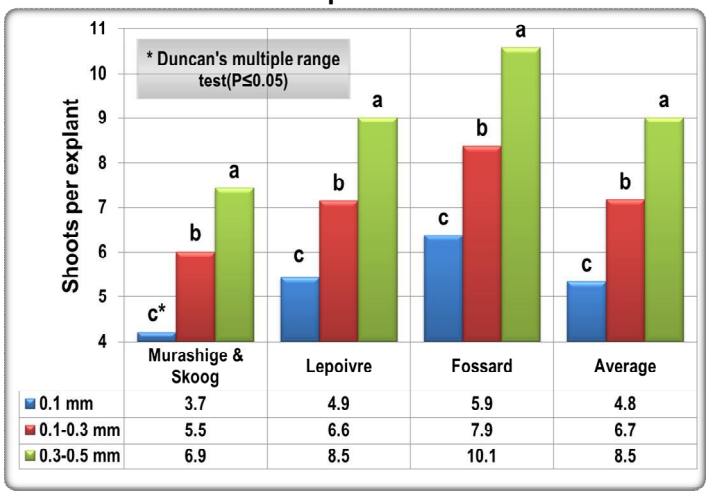


Fig. 5 *In vitro* multiplication rate variation (shoots number per explant) according to the explants dimension for different nutritive media

Fig. 6 'Viorica' cv. on multiplication media (original)

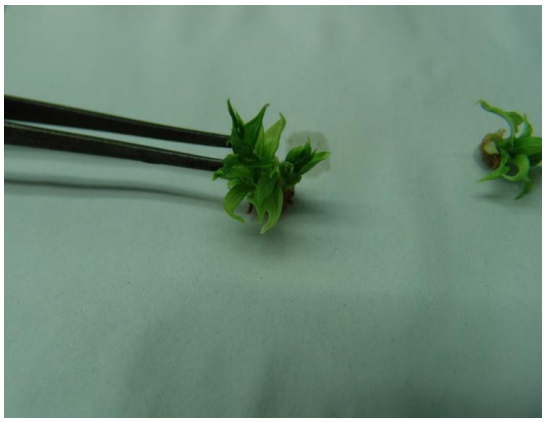


Fig. 7 Separation of the apricot shoots in multiplication phase (original)

Fig. 8 Apricot explants 2 weeks after passage on multiplication media (original)

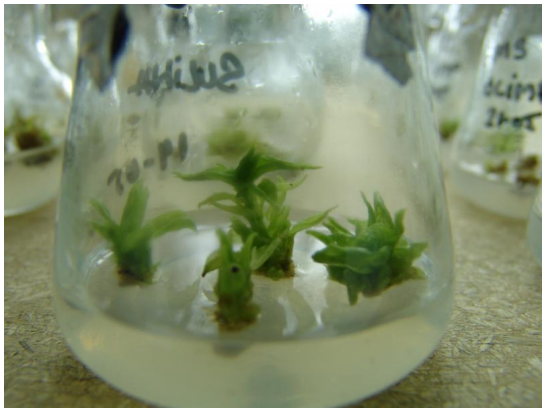


Fig. 9 'Sulina' cv. 4 weeks after passage on multiplication media (original)