CULTURE MEDIUM AND THEIR DILUTIONS ON THE IN VITRO MULTIPLICATION OF ROOTSTOCKS OF PLUM TREE 'MYROBALAN 29C' AND 'MARIANNA 2624'

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1. INTRODUCTION

Micropropagation of plum rootstocks plays a critical role in breeding programs, commercial-scale propagation, and germplasm conservation (PLOPA et al., 2012; MONTICELLI et al., 2017). Among the most widely adopted *Prunus* rootstocks are 'Marianna 2624' (*Prunus cerasifera* × *P. munsoniana*) and 'Myrobalan 29C' (*P. cerasifera*), which are favored for their vigor, adaptability to different soil types, and broad graft compatibility with commercial plum cultivars (MONTICELLI et al., 2017). The *in vitro* multiplication of these genotypes remains challenging due to genotype-specific responses and the high sensitivity of morphogenic outcomes to culture medium composition and ionic strength (PLOPA et al., 2012).

Woody species are typically propagated using basal media such as Murashige and Skoog (MS) (MURASHIGE & SKOOG, 1962), Driver and Kuniyuki Walnut (DKW) (DRIVER & KUNIYUKI, 1984), or Woody Plant Medium (WPM) (LLOYD & MCCOWN, 1980). However, full-strength MS has been associated with osmotic stress, hyperhydricity, and reduced shoot elongation in salt-sensitive plum genotypes like Myrobalan 29C (KULUS & ZALEWSKA, 2022). To mitigate these effects, dilution of the basal medium (e.g., ½ or ¾ MS) has been proposed and shown to improve shoot proliferation, reduce physiological abnormalities, and enhance rooting and survival rates (PLOPA et al., 2012).

Given these considerations, this study investigates the effects of different basal media and their dilutions on the *in vitro* multiplication efficiency of 'Marianna 2624' and 'Myrobalan 29C', with the goal of optimizing shoot proliferation protocols for commercial application.

2. METHODOLOGY

Shoots of the plum rootstocks 'Marianna 2624' and 'Myrobalan 29C' provided the plant material. These plants had previously been grown in a medium DKW (DRIVER & KUNIYUKI, 1894), to which 0.2 mg L^{-1} of 6-benzylaminopurine (BAP) was added, and pH 5.6. Every shoot was normalized according to its length (2.5 cm) and inoculated at a 45° angle to the medium's surface. Different culture media were tested in this study, including DKW, full-strength MS powder medium, ¾-strength MS powder medium, full strength and 50% of MS solution medium. All media were supplemented with 0.6 mg L^{-1} BAP, 0.2 mg L^{-1} gibberellic acid (GA_3), and 0.015 mg L^{-1} indole-3-butyric acid (IBA). The pH of the media was adjusted to 5.6 before autoclaving.

Several morphological parameters were assessed at the conclusion of the multiplication phase in order to assess the effects of various culture mediums and their dilutions. These included the height of the main shoot (cm), the height of newly generated shoots (cm), the number of new shoots per explant, the number of leaves per shoot, and the fresh weight (g) of the plantlets. Data was recorded to evaluate shoot proliferation and overall growth performance, and all measurements were made in an aseptic environment.

Each treatment had four repetitions, each represented by a flask holding four explants, and the experimental design was fully randomized, using a 2x4 factorial (2 rootstocks x 4 different culture media) with 4 replicates were examined and then the analysis of variance was conducted. The Tukey test was used with Sisvar 5.6 software when the data were significant (FERREIRA, 2019).

3. RESULTS AND DISCUSSION

Based on the results of the analysis of variance, Explants' fresh weight varied considerably depending on their growing medium and genotype, without interaction between factors (Table 1). In full-strength MS, ‘Myrobalan 29C’ produced the maximum biomass accumulation (7.56 g), whereas ‘Marianna 2624’ had the lowest in MS ¾ (2.03 g). According to GÜNEY (2019), ‘Myrobalan 29C’ cultivated on full-strength MS supplemented showed enhanced shoot biomass. Similar to this, BORKOWSKA (2001) showed that increased salt content in MS can increase biomass but can also cause physiological stress if it persists, highlighting the significance of genotype-specific optimization.

Table 1. Effect of culture medium and their dilutions on fresh mass of 'Myrobalan 29C' (Myr-29C) and 'Marianna 2624' (M-2624) rootstocks grown in vitro

Fresh Mass (g)						
	Culture medium					
Cult.	DKW	MS Full	MS 50%	MS P. Full	MS ¾	Average
'My29C'	4.30 ± 0.47	7.56 ± 0.68	4.57 ± 0.51	3.91 ± 1.00	2.91 ± 0.55	4.65 ± 0.42 a
'M2624'	3.02 ± 0.31	5.14 ± 0.27	3.57 ± 0.13	2.83 ± 0.24	2.03 ± 0.23	3.32 ± 0.23 b
Average	3.66 ± 0.34BC	6.35 ± 0.53A	4.07 ± 0.30B	3.37 ± 0.52BC	2.47 ± 0.32C	
CV (%)	28.28					

Means ± standard error (n=5) followed by different letters, uppercase in the row and lowercase in the column, differ from each other by the Tukey Test, at 5% probability *(Pÿ0.05);* CV (%) – Coefficient of Variation; Cult. – Cultivars.

Except for fresh mass, all other variables showed significant interactions between the factors. ‘Myrobalan 29C’ performed at its best in MS ¾, while ‘Marianna 2624’ showed the most shoot proliferation in MS P. Full, with every explant generating shoots. In comparison, ‘Marianna 2624’ (MS ¾) produced only 42.7% of the maximum on the lowest-performing medium, while ‘Myrobalan 29C’ (DKW) produced 40.6%. This study aligns with those of DAL ZOTTO & DOCAMPO (1997), who used MS medium to report ~1.6 shoots per explant in ‘Marianna’ 2624, and RADMANN et al. (2009), who demonstrated that cytokinin type and medium strength have a substantial impact on shoot initiation in peach rootstocks.

‘Marianna 2624’ reached its maximal elongation in DKW and MS P. Full for main shoot height, whilst ‘Myrobalan 29C’ exhibited maximum in MS Full. The treatments that performed the worst (MS 50%) were less than 55% of the maximum in both genotypes. This supports earlier research by GÜNEY (2019) and

GAMBORG et al. (1976), who showed that elongation can be encouraged by mild salt dilution without resulting in hyperhydricity.

The highest number of newly formed lateral shoots for ‘Marianna 2624’ was recorded in MS P. Full, whereas the lowest medium (MS ¾) only reached 19.6% of this value. The highest performance of ‘Myrobalan 29C’ was observed in MS Full, with a maximum DKW of 88.5%. These findings are consistent with those of RADMANN et al. (2009), who discovered that elongation increased when shoots were moved to hormone-free medium following multiplication. This suggests that genotype-specific responses depend on the strength of the medium.

Patterns of leaf formation were comparable. While ‘Myrobalan 29C’ attained maximum in MS ¾, the maximum leaf number was noted for ‘Marianna’ 2624’ in MS P. Full. ‘Marianna 2624’ and ‘Myrobalan 29C’ yielded only 42–48% and 40% of the maximum, respectively, of the weakest treatments. These findings correlate with those of BORKOWSKA (2001) and GAMBORG et al. (1976), who noted that leaf production can be favorably impacted by diluted MS media and lower ammonium nitrate contents.

Table 2 Effects of different culture media and their dilutions on in vitro growth parameters of 'Myrobalan 29C' (Myr-29C) and 'Marianna 2624' (M-2624) rootstocks

Nº of New shoots per explants					
		Culture medium			
Cult.	DKW	MS Full	MS 50%	MS P. Full	MS ¾
'My29C'	2.32 ± 0.29 Aa	4.88 ± 0.80 Aa	4.40 ± 1.20 Aa	3.04 ± 1.12 Ab	5.72 ± 1.19 Aa
'M2624'	3.35 ± 0.35 Aa	4.65 ± 0.49 Aa	3.90 ± 0.40 Aa	5.85 ± 0.74 Aa	2.50 ± 0.36 Ab
CV (%)			26.82		
Height of Main shoot (cm)					
Cult.	DKW	MS Full	MS 50%	MS P. Full	MS P. ¾
'My29C'	3.04 ± 0.16 Abb	3.42 ± 0.12 Aa	2.67 ± 0.14 Ba	2.38 ± 0.17 Bb	3.40 ± 0.26 Aa
'M2624'	3.56 ± 0.20 Aa	2.01 ± 0.20 Bb	1.75 ± 0.11 Bb	3.56 ± 0.09 Aa	1.92 ± 0.19 Bb
CV (%)			13.80		
Height of New shoot (cm)					
Cult.	DKW	MS Full	MS 50%	MS P. Full	MS ¾
'My29C'	1.61 ± 0.16 Ab	1.82 ± 0.06 Ab	1.21 ± 0.34 Ab	1.02 ± 0.24 Ab	1.35 ± 0.14 Aa
'M2624'	5.32 ± 0.60 Ba	4.95 ± 0.68 Ba	4.08 ± 0.31 Ba	8.38 ± 0.91 Aa	1.64 ± 0.30 Ca
CV (%)			32.38		
Nº of Leaves per shoot					
Cult.	DKW	MS Full	MS 50%	MS P. Full	MS ¾
'My29C'	21.64 ± 5.28 Ba	32.32 ± 2.89 ABa	28.88 ± 7.18 Ba	25.88 ± 5.82 Bb	53.88 ± 9.07 Aa
'M2624'	33.30 ± 3.11 Aba	29.32 ± 1.73 ABa	26.18 ± 2.06 ABa	47.34 ± 4.53 Aa	20.28 ± 2.32 Bb
CV (%)			20.03		

Means ± standard error (n=5) followed by different letters, uppercase in the row and lowercase in the column, differ from each other by the Tukey Test, at 5% probability *(Pÿ0.05);* CV (%) – Coefficient of Variation; Cult. – Cultivars.

4. CONCLUSION

This study demonstrated that the in vitro multiplication of ‘Myrobalan 29C’ and ‘Marianna 2624’ is significantly influenced by the type of media and the concentration of nutrients. Depending on the genotype, diluted MS media (¾ and P. Full) enhanced shoot proliferation and leaf development, whereas MS full medium

encouraged greater biomass. For plum rootstock micropropagation to be effective, media strength and growth regulator combinations must be optimized**.**

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