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# SELECTION OF NUTRIENT MEDIUM AND GROWTH REGULATORS IN IN VITRO MICROCLONAL PROPAGATION OF PLUM ROOTSTOCK MYROBALAN 29C (PRUNUS CERASIFERA.L)

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**Abstract:** This study focuses on the in vitro microclonal propagation of the plum rootstock *Myrobalan 29C (Prunus cerasifera L.)*, emphasizing the effectiveness of different nutrient media and growth regulators. The experimental results revealed that the optimized nutrient medium protocol ensured high explant viability and vigorous development, resulting in an increased number and length of shoots. Microclones were successfully obtained using phytohormones in Murashige and Skoog (MS) medium. The findings of this research are recommended as an effective approach for large-scale propagation and creation of microclones of *Myrobalan 29C* rootstock.

**Keywords:** Prunus cerasifera.l, subculture, 6-benzylaminopurine, indole 3-butyric acid, number of shoots, shoot length

**Аннотация:** Целью данного исследования было микроклональное размножение подвоя сливы Миробалан 29С (Prunus cerasifera.l) in vitro с акцентом на эффективность эксплантатов на различных питательных средах и регуляторах роста. Результаты эксперимента показали высокую жизнеспособность и развитие эксплантатов при оптимальном режиме питательной среды, а также высокую длину и количество побегов. Микроклоны были успешно получены с использованием фитогормонов на питательных средах Мурасиге и Скуга (МС). Результаты данного исследования рекомендуются в качестве эффективного подхода для массового размножения и создания микроклонов подвоя Миробалан 29С.

**Ключевые слова:** Prunus cerasifera.l, субкультура, 6-бензиламинопурин, индолил-3-масляная кислота, количество побегов, длина

**Annotasiya:** Ushbu tadqiqotda Myrobalan 29C (Prunus cerasifera.l) olxo‘ri payvantagini in vitro sharoitida mikroklonal ko‘paytirishga qaratilgan bo‘lib, eksplantlarni turli xil ozuqa muhitlar va o‘stiruvchi regulyatorlari samaradorligiga e’tibor qaratilgan. Tajriba natijalari optimal ozuqa muhit pratakoli eksplantlarning yashovchanligi va rivojlanishi yuqori darajada bo‘lib, kurtaklar soni va uzunligi yuqori darajada rivojlandi. Murashige va Skoog (MS) ozuqa muhitida fitogormonlar yordamida mikroklonlar muvaffaqiyatli hosil qilindi. Ushbu tadqiqot natijalari Myrobalan 29C payvantagni ommaviy ko‘paytirish va mikroklonlarini yaratish uchun samarali yondashuv sifatida tavsiya etiladi.

**Kalit so‘zlar:** Prunus cerasifera.l, subkultura, 6-benzilaminopurin, indol 3-moy kislotasi, kurtaklar soni, uzunligi.

**Introduction**. The genus *Prunus* includes *Myrobalan (P. cerasifera L.)*, one of the most widely used rootstocks for the propagation of plum, apricot, and other stone fruit species [1]. Its unique features—such as graft compatibility, resistance to nematodes, and environmental adaptability—have made *Prunus* rootstocks popular worldwide for vegetative propagation [2]. Additionally, *Myrobalan*-based rootstocks have been shown to improve survival rates, vegetative growth, yield, nutrient uptake, and fruit quality in both plum [3] and apricot [4] cultivars.

*Myrobalan 29C (Prunus cerasifera L.)* is widely used as a rootstock for plums and apricots because of its adaptability to different soil conditions and strong tolerance [5]. This rootstock is particularly suitable for high-density plum orchards. Moreover, *Myrobalan* has been used as a parental genotype in breeding programs for rootstocks and edible plum cultivars [6]. Many modern plum varieties originate from it due to its superior graft compatibility.

In vitro propagation techniques can improve propagation efficiency, although results may vary depending on the species and environmental conditions. Microclonal propagation is considered one of the most effective methods for developing root systems [7]. Research has shown that *Myrobalan 29C* rootstocks cultured on MS medium supplemented with 0.5 mg/l benzyladenine (BA) demonstrate a high multiplication rate [8]. Further studies reported that the best shoot growth was obtained with 1 mg/l BA, while optimal rooting occurred at 0.1 mg/l IBA [5, 9].

Other studies confirmed that MS medium produced the highest rooting rates in *Myrobalan* rootstocks. Cultures of *Myrobalan* grown on MS medium with BAP and NAA showed better growth and rooting performance [10]. For *Prunus avium* micropropagation, 0.5 mg/l BAP combined with 0.05 mg/l TDZ yielded the best multiplication rate, while 0.3 mg/l IBA was most effective for root induction [7,11]. Previous research indicates that MS or WPM basal media are commonly used for *Prunus* regeneration. Cytokinins such as TDZ and BA are the most frequently used, while IBA acts as an effective auxin for root induction [12]. According to several studies, plantlets acclimatized after 6 weeks of in vitro culture exhibited a survival rate of 76–84% under greenhouse conditions [13,14].

Therefore, developing an efficient microclonal propagation protocol for *Myrobalan 29C* is essential for large-scale rootstock production. The main goal of this study was to determine the optimal nutrient medium and growth regulator combinations for the microclonal propagation of *Myrobalan 29C*.

**2. Materials and Methods.**

***2.1. Explant Sterilization:*** Healthy meristem tissues of *Myrobalan 29C* rootstock were selected as explants. Sterilization was performed according to the Butenko method [15], which produced good results when applied to the selected plant material. The sterilization process consisted of the following stages:

Washing in tap water for 30 minutes, Immersion in soapy water (40–45°C) for 1–10 minutes, Rinsing three times with sterile distilled water. Immersion in 70% ethanol ($C_2H_5OH$) for 1.5 minutes. Treatment in 1% sodium hypochlorite solution (NaOCl, Bio Chloractive) for 10 minutes. Immersion in 0.1% silver nitrate ($AgNO_3$) solution for 3 minutes, followed by treatment with ascorbic acid. Treatment with 0.5% hydrogen peroxide ($H_2O_2$) solution for 1 minute. After each chemical treatment, the explants were rinsed several times with sterile distilled water [16].

***2.2. Nutrient Media Types:*** For the in vitro culture of *Myrobalan 29C* lateral buds, three types of media were tested: Murashige and Skoog (MS, 1962), Woody Plant Medium (WPM, Lloyd & McCown, 1980), and Driver & Kuniyuki Walnut Medium (DKW, 1984). Each medium contained specific compositions of macro- and microelements, as well as vitamin complexes (Table 1).

Plant growth regulators used were 6-benzylaminopurine (BAP) and indole-3-butyric acid (IBA) at various concentrations. The cultures were subcultured three times, each lasting 28 days. After every subculture, the total number of shoots, shoot length, and shoot quality were measured.

This stage was critical for determining the most effective nutrient medium and growth regulator combination for *Myrobalan 29C* proliferation.

| Components | Murashige & Skoog (1962). | Lloyd & McCown (1980) | Driver & Kuniyuki (1984) |
|---|---|---|---|
| *Macro elements, mg/l* | | | |
| $CaCl_2$ | 332.02 | 72.5 | 112.5 |
| $Ca(NO_3)_2x4H_2O$ | 0 | 471.26 | 1664.64 |
| $KH_2PO_4$ | 170 | 170 | 265 |
| $K_2SO_4$ | 0 | 990 | 1559 |
| $KNO_3$ | 1900 | 0 | 0 |
| $MgSO_4$ | 180.54 | 180.54 | 361.49 |
| $NH_4NO_3$ | 1650 | 400 | 1416 |
| *Micro elements, mg/l* | | | |
| $CuSO_4x5H_2O$ | 0.025 | 0.25 | 0.25 |
| $CoCl_2x6H_2O$ | 0.025 | 0 | 0 |
| FeNaEDTA | 36.7 | 36.7 | 44.63 |
| $H_3BO_3$ | 6.2 | 6.2 | 4.8 |
| KJ | 0.83 | 0 | 0 |
| $MnSO_4xH_2O$ | 16.9 | 22.3 | 33.8 |
| $Na_2MoO_4x2H_2O$ | 0.25 | 0.25 | 0.39 |
| $ZnSO_4x7H_2O$ | 8.6 | 8.6 | 17 |
| *Vitamins, mg/l* | | | |
| Glycine | 2 | 2 | 2 |
| Myo-inositol | 100 | 100 | 100 |
| Nicotinic acid ($B_3$) | 0.5 | 0.5 | 1 |
| Pyridoxine HCl ($B_6$) | 0.5 | 0.5 | 0 |
| Thiamine HCl ($B_1$) | 0.1 | 1 | 2 |
| ***Total :*** | ***4405.19*** | ***2462.6*** | ***5584.5*** |

**Table 1. Composition of MS, WPM, and DKW nutrient media for Myrobalan 29C micropropagation**

**3.Results.** The experiments conducted to study the effects of nutrient media and phytohormones on *Myrobalan 29C* explants focused on evaluating parameters such as shoot induction, growth, development, viability, and adaptability. Explants were cultured on three different nutrient media (MS, DKW, and WPM) using four BAP concentrations (0.5–2.0 mg/l), each replicated four times for 28 days. The results of each variant are summarized and analyzed below (Table 2).

**Table 2. Effect of nutrient media and BAP concentration on the proliferation of Myrobalan 29C**

| Nutrient medium | BAP concentration mg/l | Average number of shoots per explant | Average shoot length (cm) |
|---|---|---|---|
| MS | 0.5 | 1.7 | 1.10 |
| | 1 | 1.7 | 1.53 |
| | 1.5 | 2.75 | 0.93 |
| | 2 | 3.25 | 0.75 |
| DKW | 0.5 | 2.25 | 1.10 |
| | 1 | 2.25 | 0.98 |
| | 1.5 | 2.75 | 0.73 |
| | 2 | 2 | 0.83 |
| WPM | 0.5 | 2 | 0.70 |
| | 1 | 2.25 | 0.78 |
| | 1.5 | 2.25 | 0.60 |

| | | | |
|---|---|---|---|
| | 2 | 3.25 | 0.38 |
| | LSD $_{BAP}$ | 1.2* | N.S. |

*LSD for BAP = 1.2* ($p < 0.05$); *N.S.* = Not significant.

LSD (Least Significant Difference) test was used to determine statistical significance at $P < 0.05$ and $P < 0.01$. N.S. indicates no significant difference among comparisons.

**Effect of nutrient media.** As shown in Table 1, the composition of the nutrient media, particularly the presence of 6-benzylaminopurine (BAP) andindole-3-butyric acid (IBA), played a crucial role in shoot formation. Propagation was performed in three nutrient media - Murashige and Skoog (MS), Woody Plant Medium (WPM), and Driver & Kuniyuki (DKW) - using BAP and IBA at concentrations of 0.5, 1.0, 1.5, and 2.0 mg/l.

Explants grown on MS and DKW media exhibited faster shoot elongation, thicker stem formation, and better shoot vigor compared to WPM. The increase in BAP concentration enhanced the number of new shoots and improved explant proliferation.

The highest shoot growth was observed in MS medium containing 1 mg/l BAP and 0.1 mg/l IBA (Figure 1). However, at the same BAP concentration (1 mg/l), the total number of newly formed shoots was lower compared to higher concentrations (Figure 2). The pH level of the media (5.6–5.8) and sucrose content (30 g/l) were optimal for cell division and growth.

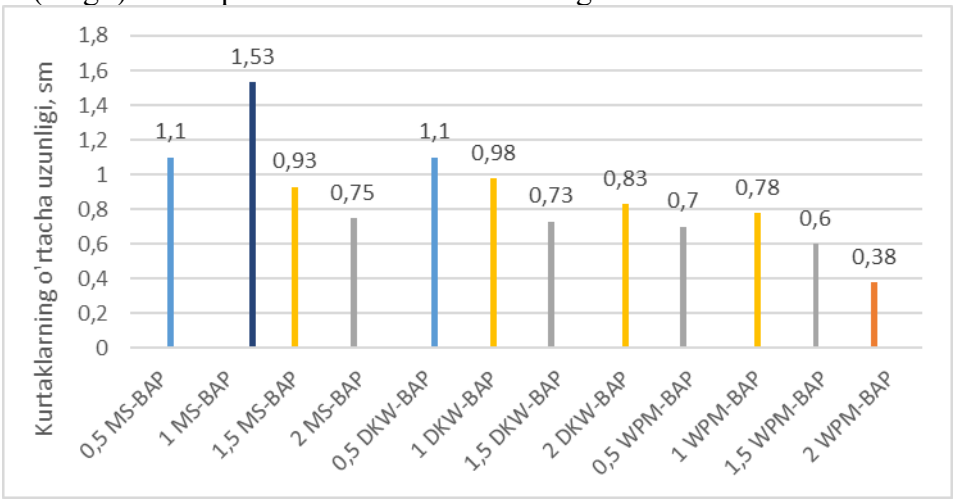



**Figure 1. Average shoot length (cm) of new shoots formed under different media and BAP concentrations.**

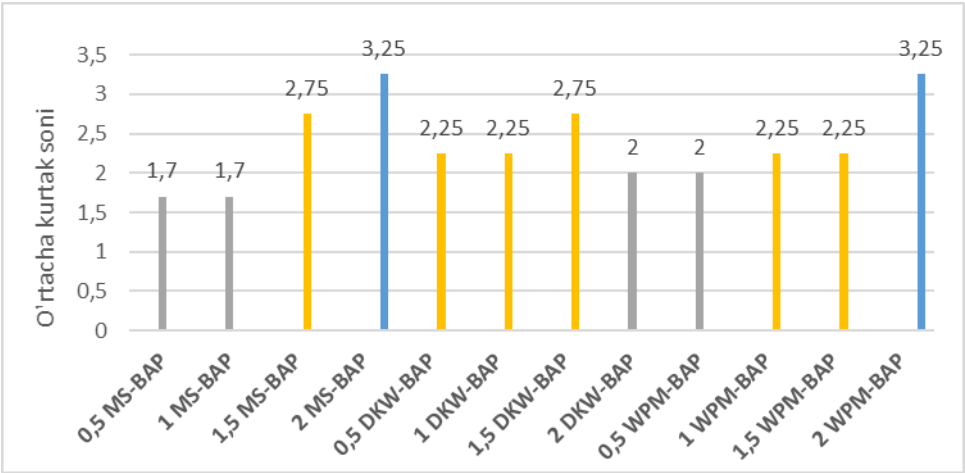



**Figure 2. Average number of shoots per explant under different nutrient media and BAP concentrations.**

**Viability and Development.** Explants cultured on the three media (four variants, four replications) showed a survival rate of 95–98% during the subsequent subculture stage. Over a period of 21–28 days, shoot length, number, and quality were monitored regularly. Culture conditions were

maintained at 23–24°C temperature, 58–60% humidity, and a photoperiod of 16 hours light and 8 hours dark with 2500 lux illumination.

**4.Discussion.** The obtained results demonstrated that sterilization and in vitro cultivation of *Myrobalan 29C* explants were highly effective in all three media tested. Although explants grew well in MS, WPM, and DKW media, the Murashige and Skoog (MS) medium produced the most vigorous and high-quality plantlets. The influence of 1–2 mg/l BAP in MS medium resulted in a significantly higher number and length of new shoots compared to other treatments (Figure 3).

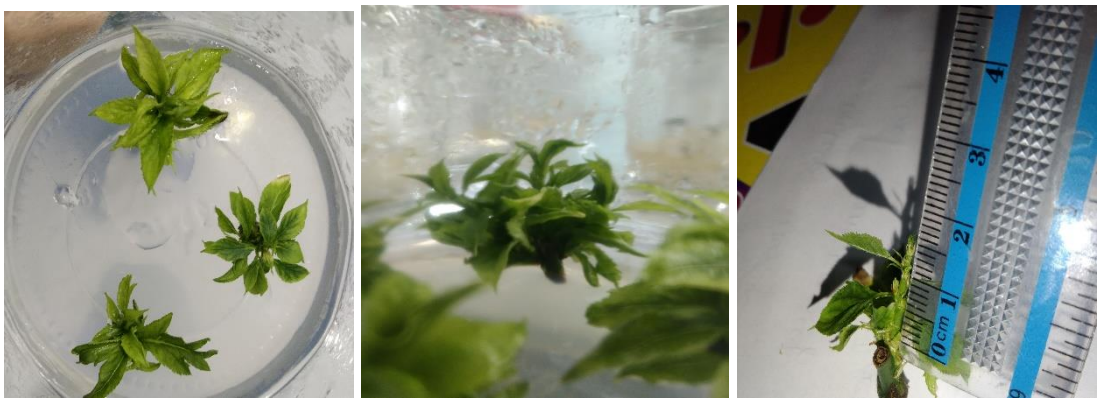


**Figure 3. *In vitro* cultured explants of *Myrobalan 29C*.**

Data were analyzed using the JMP statistical software, and differences between treatments were compared using the LSD (Least Significant Difference) test. Shoot multiplication efficiency was significantly influenced by the growth regulators and the nutrient medium composition at $p < 0.05$.

This study revealed that increasing the BAP concentration resulted in a proportional increase in the number of shoots. A concentration of 2 mg/l BAP provided the optimal multiplication rate, while higher concentrations suppressed shoot formation [17]. Similar findings were reported for *Prunus mitti* rootstocks, where 1.5 mg/l BAP increased proliferation by 50% [18,19]. The addition of cytokinins to the culture medium stimulated cell division and enhanced morphogenetic activity [20]. However, it has been suggested in some studies that auxins can regulate cytokinin biosynthesis by inhibiting its synthesis and reducing overall cytokinin levels [21]. According to various researchers, increasing the amount of exogenous cytokinins may elevate endogenous auxin concentrations, possibly because exogenous cytokinins inhibit free IAA conjugate formation [22].

The ratio of auxins to cytokinins plays a crucial role in determining cell fate and organ formation. This hormonal balance influences plant morphogenesis by either stimulating or suppressing shoot formation.

Auxins activate cell division and promote meristem formation, which can lead to organ initiation or, under certain imbalances, uncontrolled tissue growth.

High BAP concentrations, however, may cause leaf necrosis and weak shoot elongation [13]. Among the tested media, MS medium contained higher levels of chloride ($Cl^-$) and calcium ($Ca^{2+}$) compared to WPM and DKW. These elements are known to improve plant structural integrity and physiological stability, which may explain the superior results obtained with MS medium.

**Conclusion.** During this study, an optimal in vitro propagation protocol for the *Myrobalan 29C* plum rootstock was developed. The microclones demonstrated relatively rapid growth and development when cultured on MS medium. MS medium, supplemented with phytohormones, enabled the formation of well-developed microclones within 26–28 days.

This protocol can be considered a reliable and efficient method for the large-scale microclonal propagation of *Myrobalan 29C* rootstock. It provides a consistent basis for mass production and genetic stability of propagated plantlets, making it suitable for commercial propagation and breeding programs.

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