Anadolu, J. of AARI
ISSN:1300-0225 (Print)
E-ISSN: 2667-6087 (Online)
2024, 34 (1):90-96
DOI: 10.18615/anadolu.1494423

Comparative Biplot Analysis of Micropropagation of Viking Aronia Cultivar in Different Plant Tissue Culture Media

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Received (Geliş tarihi): 02.06.2024 Accepted (Kabul tarihi): 25.06.2024

ABSTRACT: *In this study, the effect of different concentrations of some plant growth regulators on the in vitro micropropagation of the Viking aronia variety was investigated. In this context, 15 different culture media were prepared by adding plant growth regulators (BAP, IBA, GA3, TDZ) in various concentrations and combinations to the MS medium (Murashige and Skoog, 1962). Sterilised shoot tips were transferred to the relevant plant tissue culture media in three replications according to the randomized parcels trial pattern, with three explants in each replication. At the end of the four-week development period, average vitrification rate (VIR), average shoot length (SHL) and average number of nodes (NON) parameters were evaluated. Biplot analysis was employed to analyze the data obtained. According to the biplot analysis, B1I002G1T1: (1.0 mg L^{-1} BAP+0.02 mg L^{-1} IBA+1.0 mg L^{-1} GA_3+1.0 mg L^{-1} TDZ), B1I002G1T05: (1.0 mg L^{-1} BAP+0.02 mg L^{-1} IBA+1 mg L^{-1} GA_3+0.5 mg L^{-1} TDZ) and B1I002G05T1: (1.0 mg L^{-1} BAP + 0.02 mg L^{-1} IBA+0.5 mg L^{-1} GA_3+1.0 mg L^{-1} TDZ) had a more positive effect on the number of nodes and shoot length. This study showed that the Viking aronia variety can be easily grown from cuttings by adjusting the plant growth regulator concentrations and combinations. Different media and plant growth regulators are needed for each aronia variety, so more studies are needed to improve success rates.*

Keywords: *Aronia melanocarpa (Michx.) Elliott, explant, plant growth regulators, plant tissue culture, micropropagation.*

Viking Aronya Çeşidinin Farklı Bitki Doku Kültürü Ortamlarında Mikroçoğaltımının Karşılaştırmalı Biplot Analizi

ÖZ: *Bu çalışmada, bazı bitki büyüme düzenleyicilerinin farklı konsantrasyonlarının Viking aronya çeşidinin in vitro mikro çoğaltımı üzerine etkisi araştırılmıştır. Bu kapsamda, MS ortamına (Murashige and Skoog, 1962) farklı konsantrasyon ve kombinasyonlarda bitki büyüme düzenleyicileri (BAP, IBA, GA3, TDZ) ilave edilerek 15 farklı kültür ortamı kullanılmıştır. Sterilize edilmiş sürgün uçları, her tekerrürde 3 eksplant olacak şekilde, tesadüf parselleri deneme desenine göre üç tekerrürlü olacak şekilde ilgili bitki doku kültürü ortamlarına aktarılmıştır. Dört haftalık gelişme süresi sonunda ortalama vitrifikasyon oranı (VIR), ortalama sürgün uzunluğu (SHL) ve ortalama boğum sayısı (NON) parametreleri değerlendirilmiştir. Elde edilen veriler Biplot analizi ile değerlendirilmiştir. Biplot analizine göre B1I002G1T1: (1,0 mg L^{-1} BAP+0,02 mg L^{-1} IBA+1,0 mg L^{-1} GA_3+1,0 mg L^{-1} TDZ), B1I002G1T05: (1,0 mg L^{-1} BAP+0,02 mg L^{-1} IBA+1 mg L^{-1} GA_3+0,5 mg L^{-1} TDZ) ve B1I002G05T1: (1,0 mg L^{-1} BAP + 0,02 mg L^{-1} IBA+0,5 mg L^{-1} GA_3+1,0 mg L^{-1} TDZ) ortamlarının boğum sayısı ve sürgün uzunluğu üzerine daha olumlu etki gösterdiği tespit edilmiştir. Bu çalışma ile Viking aronya çeşidinin mikroçoğaltımının farklı bitki büyüme düzenleyici konsantrasyonları ve kombinasyonları optimize edilerek kolaylıkla gerçekleştirilebileceği belirlenmiştir. Ancak her aronya çeşidinin mikroçoğaltımında farklı kültür ortamına ve bitki büyüme düzenleyicilerine ihtiyaç duyulması başarı oranlarının artırılmasında daha fazla çalışma yapılmasını gerektirmektedir.*

Anahtar kelimeler: *Aronia melanocarpa (Michx.) Elliott, eksplant, bitki büyüme düzenleyicileri, bitki doku kültürü, mikroçoğaltım.*

INTRODUCTION

The rapid increase in the world population and the ever-developing and expanding global economy are placing increasing pressure on natural resources. It is therefore of great importance to use existing resources in the optimum and most efficient way. Properly establishing an orchard and complying with technical principles in fruit growing is of great importance for profitable production (Sattler and Nagel, 2010; Kunzekweguta *et al.*, 2017). It is well established that fruits, particularly berries, are rich in natural antioxidants and possess a high antioxidant capacity and anthocyanin content.

The rise in global consumption levels over recent years has elevated the significance of berries in both domestic and international trade (Yang and Kortesniemi, 2015; Golovinskaia and Wang, 2021; Kırmızıkuşak, 2023). Studies have shown that there is an increasing trend towards the consumption of Aronia [*Aronia melanocarpa* (Michx.) Elliott], a type of fruit rich in natural antioxidants (Kapci *et al.*, 2013; Bolling *et al.*, 2015; Jurikova *et al.*, 2017; Vagiri *et al.*, 2017; Çulhacı and Yıldırım Yalcın, 2022; Gao *et al.*, 2024).

Aronia, belonging to the *Rosaceae* family, is categorized under the Aronia genus, which encompasses three acknowledged species (Kulling and Rawel, 2008). These species include *Aronia melanocarpa* (Michaux) Elliot (black chokeberry), *Aronia prunifolia* (Marshall) Rehder (purple chokeberry), and *Aronia arbutifolia* (Linnaeus) Persoon (red chokeberry). Aronia berries (*Aronia melanocarpa*) are rich in various bioactive compounds such as anthocyanins, carotenoids, phenolic compounds, fatty acids, flavonoids and vitamins (Rauf *et al.*, 2019; Shahin *et al.*, 2019; Lackner *et al.*, 2024). Aronia berries originating from the eastern regions of North America have been cultivated and utilized in traditional medicine within the former Soviet Union since the mid-20th century (Fidancı, 2015; Yilmaz *et al.*, 2021; Kadıoglu and Yilmaz, 2023).

Presently, aronia is grown in Eastern European nations and Russia, where it is utilized in the crafting of homemade or commercial juices, preserves, fruit infusions, wines, and organic food dyes (Jurikova *et al.*, 2017; Mertoglu *et al.*, 2021). A study of the effects of aronia (*Aronia melanocarpa*) on human health revealed that its fruits have the highest antioxidant capacity and anthocyanin amount of all berries (Sharif *et al.*, 2012; Szopa *et al.*, 2017). It has been demonstrated that regular consumption of this type of fruit provides protection against cardiovascular diseases, digestive system diseases and some cancers (Daskalova *et al.*, 2019). Aronia berries have been accepted as medicinal plants in Russia due to their biochemical properties, which have been the subject of extensive research (Martin *et al.*, 2014; Bakır, 2019). Aronia fruits are designated as functional foods due to their high antioxidant activity, and their use and cultivation are becoming increasingly prevalent across the globe. Aronia has gained importance due to its bioactivity, which is evidenced by the presence of dietary fibre, organic acids, sugar, fat, protein, minerals and vitamins, antioxidants, anthocyanins, flavonoids, proanthocyanidins, flavanols, phenolic acids and flavonol polyphenols (Chrubasik *et al.*, 2010).

Plant tissue culture techniques are employed extensively in the propagation and breeding of perennial plants. However, due to the problem of nonviable seeds, there is a need for tissue culture techniques that can increase the propagation rate by using explants taken from various parts of the plant with the help of plant growth regulators, especially for shoot and root induction (Kaya *et al.*, 2023). Although numerous studies have yielded promising results when employing plant tissue culture techniques on diverse perennial plant species, there is a paucity of literature addressing the optimization of propagation protocols and the advancement of biotechnology research (Singh, 2015; Dogan and Emsen, 2018).

Auxins, gibberellins, cytokinins and indole butyric acid group phytohormones, also known as plant growth regulators, are employed in isolation or in various combinations for specific purposes in plant tissue culture studies (Kumlay and Eryigit, 2011).

Aronia, a plant species cultivated in Türkiye, has gained popularity in recent years. It thrives particularly well in the climate and soil conditions of the Black Sea region (Yilmaz, 2020). Although not naturally found in Türkiye's flora, aronia has been widely introduced through agricultural production projects and government incentives (Demir, 2019). The production process has accelerated with the education and support provided to farmers, yielding productive results (Kaya, 2021). In terms of consumption, aronia berries are noted for their high antioxidant content and health benefits (Özturk, 2020). They are consumed in various forms such as juice, jam, dried fruit, and dietary supplements, making aronia a valuable product in both domestic and export markets (Çelik, 2022). Consequently, while aronia production in Türkiye is

rapidly increasing, consumption rates are also rising in parallel (Aydin, 2023).

The aim of this study was to investigate the effects of different levels of plant growth regulator combinations in *in vitro* plant tissue culture environment on aronia, which is newly known in Türkiye but has a high economic importance with different usage areas around the world.

MATERIAL AND METHOD

Plant material

In the study, shoot tips of the Viking variety *(Aronia melanocarpa)* were employed as explant sources during the active development period between April and May. The plant materials were obtained from four-year-old healthy plants cultivated in accordance with the ecological conditions of Çanakkale.

Surface sterilization of explants

The collected shoot tips were transferred to the laboratory and subjected to a preliminary cleaning under tap water. During the sterilization phase, explants were subjected to sterilization procedures developed by Soylu and Erturk (1999). Initially, the shoot tips were washed under running water for a period of five minutes. Thereafter, the explants were immersed in a 3% $CuSO_4$ solution for a duration of 20 minutes, with intermittent agitation, and subsequently in a 70% ethyl alcohol solution for a period of one minute. Subsequently, the explants were rinsed with sterile pure water and placed in a solution containing 15% (v/v) NaOCl and a few drops of Tween 20. After a 15-minute incubation period, the explants were rinsed with sterile pure water three times for 5 minutes each, and the surface sterilization process was completed.

Contents of plant tissue culture media

A total of 15 different propagation media (Table 1) were employed in the study. The plant growth regulator substance combinations utilised in the trial for the propagation media are presented in Table 1. Following sterilization, the explants were excised from the upper and lower parts of the bud, leaving a 0.5-0.7 cm segment, and planted in the culture media so that the bud remained on the surface. In the initial culture phase, Murashige and Skoog (MS; M-5524, Sigma) culture media was employed, with the addition of 30 g/L sucrose and 7 g/L agar. The pH of the culture media was adjusted to 5.8 using 0.1 N HCl and 0.1 N NaOH. The prepared media were then placed on the heater until they reached boiling point (92°C), after which they were decanted (approximately 15 ml) into 75x75x100 mm magenta boxes and sterilised for 15 minutes at 121°C at 1.2 atm pressure. After planting, the magenta boxes were cultured for four weeks at 25±1°C, under a 3000 lux fluorescent lamp with a 16-hour photoperiod. The following parameters were evaluated at the end of the four-week development period. The protocol stated by Şengül (2012) was followed during the preparation of the culture media.

The experiment was set up according to the randomized parcels trial pattern, with three replications and three explants in each replication. Biplot analysis (SAS Institute Inc. JMP 15.1, 2020) was employed for data interpretation, and the findings were visualized on the graphs (Figure 1).

Features examined within the scope of the study

Vitrification rate (VIR - %): The number of vitrified plants in each magenta box was counted and expressed as a percentage.

Number of nodes (NON - piece): The number of nodes in each shoot was quantified and averaged.

Shoot length (SHL - mm): At the end of the development phase, shoot lengths were quantified in millimeters using caliper.

Table 1. Plant tissue culture media contents used in the study.#
Çizelge 1. Çalışmada kullanılan bitki doku kültürü ortam içerikleri.#

Culture Media	Media Content				
No/Code	IBA mg L^{-1}	BAP mg L^{-1}	GA_3 mg L^{-1}	TDZ mg L^{-1}	Media
1. B1I001GYTY	0.01	1.0	-	-	MS
2. B1I001GYT05	0.01	1.0	-	0.5	MS
3. B1I001GYT1	0.01	1.0	-	1.0	MS
4. B05I001G05TY	0.01	0.5	0.5	-	MS
5. B05I001G05T05	0.01	0.5	0.5	0.5	MS
6. B05I001G05T1	0.01	0.5	0.5	1.0	MS
7. B1I002G05TY	0.02	1.0	0.5	-	MS
8. B1I002G05T05	0.02	1.0	0.5	0.5	MS
9. B1I002G05T1	0.02	1.0	0.5	1.0	MS
10. B1I002G1TY	0.02	1.0	1.0	-	MS
11. B1I002G1T05	0.02	1.0	1.0	0.5	MS
12. B1I002G1T1	0.02	1.0	1.0	1.0	MS
13. B2I002G05TY	0.02	2.0	0.5	-	MS
14. B2I002G05T05	0.02	2.0	0.5	0.5	MS
15. B2I002G05T1	0.02	2.0	0.5	1.0	MS

#: IBA: Indol Butyric Acid, BAP: 6-Benzylaminopurine, TDZ: Thidiazuron, MS: Murashige and Skoog Media.

RESULTS AND DISCUSSION

Within the scope of the study, the components of plant tissue culture media are given in Table 1. Upon examination of the study results, it was found that as BAP concentrations increased, the shoot length parameter exhibited a corresponding increase. The results obtained within the scope of the study were evaluated through biplot analysis. Biplot analysis is a technique that facilitates the visualization of multiple variables on the same graph. This method is commonly employed to comprehend, visualize, and interpret multidimensional datasets. By representing relationships among variables and the impact of variables on observations within a single graph, biplot analysis serves as a powerful tool for understanding the structure of a dataset.

In the study, the interaction of plant growth regulators BAP (0.01, 0.02 mg L^{-1}), IBA (0.5, 1.0, 2.0 mg L^{-1}), GA_3 (0.5, 1.0 mg L^{-1}), and TDZ (0.5, 1.0 mg L^{-1}) accounted for 86.5% of the variation in PC1 and PC2 scores, explaining the parameters VIR (Vitrification rate), NON (Number of nodes), and SHL (Shoot length).

Parameters B1I002G1T1, B1I002G1T05, B1I002G05T1, B1I001GYT1, B1I001GYT05, and B1I001GYTY (PC1>0) were found to be positively associated with NON and SHL, while parameters B2I002G05T1, B2I002G05T05, B2I002G05TY, B1I002G1TY, B1I002G05T05, B1I002G05TY, B05I001G05TY, B05I001G05T05, and B05I001G05T1 (PC1<0) were positively associated with VIR. This indicated that parameters aligned with PC1>0 were indicative of higher levels of NON and SHL, while those aligned with PC1<0 were indicative of higher levels of VIR. The parameters B1I001GYT1, B1I001GYT05, and B1I001GYTY being negatively associated with VIR (PC1>0, PC2<0) suggested lower values of VIR for these parameters (Fig. 1).

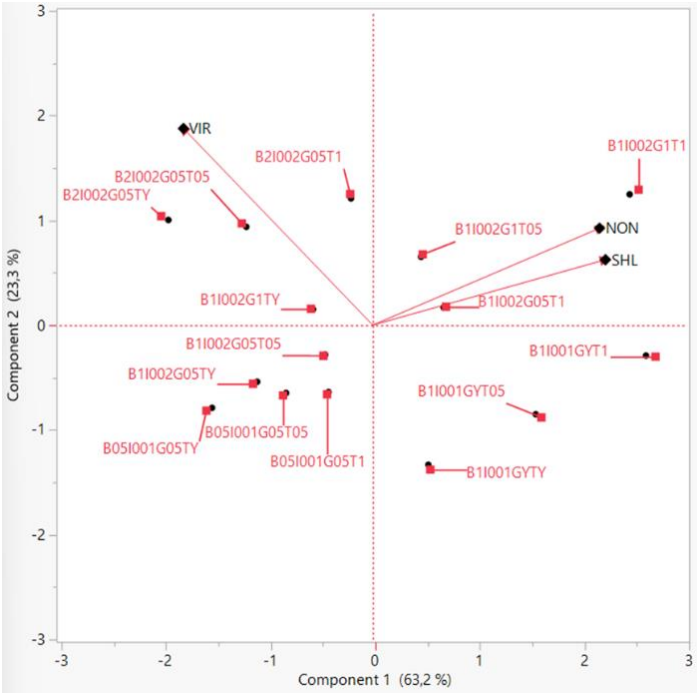

Figure 1. Grouping the effects of plant tissue culture media containing different levels of plant growth regulators on the number of nodes, vitrification rate and shoot length characteristics using the biplot analysis method.
Şekil 1. Farklı düzeylerde bitki büyüme düzenleyicileri içeren bitki doku kültürü ortamlarının boğum sayısı, vitrifikasyon oranı ve sürgün uzunluğu özellikleri üzerindeki etkilerinin biplot analiz yöntemi kullanılarak gruplandırılması.

Parameters B1I001GYT1, B1I001GYT05, and B1I001GYTY, where GA_3 was not applied, were found to be negatively associated with VIR (PC1>0, PC2<0). Conversely, parameters B1I002G1T1, B1I002G1T05, and B1I002G05T1, which involved the application of GA_3 (0.5, 1.0 mg L^{-1}), were aligned with NON and SHL (PC1>0, PC2>0). The graphical representation suggests that the combination of GA_3 with these mentioned hormones in parameters B1I002G1T1, B1I002G1T05, and B1I002G05T1 had a more favorable effect on NON and SHL (Figure 1).

Upon examination of the literature on this subject, researchers reported that shoot length increased with increasing BAP concentration (Güçlü *et al.*, 2010). Some researchers have reported that shoot lengths decrease with increasing BAP concentrations (Sayılır *et al.*, 2007). In general, the vitrification rate increases as explants remain in tissue culture environments. This situation can also be associated with high BAP concentration and environmental conditions, in line with previous information. As a matter of fact, environmental factors such as temperature change, humidity content of the environment and light intensity can be shown to be the causes of vitrification (Yilmaz-Gokdogan and Kaya, 2017). Vitrification is an undesirable physical disorder of *in vitro* tissues, characterised by a glassy, translucent, succulent or wet and often swollen appearance. This is commonly observed in leaves and occasionally in stems. The occurrence and degree of vitrification can be influenced by a multitude of intricate factors. There are numerous reasons for the occurrence of vitrification in micropropagation. These include light intensity, temperature, BAP concentration and the physiological structure of the plant (Özzambak *et al.*, 2018).

CONCLUSION

The propagation of Aronia plants through tissue culture enables the preservation of plant traits and facilitates rapid reproduction. Additionally, it simplifies the production of disease free or resistant plants and provides a significant platform for genetic transformation studies. Moreover, it allows for the cloning of plant material, ensuring stable production. Therefore, tissue culture propagation of Aronia plants

serves as a crucial tool in plant cultivation. The present study examined the effects of different levels plant growth regulators on the micropropagation of the Viking aronia variety under *in vitro* conditions.

According to the graphical representation obtained by biplot analysis, it was determined that the combination of GA_3 with these plant growth regulators mentioned in the parameters B1I002G1T1: (1.0 mg L^{-1} BAP + 0.02 mg L^{-1} IBA + 1.0 mg L^{-1} GA_3 + 1.0 mg L^{-1} TDZ), B1I002G1T05: (1.0 mg L^{-1} BAP + 0.02 mg L^{-1} IBA + 1 mg L^{-1} GA_3 + 0.5 mg L^{-1} TDZ) and B1I002G05T1: (1.0 mg L^{-1} BAP + 0.02 mg L^{-1} IBA + 0.5 mg L^{-1} GA_3 + 1.0 mg L^{-1} TDZ) had a more positive effect on the number of nodes and shoot length.

As a result of the research, it was determined that micropropagation of Viking aronia variety can be easily achieved by optimizing different plant growth regulator concentrations and combinations. As the culture media and plant growth regulators employed in the successful propagation of each aronia variety are distinct, the media to be utilised is not a singular entity. Consequently, further studies should be conducted in order to achieve success in the different aronia varieties.

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