Research Article

Ekin
Journal of Crop Breeding and Genetics

12(2):110-116, 2026

www.ekinjournal.com

Ekin International biannual peer-reviewed journal

In Vitro Regeneration Studies in Turkish Tomato Cultivars(§)

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Citation:
Bahadır S., Yapıcı P., Kavak S., Ellialtıoğlu ŞŞ., 2026. *In Vitro* Regeneration Studies in Turkish Tomato Cultivars. Ekin J. 12(2):110-116.

Received: 22.06.2026 ***Accepted: 26.07.2026*** ***Published Online: 31.07.2026*** ***Printed: 31.07.2026***

ABSTRACT

Tomato is among the most extensively studied plant species in global breeding and genetic research. In recent years, CRISPR-Cas9 technology offers extensive opportunities for integrating traits difficult to transfer through classical breeding methods into qualified genotypes or for generating precise mutations. Furthermore, the regenerative capacity of the studied plant species or genotype in tissue culture is a prerequisite for advancing genetic studies. This study aimed to induce *in vitro* regeneration from cotyledon and hypocotyl explants of four different tomato varieties (PTK-254, PTK-273, Şekerkız, Yelpare 82) developed by Petektar Seed Co. Organogenesis was achieved in MS medium using different doses and combinations of the growth regulators TDZ, BA, IAA, and Zeatin. In this study, where the interaction of nutrient medium and varieties was found to be important, the highest shoot formation was obtained from MS-1, MS-8, MS-5, and MS-6 media containing the Şekerkız variety. These combinations, in which more than 10 shoots were obtained from each explant, were followed by the Yelpare 82, PTK-254, and PTK-273 varieties cultured in MS-1 medium. The combination of 1.0 mg/L Zeatin and 0.1 mg/L IAA (MS-1) provided regeneration in all varieties, albeit to varying degrees. The use of TDZ alone at doses of 2 and 3 mg/L can also yield positive results depending on the variety. Developing shoots were subcultured and grown. For rooting, hormone-free MS medium was used, and tomato plantlets were transferred to peat pots and acclimatized. In this study, the *in vitro* regeneration system for advanced genetic applications was optimized on experimental material.

Keywords: Tomatoes, organogenesis, *Solanum lycopersicum* L., zeatin, TDZ

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most economically valuable members of the Solanaceae family and ranks as the world's second most produced vegetable after potato. Recent statistics indicate that global tomato production has reached nearly 192 million tons, with Türkiye contributing about 13.3 million tons annually. This production volume places Türkiye third worldwide, following China and India (Anonymous, 2025a). While most tomatoes are consumed domestically, approximately 590 thousand tons are exported, accounting for nearly half of the country's total revenue from fresh vegetable exports (Anonymous, 2025b).

It is widely accepted that tomato originated in the American continent. The primary center of origin is considered to be South America, particularly the western coastal highlands spanning parts of Chile, Bolivia, Ecuador, Colombia, and Peru (Bai and Lindhout, 2007). Wild tomato species occur naturally in these regions (Taylor, 1986). The cultivated tomato (*S. lycopersicum*) was introduced from Mexico to Spain during the early sixteenth century, from where it gradually spread throughout Europe (Caicedo and Peralta, 2013). According to Diez and Nuez (2008),

(§) *This study was presented as a poster presentation at the V. International Plant Breeding Congress held in Antalya, Türkiye, on December 1-5, 2025.*

Turkish merchants played an important role in its dissemination to Mediterranean and Middle Eastern countries. Bayraktar (1953) and Oraman (1968) reported that tomato was introduced into Türkiye around 1770. Initially cultivated in the Adana province and other Mediterranean regions, tomato production subsequently expanded to the Marmara region, Istanbul, and eventually throughout Anatolia (Abak and İlbi, 2022). Over time, locally adapted genotypes and landraces emerged, giving rise to numerous traditional local varieties. As in many other vegetable crops, Türkiye represents an important diversification center for tomato, with a rich collection of local populations and traditional varieties (Sönmez, 2014).

Today, tomato is consumed in a wide range of fresh and processed products and is increasingly recognized as a functional food. As one of the richest natural sources of lycopene, tomato also contains carotenoids and polyphenolic compounds that contribute not only to its nutritional value but also to its sensory and functional characteristics, including flavor and aroma (Martí et al., 2016; Sönmez and Ellialtıoğlu, 2014). Although local varieties and other open-pollinated genotypes cultivated in Türkiye exhibit considerable genetic diversity and desirable quality traits, many of them require further improvement for use in modern breeding programs. Consequently, efficient biotechnological approaches have become increasingly important to accelerate breeding and facilitate the incorporation of valuable agronomic and quality traits into elite germplasm.

Among the advanced biotechnological tools currently used in crop improvement, genetic transformation and CRISPR/Cas9-mediated genome editing have opened new opportunities for precise modification of economically important traits. However, the successful application of these technologies depends primarily on the ability to regenerate whole plants from cultured tissues. Therefore, the development of efficient and reproducible *in vitro* regeneration systems is considered a fundamental prerequisite for both functional genomics and modern breeding applications.

Extensive studies have been conducted on tomato *in vitro* regeneration through organogenesis using various explants, including cotyledons, hypocotyls, leaves, stem segments, pedicels, petioles, and inflorescences. A comprehensive review of these studies was presented by Senapati (2016). Regeneration efficiency is influenced by several factors, particularly genotype, explant source, culture medium composition, and the type and concentration of plant growth regulators. In regeneration systems, the balance between cytokinins and auxins governs organogenesis, callogenesis, and somatic embryogenesis through synergistic, antagonistic, or additive interactions depending on the plant species and tissue type. Generally, *in vitro* regeneration consists of an induction phase, a shoot elongation phase, and a rooting phase. In tomato, zeatin and BA are the most frequently used cytokinins, whereas IAA and NAA are commonly employed auxins to promote shoot regeneration (Rai et al., 2013; Seçgin, 2021; Sherkar and Chavan, 2014; Wayase and Shitole, 2014).

Since regeneration capacity is highly genotype-dependent, optimization of regeneration protocols remains essential for newly developed breeding materials. Therefore, the objective of the present study was to determine the most suitable regeneration medium for four Turkish tomato cultivars developed by Petektar Seed Co. by evaluating different explant types and combinations of plant growth regulators. The optimized regeneration protocol established in this study is expected to provide a reliable platform for future applications in genetic transformation, genome editing, and tomato breeding.

Materials and Methods

Plant material

The experiments were conducted at the Petektar Seed R&D Center, which comprises a research greenhouse and plant biotechnology laboratories located in the Kurşunlu district of Antalya. Four tomato varieties (PTK-254, PTK-273, Şekerkız, and Yelpare 82) were used as plant material.

Seed sterilization and *in vitro* germination

Seeds of four tomato (*S. lycopersicum*) varieties were placed in 50 mL Falcon tubes and first immersed in 70% (v/v) ethanol for 1 min. This was followed by surface sterilization in 20% (v/v) sodium hypochlorite (NaOCl) solution containing 2-3 drops of Tween-20 for 20 min. The seeds were then rinsed three times with sterile distilled water, each wash lasting 5 min. After washing, seeds were transferred onto sterile filter papers inside a laminar flow cabinet to remove excess moisture and then placed on germination medium in glass jars. All procedures were carried out under aseptic conditions, and cultures were incubated at 25°C under a 16/8 h light/dark photoperiod in a growth chamber.

Explant preparation and culture conditions

In all four tomato varieties, explants were prepared and transferred to regeneration media when the cotyledon leaves were positioned horizontally and the first true leaf had just emerged. For this purpose, hypocotyl tissues and longitudinally bisected cotyledon leaves were used as explant sources.

Murashige and Skoog (MS) mineral salts and vitamins (Murashige and Skoog, 1962) were used

as the basal medium. The media were supplemented with 3% (w/v) sucrose and solidified with 0.7% (w/v) agar. The pH of all media was adjusted to 5.7 before autoclaving. Media were sterilized at 121°C for 15 min in an autoclave. The explants were cultured on MS medium containing eight different combinations of plant growth regulators (PGRs). For each treatment, 10 Petri dishes were prepared, and six cotyledon explants were placed per Petri dish. In addition, approximately 30 hypocotyl explants (approximately 1 cm in length) were placed in two Petri dishes per treatment. The concentrations and combinations of PGRs used in the experiment, along with their respective code numbers, are presented in Table 1.

Rooting and acclimatization

Once regeneration had initiated, shoots approximately 2-3 cm in length were transferred to RM medium for root induction. All cultures were incubated in a growth chamber at 26 ± 2 °C under a 16/8 h light/dark photoperiod. Rooted plantlets were then transferred into pots containing peat-based substrate. The plants were gradually acclimatized and subsequently transferred to the greenhouse under healthy growth conditions (Okutan et al., 2025).

Statistical analysis

One-way analysis of variance (ANOVA) was performed to evaluate differences between media and varieties. The general linear model procedure of MINITAB 17.0 Statistical Software was used for data analysis. All main effects were considered as fixed factors. Tukey's multiple range post hoc test was employed for multiple comparisons of the means with an alpha level of 0.05.

Results and Discussion

To determine the optimal regeneration medium for tomato, the effects of explant source and plant growth regulators were evaluated using four different tomato varieties. Cotyledon and hypocotyl explants were cultured on MS media containing various combinations and concentrations of plant growth regulators. No regeneration was observed from hypocotyl tissues. The effects of eight different MS media compositions on plant regeneration from cotyledon explants are presented in Table 2.

The interaction between medium compositions and varieties was found to be statistically significant. The highest regeneration rate (82%) was obtained in the 'MS-1 × Şekerkız' and 'MS-1 × Yelpare 82' combinations. Since the mean values were subjected to angular transformation before statistical analysis, these transformed values were also used for interpretation. The next highest regeneration rates were observed in the 'MS-5 × Şekerkız' and 'MS-6 × Şekerkız' combinations (55.2%). Although numerical differences were observed among treatments, regeneration occurred to varying degrees in almost all combinations, except for 'MS-3' medium and the 'PTK-254' variety, which showed no response. Considering the fundamental principle of tissue culture that regeneration frequency is highly genotype-dependent, the interaction effect was further analyzed separately. Under this approach, statistically significant differences were also observed both among varieties and among media compositions.

The composition of the culture medium, especially the growth regulators it contains, has a decisive effect on the *in vitro* morphogenesis and shoot regeneration capacity of plants. Among the tested media compositions, 'MS-1' medium containing '1.0 mg/L Zeatin + 0.1 mg/L IAA' provided by far the highest regeneration rate (71.4%) compared with the others. This evaluation represents the mean regeneration response across all varieties (Table 2). Similarly, several researchers have also reported that the use of Zeatin has a positive effect on regeneration efficiency in tomato. According to Gubiš et al. (2005), not only the combination of zeatin and IAA but also the TDZ and IAA combination promoted shoot regeneration in tomato explants. In addition, 'MS-5' medium, containing 3.0 mg/L TDZ, achieved the second-highest average regeneration rate (approximately 27%). Cao et al. (2020) also demonstrated that trans-zeatin, a cytokinin hormone, plays an important role in the *in vivo* regeneration of tomato. This medium (MS-5), together with 'MS-8' (3.0 mg/L BA + 0.5 mg/L IAA), showed statistically similar results, falling within the same significance group. Seçgin et al. (2021) also reported that the combination of 3.0 mg/L BA + 0.1 mg/L IAA (corresponding to MS-8) was suitable for regeneration from cotyledon explants in the tomato cultivar 'Crocker'. The authors noted, however, that the use of kinetin resulted in better regeneration performance in the 'Bobcat' cultivar.

Genotype had a pronounced effect on regeneration success in tomato. It has been well documented that certain cultivars are recalcitrant and highly challenging for regeneration. Among the varieties used in this study, 'Şekerkız' exhibited the highest average regeneration rate, as 41.9% (Figure 1), followed by 'Yelpare 82' (23.3%). 'PTK-273' ranked third with 16.3%, showing no statistically significant difference from 'Yelpare 82' (Table 2). 'PTK-254' displayed the lowest regeneration response (Figure 2). For this variety, further optimization using alternative PGR combinations and concentrations appears necessary. The influence of genotype on regeneration capacity is well documented in tomato tissue culture studies. Grozeva et al. (2006)

reported significant differences among tomato cultivars in their response to *in vitro* regeneration, emphasizing that genotype plays a decisive role in morphogenic competence. Similarly, El-Shafey et al. (2017) observed that variations in shoot regeneration frequency were strongly associated with the genetic background of the tested cultivars, regardless of identical culture conditions. These findings indicate that regeneration potential is an inherent, genotype-dependent trait rather than solely a result of external culture factors. Therefore, the selection of a responsive genotype is crucial for the successful establishment of an efficient regeneration system in tomato. It is difficult to propose a single universal regeneration formula suitable for all tomato varieties. In light of the findings from this study, Zeatin combined with a low level of IAA proved to be the most effective cytokinin treatment for promoting regeneration in tomato explants. All obtained shoots were successfully acclimatized and subsequently transplanted into soil (Figure 3).

Conclusions

In this study, nutrient media supplemented with Zeatin, TDZ, and BA (cytokinins) in combination with IAA (auxin) were tested for their ability to induce shoot regeneration from cotyledon explants of four tomato varieties. The results indicated that genotype was the key factor affecting regeneration efficiency, though the composition of PGRs in the medium also contributed to the observed differences. Among the tested varieties, 'Şekerkız' demonstrated superior regenerative potential, whereas 'PTK-254' showed limited response, confirming its recalcitrant nature. Zeatin, together with BA and TDZ, promoted regeneration to different extents across varieties, highlighting the genotype-dependent nature of morphogenic response. Furthermore, cotyledon explants proved to be markedly more efficient in regeneration compared to hypocotyl explants.

The outcomes of this research provide a solid basis for future investigations, particularly for applications involving genetic transformation and accelerated breeding in tomato. This study underscores the importance of genotypic variation in tissue culture responses, suggesting that a tailored optimization of regeneration protocols for each genotype is indispensable for achieving consistent and reproducible results.

Acknowledgements

The authors used OpenAI ChatGPT solely to improve the English language, grammar, and readability of the manuscript. The AI tool was not used to generate scientific content, analyze data, interpret results, or draw conclusions. All scientific content and conclusions are entirely the responsibility of the authors.

Ethical Statement

A preliminary version of this study was presented as a poster at the "V International Plant Breeding Congress, 1-5 December 2025, Sherwood Exclusive Lara, Antalya, Türkiye". The present manuscript is an expanded and revised version prepared for full publication.

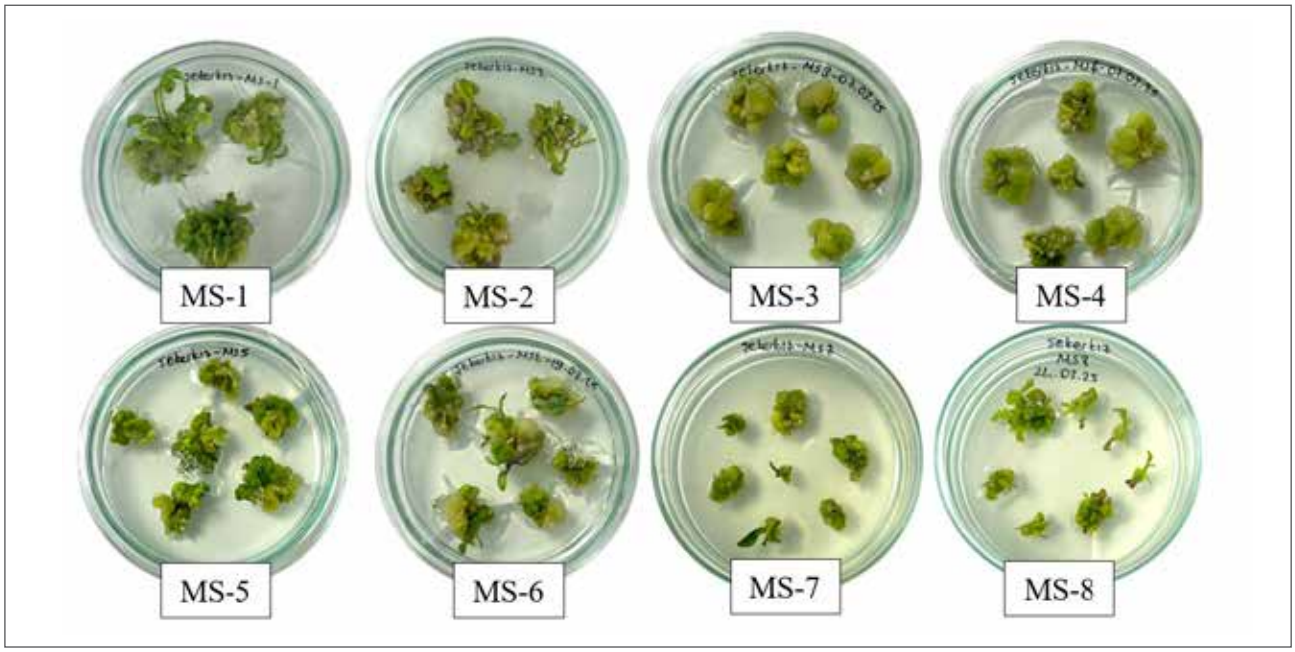

Figure 1. Shoot regeneration of 'Şekerkız' tomato cotyledon explants in eight media compositions.

Figure 2. Healthy shoots obtained from the 'PTK-254' tomato variety, which showed the lowest regeneration capacity among the tested varieties were cultured on MS-1 medium.

Figure 3. Tomato regenerants exhibiting healthy growth following the acclimatization process.

Table 1. The codes, types, and concentrations of plant growth regulators (PGRs) used in the regeneration media.

PGRs (mg/L)	Medium Code							
	MS-1	MS-2	MS-3	MS-4	MS-5	MS-6	MS-7	MS-8
Zeatin	1.0	2.0						
IAA	0.1	0.1						0.5
TDZ			1.0	2.0	3.0	0.5	1.0	
BA						0.5	1.0	3.0

Table 2. *In vitro* regeneration percentages of four tomato varieties cultured on eight different MS media compositions.

Medium Code	Varieties				
	Şekerkız	Yelpare 82	PTK-273	PTK-254	Means*
MS-1	94.4 ***82.0 a***	94.4 ***82.0 a***	61.1 ***51.5 abcde***	83.3 ***70.2 ab***	***71.4 A***
MS-2	38.9 ***38.0 abcde***	27.8 ***26.8 bcde***	22.2 ***23.0 bcde***	0 ***0 e***	***22.0 BC***
MS-3	0 ***0 e***	5.56 ***8.0 de***	0 ***0 e***	0 ***0 e***	***2.0 C***
MS-4	22.2 ***23.5 bcde***	5.56 ***8.0 de***	5.56 ***8.0 de***	0 ***0 e***	***9.9 BC***
MS-5	66.7 ***55.2 abcd***	16.7 ***19.8 bcde***	22.2 ***23.5 bcde***	0 ***0 e***	***24.6 B***
MS-6	66.7 ***55.2 abcd***	11.1 ***11.8 de***	5.56 ***8.0 de***	0 ***0 e***	***18.6 BC***
MS-7	16.7 ***15.0 cde***	0 ***0 e***	11.1 ***16.1 cde***	0 ***0 e***	***7.8 BC***
MS-8	77.8 ***67.0 abc***	33.3 ***30.0 abcde***	0 ***0 e***	0 ***0 e***	***24.2 B***
Means**	***41.9 A***	***23.3 B***	***16.3 BC***	***8.8 C***	

Variety ($p < 0.05$, Tukey's multiple comparison test)
Medium ($p < 0.05$, Tukey's multiple comparison test)
Variety × Medium ($p < 0.05$, Tukey's multiple comparison test)

* Means followed by different letters within the same column are significantly different at the 0.05 probability level.
** Means followed by different letters within the same row are significantly different at the 0.05 probability level.
Italic and bold values represent angular transformed data.

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