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Optimization of In Vitro Regeneration in Wheat and Agrobacterium-mediated Rapid Transformation

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Research Article

Keywords: 2,4-D, picloram, dicamba, IAA, qRT-PCR, Agrobacterium, in vitro regeneration, Triticum aestivum L

Posted Date: April 22nd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9359045/v1

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Additional Declarations: No competing interests reported.

Abstract

Wheat (*Triticum aestivum* L.) is a staple food that plays a significant role in human nutrition due to its high nutritional value. The development of an effective in vitro regeneration system is a prerequisite for achieving rapid Agrobacterium-mediated genetic transformation in wheat. In this study, the tested concentrations and combinations of picloram, 2,4-D, dicamba, and IAA in mature embryo cultures of Kırik and Palandöken 97. While analyzing CFR (%) and CW (%) and gene expression for 6 different media, ECR (%), RECR (%), RE (number), and the effects of 12 hormone-free and IAA-supplemented media were evaluated following somaclonal variation. Furthermore, following gene transfer optimization, the pGFPGUS*Plus* vector was introduced into 10-day-old calli via "*Agrobacterium*-mediated transformation," and *HPTII*, *GUS*, and mRNA expression were analyzed by qRT-PCR in selected T_0 plants. According to the results, MS4-1 and MS1-1 generally had the greatest effects on ECR, RECR, and RE among the media, while MS2-1 and MS2-2 generally had the least. In both varieties, the WOX4 gene response peaked at 14 DAI and decreased by 28 and 42 DAI. LEC1 expression peaked at 42 DAI, while the BBM1 and *TaSERK* genes showed high expression at 14 and 28 DAI, respectively. MS4 was selected as the most effective medium for all genes. In both Kırik and Palandöken-97, the MS1-1, MS3-1, and MS4-1 media showed the closest similarity to the parent plant. As a result of gene transfer, 12 To plants were selected. Line 11 exhibited the highest response in both GUS and HPTII. Expression levels were higher than in the control in all 12 lines. Overall, the tests revealed that Kırik and MS4 emerged as effective media for rapid gene transfer. Optimized in vitro regeneration and transformation protocols enhance the effectiveness of biotechnological approaches in wheat breeding, thereby making significant contributions to sustainable agriculture.

1. Introduction

Wheat (*Triticum aestivum* L.) is the most essential staple crop among all cereals. With its high nutritional value and contribution to a significant portion of protein intake, it is the most important product produced worldwide, especially for human nutrition [1–3].

Globally, cultivated agricultural land is decreasing due to global warming and anthropogenic impacts [4, 5]. This situation is negatively impacting wheat yield and quality. To address this problem, various agronomic practices and breeding programs have been developed and used to produce important agricultural products [6]. Meeting the increasing food supply while the world population grows is a global priority for researchers [7]. Classical breeding programs have been established to develop wheat varieties tolerant of various biotic and abiotic stress factors, and success has been achieved [8, 9]. By using classical breeding methods, genes affecting quantitative characters have been transferred. However, many problems, such as linkage, may be encountered with this method, and the breeding period in cultivar development may take a long time [10]. Plant genetic engineering or biotechnological methods offer effective alternatives to address these problems [11, 12].

In the field of genetic engineering, genes are transferred into plant cells cultured using tissue culture methods [10]. In recent years, significant advances have been made in the field of genetic engineering and biotechnology in monocotyledonous (monocot) model plants. However, the complex, polyploid genome of wheat, a monocot plant, poses challenges for achieving high transformation efficiency. Therefore, the development of an effective regeneration system is a prerequisite for achieving successful gene transfer in wheat via *Agrobacterium* [13–16]. Somatic embryogenesis (SE) and organogenesis are the two primary regeneration systems in plant tissue culture [12, 17, 18]. SE is the most effective regeneration system in genetic transformation studies. This system involves processes regulated by a set of genes (*WOX*, *LEC*, *BBM*, and *SERK*) that confer embryogenic capacity to somatic cells [19, 20]. It has been reported that the SERK, LEC, and WUS-like genes play significant roles in transcriptional regulation and signaling pathways during SE [21]. In particular, *LEC1* and *BBM1* are crucial for embryo differentiation and maturation [22].

Explant type, genotype, and nutrient medium content are the most important factors influencing the success of plant regeneration [18, 23]. In wheat genetic transformation studies, immature and mature embryos, anthers, and young spikes are commonly used as explant types. Another important factor affecting the success of plant regeneration studies is the composition of the culture medium. The culture medium generally contains macroelements, microelements, plant growth regulators (auxin, cytokinin, gibberellin, ethylene, etc.), vitamins, organic components, solidifying agents (if semi-solid), and sucrose [24]. Plant growth regulators (PGRs) play a significant role among all these components. Indole-3-acetic acid (IAA), 2,4-dichlorophenoxyacetic acid (2,4-D), picloram, and dicamba are the most commonly used PGRs in wheat [25, 26]. These PGRs play a role in processes such as cell division, callus formation, tissue differentiation, and the development of adventitious roots and shoots from callus. They are also known to be effective in regulating the propagation and regeneration of roots and shoots [13, 27].

In plants grown in vitro, components of the culture medium are a common source of genetic or epigenetic changes [28]. While genetic changes refer to cytogenetic abnormalities and alterations in DNA sequences, epigenetic changes involve modifications in gene expression. This phenomenon is defined as somaclonal variation in plant tissue culture systems [29]. Somaclonal variation is a critical parameter that must be considered in terms of micropropagation and genetic engineering when selecting an effective regeneration system [30]. Somaclonal variation may limit the use of transgenic plants in the functional analysis of cloned genes whose functions have not yet been defined [31]. To this purpose, somaclonal variation has been identified to date using various molecular marker methods (RFLP, RAPD, ISSR, SSR, and SCOT) [32, 33]. Among these, the sequences surrounding the ATG start codon in the plant genome provide a simple, highly reproducible, and practical molecular marker system that has been successfully used in many plant species [34–36].

This study aims to develop a reliable and effective in vitro regeneration system for wheat using mature embryos as explants, supported by endosperm. In this context, the effects of different culture media on regeneration were evaluated comparatively for two different wheat varieties, and MS4 was determined to

be the most suitable regeneration medium. Among the two varieties, a rapid, efficient gene transfer system was implemented to enhance reproducibility in transformation and breeding studies. In this context, the focus was on evaluating tissue culture parameters, gene expression, and the genetic stability of plants regenerated. Furthermore, the plants obtained through gene transfer were evaluated.

2. Materials and methods

2.1. Optimization of In Vitro Plant Regeneration

2.1.1. Plant material and sterilization

In this study, dry seeds of Kırik bread wheat and Palandöken-97 varieties were used as explant sources. Mature embryos with endosperm support obtained from dry seeds were used as explants. To begin the sterilization process, the seeds were first treated with 70% ethanol for 5 minutes, followed by a 20% commercial bleach solution containing 5% sodium hypochlorite for 25 minutes [37].

2.1.2. In Vitro Regeneration Protocol and Culture Conditions

The mature embryos of the seeds were divided into six parts (2 horizontal, 1 vertical) without separating from the endosperm. These were then placed in six different medium (MS1, MS2, MS3, MS4, MS5, and MS6) (Table 1) containing MS [38] medium, 20 g/l sucrose, 2 g/l phytagel, and 1.95 g/l MES [(2-(N-morpholino) ethanesulfonic acid)] and different concentrations and combinations of plant growth regulators, to optimize in vitro regeneration. These media were cultured in the dark at 25 ± 1°C for 21 days. At the end of this period, callus formation rate (CFR) (%) [(number of calluses/number of explants) x100], fresh callus weight (CW) (%) (total callus weight/number of calluses) were calculated. Callus samples showing embryogenic characteristics at the end of day 21 were cultured for 16 hours at a photoperiod of 60 µmol/m2 s and a temperature of 25 ± 1°C in two different regeneration media (hormone-free callus formation medium and callus formation medium containing 1 mM IAA). Since embryogenic structures were more clearly evaluated a few days after the samples were placed in this medium, the embryogenic callus ratio (ECR) (%) [(number of embryogenic calluses/number of explants) x 100] was calculated at this stage. On the 14th day of continuing the same culture, green formations were observed on the embryogenic calluses, and on this day, the responding embryogenic callus ratio (RECR) [(number of embryogenic calluses forming seedlings / number of explants) x 100] was measured. Plant number per explant (regeneration efficiency) (RE) (number) was calculated.

Table 1
The medium used and the content of that medium

Medium Code	Composition
MS1	MS + 12.0 mg/L Dicamba + 0.5 mg/L IAA
MS2	MS + 8.0 mg/L 2,4-D
MS3	MS + 2.2 mg/L Picloram + 5.0 mg/L 2,4-D
MS4	MS + 4.4 mg/L Picloram + 1.0 mg/L 2,4-D
MS5	MS + 5.0 mg/L Picloram + 2.2 mg/L 2,4-D
MS6	MS + 6.6 mg/L Picloram + 1.5 mg/L 2,4-D

2.1.3. Gene Expression Analysis

Expression of *WOX4*, *LEC1*, *BBM1*, and *TaSERK* genes was detected by RT-qPCR in samples collected 14, 28, and 42 days after the initiation of the in vitro regeneration process (DAI). Total RNA isolation was performed using Trizol® Reagent (Invitrogen Life Technologies). At the end of RNA isolation, RNA was qualitatively and quantitatively determined by measuring the $A_{260/280}$ O.D. on a Nanodrop and visualized on a 1.2% agarose gel. For the purpose of generating cDNA from total RNA, the RevertAid First Strand cDNA Synthesis Kit (Catalog Number: K1621, Thermo Scientific, United States) was effectively applied. To determine gene expression levels, the generated cDNA was used. The Rotor-Gene Q system (Qiagen, Germany) was used in conjunction with the EnTurbo™ SYBR Green PCR SuperMix (ELK Biotechnology, Denver, USA, #EQ001). The final reaction mix (20 µL), consisting of 2 µL of diluted cDNA sample, 10 µL of EnTurbo™ SYBRGreen, 0.4 µL of forward and reverse primers (100 pmol), 0.4 µL of ROX, and RNase-free H_2O, was used for the qPCR experiment. The reaction conditions were set to 40 cycles of 95°C for 5 min, 95°C for 5 s, 50–65°C for 30 s, and 72°C for 30 s. A list of the genes used in the research is provided in Table S1. Primers were designed according to the data obtained from the study by Sharma and Khurana [39], Singh et al [40], and Ucar et al (2025). *GUS*, *HPTII*, and *NPTII* primers were designed using the web-based Integrated DNA Technologies (IDT) PrimerQuest Tool. The size and specificity of each amplicon were determined using agarose gel electrophoresis and melting curve analysis. The actin gene was used to assess normalization of expression for *WOX4*, *LEC1*, *BBM1*, *TaSERK-1*, *-2*, *-3*, *-4*, *-5*, *-6*, *-8*, *-10*, *-11*, and – *12* genes. Melting curve analysis was used to successfully validate specific PCR results. Gene expression levels were measured using the $2^{-\Delta\Delta Ct}$ method by Livak and Schmittgen [41]. The data's means and standard deviation values were obtained using four replicates, and three biological replicates were visualized with Origin 2021b software (OriginLab Corporation, Northampton, MA, USA).

2.1.4. Assessment of Somaclonal Variation

The genetic stability of the plants obtained in the study was determined using the SCoT molecular marker technique. For SCoT analysis, random leaf samples were selected and collected from four replicates of 12 media. Plant samples obtained from the germination of both varieties were used as a

control group. A bulk DNA sampling strategy was applied in the study (Reyes-Valde et al. 2013). Leaf samples were frozen in liquid nitrogen and stored at -80°C until DNA isolation. DNA isolation was performed using the cetyltrimethylammonium bromide (CTAB) technique [42]. DNA purity and quantity were determined using a Nanodrop spectrophotometer (Thermo Scientific Multiskan Go), and the DNA samples were stored at -20°C. SCoT primers were determined as a result of a literature search [43]. A total of 14 SCoT primers were used in the study (Table S2). These primers were those that showed a high-quality amplification profile in the test of 30 SCoT primers (Xia et al. 2016). SCoT-PCR was performed according to Taq DNA Polymerase 2X Master Mix (AMPLIQON, APQ-A140301). The PCR reaction was performed using a total volume of 15 µl containing 8 µL Taq DNA Master Mix, 1 µL (10 ng) genomic DNA, 5 µL ddH_2O, and 1 µL primer. The SCoT-PCR conditions were as follows: 95°C for 5 min, 94°C for 1 min (for 40 cycles), 54–63°C for 1 min (primer binding phase), 72°C for 1.5 min, and 72°C for 10 min extension phase, and finally the program was terminated at 4°C for 10 min. Then, 10 µl of amplified PCR products were mixed with 3 µl of 6× gel loading dye and loaded onto a 1.5% agarose gel (containing 5 µl of ethidium bromide). Electrophoresis was then performed in 1X TBE (Tris-borate-EDTA) buffer for 1.5 hours at 70 V, and DNA bands were visualized by UV transillumination.

2.2 *Agrobacterium tumefaciens*-mediated transformation

2.2.1. Preparation of component cells of Agrobacterium tumefaciens AGL1 strain and transformation of the pGFPGUSPlus vector into the AGL1 strain

Bacteria obtained from a single bacterial colony were propagated overnight. A 1 ml aliquot was taken from this suspension culture, 40 ml of LB low-salt medium was added, and the mixture was incubated at 28°C at 200 rpm for 4 hours. Then, when the culture OD_{600} value reached 0.8-1.0, it was placed on ice and centrifuged at 4°C to collect the cells. The resulting pellet was resuspended in 10% sterile glycerol to prepare competent cells. Next, 80 µl of AGL1 competent cells and 5 µl of plasmid were combined in a cuvette. The pGFPGUS*Plus* plasmid (Vickers et al. 2007) was transferred to AGL1 cells via electroporation using the Bio-Rad GenePulser system (2.5 kV, 25 µF, 400 Ω). The entire cuvette was then transferred into a 1.5 ml microcentrifuge tube and incubated at 28°C for 3 hours at 200 rpm in a shaker. Then, 150 µl was dropped onto LB agar containing 50 mg/ml kanamycin at different points, and the plates were separated in Drigalski's medium. It was incubated at 28°C for 2 days. The first colonies began to appear after 36 hours. Colony PCR was performed using *HPTII* primers to identify positive colonies containing the pGFPGUS*Plus* vector, with Taq DNA Polymerase 2X Master Mix (Ampliqon, APQ-A140301) according to the manufacturer's instructions. The identified positive colonies were grown in LB low-salt medium at 28°C for transformation into wheat plants.

2.2.2. Gene Transfer to the wheat (*Triticum aestivum L.*) plant using the *Agrobacterium-mediated* transformation method

Based on data from in vitro regeneration optimization studies conducted in the first phase, two varieties and six methods were compared for plant regeneration via callus induction. The evaluations revealed that the variety with the highest success rate was Kırik, and the most effective method was MS4. This combination was used throughout the gene transfer studies. Furthermore, the media and their components used in the gene transfer process in wheat are shown in Table 2. Kırik wheat seeds were sterilized and cultured in MS4 medium. Ten-day-old calluses were used for gene transfer. The calluses were separated from the seeds and transferred to an inoculation medium containing *Agrobacterium* (OD_{600}=0.8) and incubated in the dark at room temperature for 15 minutes. At the end of the incubation period, the calluses were dried for 5 minutes using Whatman filter paper moistened with the co-cultivation medium (Table 2). The calluses were then transferred to the restring medium and cultured for 5 days at 25°C in the dark. Then, cowered with thin paper (to reduce light intensity), they were placed in selection medium at a 16:8 light cycle and left for 5 days. After 5 days, the papers were removed, and calluses with green dots were transferred to regeneration medium. In this medium, they were cultured at 25°C under a 16/8 hour light cycle until green shoots 5 cm long were obtained. To encourage rooting of the resulting shoots, they were transferred to magenta boxes containing rooting medium and cultured at 25°C under a 16/8-hour light cycle.

Table 2
Components and their compositions used in the gene transfer process in wheat

Components	**CFM**	**IM**	**CCM**	**RM**	**SM**	**PRM**	**RM**
MS Salt (g)	4.3	0.43	0.43	4.3	4.3	4.3	4.3
MS Vitamin (1000x) (mL)	1	1	1	1	1	1	1
Sucrose (g)	30	15	15	30	30	30	30
Glucose (g)	-	10	10	-	-	-	-
Picloram [mL (1 mg/mL)]	4.4	-	-	4.4	-	-	-
2.4-D (mg)	1.0	-	-	1.1	-	-	-
IAA [mL (1 mg/mL)]					5.75		1
MES Hydrate (g)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Hygromycin (mg)	-	-	-	-	25	25	25
Cefotaxime (mg)	-	-	-	150	150	150	150
Acetosyringone (µM)		200	200				
Phytagel (g)	2	-	-	-	2	2	2
Agar (g)	-	-	-	7	-	-	-

CFM; Callus Formation Medium, IM; Inoculation Medium, CCM; Co-cultivation Medium, SM; Selection Medium, RM; Restring Medium, PRM; Plant Regeneration Medium, RM; Rooting Medium

2.2.3. Verification of positive plants

The presence of T_0 plants in the selection medium was verified by PCR and RT-qPCR using *HPTII* primers (Table S1). The PCR reaction components and conditions were as previously described.

2.3. Statistical Analysis

The study was planned according to a Completely Randomized Design with four replicates, each petri dish containing 15 endosperm-supported embryos. Statistical analysis of tissue culture parameter data was performed using Minitab 19. Differences between means were determined at the 5% significance level using the Tukey multiple-comparison test. All graphical presentations, including bar graphs and correlation heat maps, were prepared using Origin 2025b software and GraphPad Prism 9 to present the findings more effectively. For the SCoT amplification bands, a binary matrix of presence (1) or absence (0) was generated using the Total Lab TL120 computer program. The obtained data were analyzed using the NTSYSpc 2.11 software package. The similarity coefficient using the SIMQUAL module. A dendrogram was constructed from the similarity coefficients using the UPGMA (unweighted pair-group method using arithmetic mean) method in the SAHN module. The polymorphism information content (PIC), discrimination power (D), and resolution power (R) were calculated for each primer.

3. Result

3.1. Plant Tissue Culture Characteristics

CFR (%) was found to be insignificant in terms of variety, but significant ($p < 0.01$) in terms of M and V × M (Table S3). When comparing all media in the Kırik in terms of CFR (%), 150% was observed in MS1, MS4, MS5, and MS6, while 36.6% was observed in MS2 and 76.66% in MS3. When Palandöken-97 was evaluated, a CFR of 150% was observed in MS1, MS3, and MS4 media, whereas a decrease in CFR was observed in MS2 and MS6 (Fig. 1).

According to Table 3, CW (%) was found to be insignificant for M, but significant ($p < 0.01$, $p < 0.05$) for V and V × M interaction. In the Kırik variety, MS4 medium had the highest CW value at 1.4 mg, while in the Palandöken-97 variety, MS1 and MS3 media had the highest values at 1.23 mg (Table S3).

Embryogenic calli have a compact, dispersible, and shiny appearance. Calli with this structure were considered embryogenic. The interaction effect of media on ECR was significant ($p < 0.01$) (Table S3). In both varieties, the highest ECR value was measured in MS4-1 medium. When the varieties were evaluated within themselves, the ECR value (146,66%) was higher in the Kırik. The lowest ECR value was observed in the Kırik under MS1-2 medium (36,66%), while the Palandöken-97 showed the lowest value under MS2-2 medium (30%) (Fig. 2).

While V and M alone were found to be significant ($p < 0.01$) in terms of RECR, the V x M interaction was insignificant (n) (Table S3). In both varieties, the highest RECR value was recorded in the MS4-1 medium,

and this value was higher in the Palandöken-97 variety. The highest RECR value was followed in decreasing order by the MS4-2 medium and the Palandöken-97 variety (133.33%) in both genotypes, and by the MS1-1 medium (123.33%) in one genotype. The lowest RECR value was measured in the Palandöken-97 variety in the MS2-1 and MS2-2 media (Fig. 2).

Regarding RECR, the interaction between V, M, and V × M was recorded as significant ($p < 0.01$) (Table S3). In both varieties, the highest RE value was found in MS4-1 (4.7 number) and MS4-2 (4.5 number) media for the Kırık, and in MS2-1 medium for the Palandöken-97 (Fig. 2).

The relationships between CFR (%) and CW (mg), and between ECR (%) and RECR (%) and RE (number) are shown using a scatter matrix (Fig. 4A, B, C, and D). The distribution and correlation of these parameters were examined across media and varieties (Kırık and Palandöken-97). The histograms in the graphs show the distributions of the variables, while the scatter plots and confidence ellipses reveal the relationships between the two parameters. The relationships between CFR (%) and CW (mg) are shown across media and varieties. When the results obtained in the media were examined, it was found that the relationship between CFR and CW varied across media. Pearson correlation coefficients showed a weak negative relationship in MS1 and MS2 media with r = -0.35 and r = -0.27, respectively, while a positive relationship was detected in MS3 (r = 0.35), MS4 (r = 0.54), MS5 (r = 0.32), and especially MS6 (r = 0.83) media. These results indicate that the CFR (%) ratio also increases. When considered in terms of varieties, a moderately positive relationship (r = 0.61) was determined between CFR and CW in the Kırık, while a strong negative relationship (r =-0.72) was found in the Palandöken-97 (Fig. 4A, B).

The relationships between ECR (%), RECR (%), and RE (Number) are shown by media and variety. When examining results across media, it was observed that the relationships between ECR and RECR varied by medium. When Pearson correlation coefficients were examined, a positive relationship was observed among medium MS1 (r = 0.45), MS2 (r = 0.39), MS3 (r = 0.36), MS4 (r = 0.33), MS5 (r = 0.29), and MS6 (r = 0.27). The correlation coefficients between ECR and RE were generally lower. This decrease varied across media. Similarly, the relationships between RECR and RE appeared as weak positive correlations. A strong positive correlation was observed between ECR and RECR in both varieties (Kırık: r = 0.92; Palandöken-97: r = 0.91). The relationship between ECR and RE was found to be moderately positive in the Kırık (r = 0.51) and more positive in the Palandöken-97 (r = 0.69). The correlation between RECR and RE was r = 0.61 in the Kırık and r = 0.71 in the Palandöken-97 (Fig. 4C, D).

3.2. Relative Expression Analysis of Genes

Somatic embryonic structure first appeared on day 14 DAI in almost all media in both varieties, and *WOX4* gene expression was observed to increase at this time in all media. Moreover, a significant decrease in expression level was observed at 28 and 42 DAI compared to 14 DAI. Conversely, *LEC1* gene expression level was generally higher at 42 DAI. In both varieties, the highest *LEC1* gene expression levels were determined in MS4 medium 14, 28, and 42 DAI. *BBM1* gene expression levels were almost similar in both varieties, with the highest expression levels at 28 DAI in MS4 medium. The lowest expression levels were observed in MS2 and MS3 medium (Figs. 5 and 6).

The expression of *TaSERK1*, *TaSERK2*, *TaSERK3*, and *TaSERK4* genes was higher at 28 DAI compared to other days. The increases and decreases in these genes were almost similar in the Kırik and Palandöken-97 varieties. In the Kırik, *TaSERK1*, *TaSERK2*, and *TaSERK4* showed the highest expression at 28 DAI in the MS4 medium, whereas TaSERK3 showed higher expression in the MS1 medium. In Palandöken-97, all these genes showed their highest expression at 28 DAI in MS4 medium. Basal expression of these genes was low at 42 DAI (Fig. 7, 8).

The expression levels of *TaSERK-5*, *TaSERK-6*, and *TaSERK-8* genes showed significant variation in both varieties, depending on medium and time factors. The highest expression was observed at 28 DAI, followed by a significant decrease at 42 DAI. In particular, *TaSERK-6* expression levels decreased considerably in MS2 in the Palandöken-97. Overall, it was determined that MS4 was the medium where these genes were most active (Figs. 9 and 10).

The expression profiles of *TaSERK-10*, *TaSERK-11*, and *TaSERK-12* genes were similar in both varieties. Similar increases in gene expression were observed at 14 and 28 DAI. The MS4 medium significantly affected the expression levels of *TaSERK-10*, *TaSERK-11*, and *TaSERK-12*. This medium was prominent at all culture times (14, 28, and 42 DAI). A significant decrease in the levels of these genes was observed at 42 DAI in almost all varieties and in the medium (Figs. 11 and 12).

Correlations were established among *WOX4*, *LEC1*, *BBM1*, and members of the *TaSERK* gene family (Fig. 13A). An examination of the correlation matrix revealed that the *WOX4* gene showed positive correlations with several *TaSERK* genes ($r = 0.22–0.56$). Similarly, *WOX4* also showed negative correlations with *LEC1* and *BBM1* ($r = -0.50$ and $r = -0.11$, respectively). Furthermore, the relationship between *WOX4* and the other genes was statistically significant ($p \leq 0.05$). While *LEC1*, *BBM1*, and *TaSERK-1*, *-2*, *-3*, and *– 5* showed a negative relationship, the relationships with other genes were positive. It was found that the BBM1 gene had a positive correlation ($r = \approx 0.46–0.68$) with most members of the *TaSERK* gene family. When the *TaSERK* genes were examined, Pearson correlation coefficients ranged from 0.60 to 0.96, indicating a strong positive correlation among them. Most of these relationships among the *TaSERKs* were statistically significant ($p \leq 0.05$).

Two different scatter plots were created to examine the distribution of genes (Fig. 13B). Figure 14 shows that the gene data exhibit a wide distribution. A large portion is concentrated in the 0–80 range, while some exceed 100. It is noteworthy that the *TaSERK* genes have a much wider range than the *WOX4*, *LEC1*, and *BBM1* genes, reaching values up to 120–180. Values for *WOX4*, *LEC1*, and *BBM1* genes are concentrated in the low and medium ranges. Overall, this distribution reveals variation heterogeneity in gene expression levels. In the graph, each symbol represents up to 11 observations with the same value.

Another dot plot (Fig. 13C) shows that, when considered, there are striking differences in gene expression levels. The *TaSERK-1*, *TaSERK-2*, and *TaSERK-3* genes have a wide distribution, while the *WOX4*, *LEC1*, and *BBM1* genes are confined to a limited area. In the graph, each symbol represents up to 7 observations with identical values.

3.3. Somaclonal variation

The genetic stability of the Kırik and Palandöken-97 varieties was evaluated using the SCoT molecular marker technique. In the study, 20 primers were tested on the control plant, and 14 SCoT primers showing the best amplification (Table S2) were used to evaluate the genetic stability of regeneration plants from 12 different media, including one control. The gel image of some SCoT primers used in the study is shown in Table S2. When the genetic stability of the Kirik variety was evaluated, an average of 26.28 bands per primer and a total of 368 DNA bands were obtained. The highest number of bands, 33, was obtained with the SCoT-6, -8, and – 17 primers, while the lowest, 18, was observed with the SCoT-18 primer. The number of polymorphic bands (PB) ranged from 17 to 31, while the number of monomorphic bands (MB) ranged from 1 to 8. The polymorphism rates (P%) of the primers were highest in SCoT-27 with 96.88% and lowest in SCoT-6 with 75.76%. The PIC values of the primers ranged from 0.342 to 0.512. The highest PIC value, 0.512, was observed for primer SCoT-28. On the other hand, primer SCoT-27 had the highest resolution power (R) with 4.50, while primer SCoT-21 had the highest discrimination power (D) (Table S4). Regarding the genetic stability of the Palandöken-97 variety, a total of 367 DNA bands were detected, averaging 26.21 per primer. The highest number of bands was measured in SCoT-6 and SCoT-8 (33). The total number of monomorphic bands (MB) was 4, with an average of 0.28. The number of polymorphic bands was calculated as 25.92. The polymorphism rate was quite high in the Palandöken-97 variety, reaching 100% with all SCoT primers except SCoT-12, -10, and – 24. The PIC values ranged from 0.29 to 0.33, and those for all primers were similar. On the other hand, the highest discrimination power (D) was observed with the SCoT-21 primer (0.71), and the highest resolution power was observed with the SCoT-8 primer (5.41) (Table S5).

The genetic relationship between the media was obtained using a dendrogram generated with NTSYS-pc 2.1 software, based on the UPGMA algorithm with a SIMQUAL similarity coefficient (B). In the Kırik, the similarity coefficient between the media ranged from 0.62 to 0.73. The similarity coefficients between the plants grown on the media and the parent ranged from 0.63 to 0.73. The highest similarity to the parent plant was observed in MS1-1 medium (0.73), followed by MS3-1 and MS4-1 (0.72). The least similarity was shown in the MS2-2 medium (0.63) (Table S6). In Palandöken 97, the similarity coefficient between the plants obtained from the media and the parent plant ranged from 0.63 to 0.74. Again, among these plants, the closest medium to the parent plant in terms of similarity was medium MS1-1 (0.74), while the furthest was medium MS4-2 (0.63) (Table S7).

Figures A and B show the Principal Coordinates Analysis (PCoA) results for the media belonging to the Kırik and Palandöken-97. Examining the graph, it is noteworthy that samples MS3-1 and MS0, as well as MS4-1 and MS1-1, are close together in the Kırik variety. In contrast, samples MS2-2 and MS6-1 are located further apart from the others. Among the media belonging to the Palandöken-97 variety, samples MS3-1 and MS0, as well as MS5-1 and MS5-2, are particularly close. This indicates a high similarity between these media. However, the further apart the medium MS2-2 is, the more different its distribution is within the dataset (Figure S1A, B).

3.4 Molecular Confirmation of Positive Transgenic Plants Using HPTII Gene-specific Primers

3.4.1. Confirmation of the presence of the *HPTII and GUS* sequence by PCR

To confirm T-DNA integration in the plants, PCR analysis was performed using genomic DNA from 12 T_0 lines (regenerated plants) derived from different explants (Fig. d). PCR amplification using HPTII primers revealed the presence of a 191-bp fragment for *NPTII* and a fragment ranging from 200 to 500 bp for *HPTII* (Fig. b). To validate the test's accuracy, control DNA from wheat that had not undergone any transgenic manipulation was used, and the PCR results showed no bands (Fig. b).

3.4.2. Expression analysis of HPTII gene in transgenic lines by RT-PCR

RNA was isolated from the plants identified as positive in the PCR validation, and *HPTII* expression was examined by RT-PCR. The results indicated that *HPTII* elicited a response in all 12 T_0 lines, except the NT (non-transformed control), and that the highest response to *HPTII* was observed in Line 11. Furthermore, the results showed that Line 8 (1.32) exhibited the lowest response to GUS, while Line 9 (2.42) and Line 12 (1.43) exhibited the lowest responses to HPTII compared to the others (Fig. 14).

4. Discussion

Food security is one of the key challenges the world will face in the coming years. To mitigate the adverse effects of climate change and other factors, food production on existing arable land must be sustainably increased [44, 45]. Although breeding programs in recent years have led to a steady increase in production, the desired yield improvements have not yet been fully achieved. Therefore, biotechnological and molecular genetic methods are increasingly used to support breeding programs aimed at developing higher-yielding crops [46–48]. Plant tissue culture techniques play a significant role in facilitating genetic engineering, enabling rapid development of new breeding lines and varieties, and increasing crop productivity. Research on improving wheat using biotechnological tools is limited [49, 50]. The development of a reliable in vitro plant regeneration procedure for this important cereal is a prerequisite for its improvement through genetic transformation [51–53].

The success of an effective regeneration system depends on many factors, including genotype, explant type, culture medium content, and PGRs [54, 55]. Explant type directly affects the plant's growth capacity and morphogenetic response under in vitro conditions. In this study, endosperm-supported mature embryos were used as explants due to their structural stability, ability to maintain physiological integrity from the start of culture, and availability throughout the year (Figs. 1 and 2). The presence of endosperm tissue creates a suitable microenvironment by supporting nutrient transport, hormonal balance, and cellular signal transduction between the embryo and surrounding tissues, thus effectively increasing

culture success [56, 57]. Furthermore, this physical and chemical linkage maintains intrinsic auxin and cytokinin gradients, thereby conferring totipotency [58]. This result will facilitate the transformation of embryogenic tissues into complete plantlets.

The presence of PGRs in the medium affects every stage of the in vitro regeneration process [59]. Takamori et al. [60] reported promoting callus development by rapidly altering cellular metabolism. Our results were consistent with this scientific evidence. In our study, auxin-rich media induced callus formation in mature embryos supported by endosperm (Figure). In particular, the Kırik variety had the greatest effect on the MS4 medium's CFR (%) and CW (mg). Embryogenesis is induced by transferring callus obtained in an auxin-supplemented medium to a fresh culture medium containing low concentrations of PGRs or none at all [61]. In our study, callus tissues exhibited a greater number of embryogenic structures in a hormone-free medium (Fig. 1).

It was observed that callus tissues derived from the MS4 medium (4.4 mg/L picloram + 1.0 mg/L 2,4-D) exhibited high ECR (%), RECR (%), and RE (number) values in both the Kırik and Palandöken-97. In the second stage, the hormone-free and low-auxin (IAA)-containing MS4-1 and MS4-2 media increased embryogenic induction (Fig. 2). In contrast, although the MS6 medium contained 6.6 mg/L picloram + 1.5 mg/L 2,4-D, the efficacy of ECR and RECR was reduced in this medium. This finding was considered a significant indicator of how different combinations and concentrations of PGRs affect in vitro regeneration efficiency. Picloram is a synthetic auxin analog of indole-3-acetic acid (IAA), an endogenous plant hormone. Intracellularly, it activates the auxin signaling pathway by binding to the TIR1/AFB receptor complex. As a result of this binding, Aux/IAA proteins, which suppress the expression of auxin response genes, are rapidly degraded by ubiquitin-proteasomes. Thus, Auxin Response Factors (ARFs) are released, increasing the expression of auxin-sensitive genes. High concentrations of picloram activate this signaling pathway uncontrollably, leading to an imbalance in hormonal intermodulation and disrupting tissue differentiation [62–65]. It has been suggested that this situation may be one reason the RECR (%) ratio was low in our study, despite the high auxin levels in the IAA and MS4-2 media used for the Kırik variety. Therefore, picloram, when applied exogenously at appropriate concentrations, can effectively promote cell proliferation and, ultimately, be used effectively in embryogenesis processes and plant regeneration. In other plant species, picloram has also been effective in inducing embryogenesis at different concentrations: 2–4 mg/L in peach palm [66] and 7.5 mg/L in barley [67]. Barro et al. [68] reported in their study on wheat and barley that picloram induced more callus formation than 2,4-D. Furthermore, Aydin et al. [37] and Mahmood et al. [69] reported that various plant growth regulators (2,4-D, dicamba, IAA, NAA, picloram, BAP, and TDZ) in wheat have significant effects on all vital stages of plant regeneration (callus formation, embryogenesis, and plant establishment).

On the other hand, a positive relationship has been observed between the media in terms of ECR (%), specifically [MS1 (r = 0.45), MS2 (r = 0.39), MS3 (r = 0.36), MS4 (r = 0.33), MS5 (r = 0.29), and MS6 (r = 0.27)] (Fig). This result may indicate that an increase in the ECR ratio enhances the media's regeneration capacity. A decrease in correlation coefficients has been observed between ECR and RE, and between

RECR and RE. This situation demonstrates that RE is not solely dependent on ECR or regeneration capacity but can also be influenced by factors such as culture medium and genotype (Fig. 4).

The finding showed a high positive correlation between ECR and RECR across varieties (Kırik r = 0.92; Palandöken-97 r = 0.91), while moderate positive correlations were observed between ECR and RE and between RECR and RE. These findings were interpreted as indicating a strong relationship between ECR and RE, which varied depending on the genotype (Fig. 4).

The SE technique has been successfully applied in the *Agrobacterium*-mediated gene transfer system. Genes such as *SERK*, *WOX*, *LEC*, and *BBM1* regulate the SE process (totipotency, redifferentiation) [70–72]. Part of the research has shown that *WOX* regulates gene transcription during the initial induction phase of embryogenesis and, through this key role, acts as an inducer of somatic embryogenesis; that the *BBM1* gene is involved in the formation and development of somatic embryos; and that LEC genes initiate the transition from somatic embryo to plant [19, 71, 73–75]. Our results were consistent with these studies. In particular, it was observed that *WOX* exhibited distinct responses at 14 DAI in both varieties and under nearly all conditions, whereas the response decreased as callus age increased (28 and 42 DAI). Similarly, Nic-Can et al. [76] and Haecker et al. [77] reported *WOX4* expression at the onset of embryogenic development. The *BBM1* gene generally showed high expression during the mature embryo stage (28 DAI), whereas the *LEC1* gene showed high expression during the transition to the plant stage (42 DAI). Ucar and Aydın [78] found nearly identical responses of these genes in the Kırik wheat variety across callus ages.

Many studies have demonstrated that the *SERK* gene family (*TaSERK-1*, *-2*, *-3*, *-4*, *-5*, *-6*, *-7*, *-8*, *-9*, *-10*, *-11*, and *− 12*) serves as a tissue-level marker for cells capable of somatic embryogenesis in monocotyledonous plants [79–81]. In this study, embryogenic cell formations were obtained at 14 and 28 DAI, and *TaSERK* gene expression was highest in both varieties on these days. This result demonstrated that the MS4 medium, which had the highest ECR and RECR ratios, promoted the expression of *TaSERK* genes. Previous studies [80, 82–84] supported our findings. Singla et al. [85] emphasized that the *TaSERK1* gene in wheat is expressed during the embryogenic stage and in somatic tissues. Several studies [86–89] supported these findings by stating that the presence of auxin-based PGRs (2,4-D, picloram, dicamba, and IAA) transforms calli into a totipotent state capable of embryogenesis, and that *SERK* genes, by being expressed at this stage, preserve the pluripotent capacity in vascular tissues. According to our results, the expression profiles of SERK genes generally decreased at 42 DAI, the plant transition stage, in both varieties. This result provided evidence that *SERK* genes play a role in embryogenic development.

WOX4 generally showed a negative correlation with other genes (*LEC1* r = -0.50; *BBM1* r = -0.11). *LEC1* showed a negative correlation with *BBM1* and some *TaSERK* genes. In contrast, a positive correlation (r ≈ 0.39–0.79) was found between *BBM1* and most *TaSERK* genes (Fig.). Again, *TaSERK* genes largely exhibited positive correlations. These results suggest that genes exhibiting genotype-, callus age-, and

medium-condition-dependent positive correlations may be part of common regulatory networks in SE and regeneration processes.

One key factor in the development of an effective regeneration system is the occurrence of somaclonal variation in regenerative plants [90]. This is an undesirable situation, particularly in gene transfer studies, as it can lead to random and uncontrolled genetic changes beyond the intended gene transfer. The resulting variations may mask the effect of a specific gene or lead to mis-expression, causing genotypic and phenotypic inconsistencies and the emergence of undesirable morphological traits in plants regenerated from calli, somatic embryos, and organ cultures [91]. In the study, somaclonal variation was evaluated separately within each variety across growth media. Regenerative plants obtained from various media were screened for genetic variation using the SCoT approach. SCoT markers were used to detect somaclonal variations in species such as *Stevia rebaudiana* Bertoni [90], *Calligonum polygonoides* [92], *Rheum ribes* L. [36], and *Rumex hastatus* [9].

The polymorphism rate observed in this study ranged from 96.88% to 75.76% (Table S4). It is believed that the likely cause of polymorphism is the stress induced in the regenerative plants by all components present under in vitro culture conditions [31, 93]. Reactive oxygen species (ROS) generated by stress lead to genomic instability [94, 95]. In both the Kırik and Palandöken-97 varieties, the MS1-1 (12.0 mg/L dicamba + 0.5 mg/L IAA), MS3-1 (2.2 mg/L picloram + 5.0 mg/L 2,4-D), and MS4-1 (4.4 mg/L picloram + 1.0 mg/L 2,4-D) media. The other media showed less similarity. It was hypothesized that this result was due to an excess of exogenous auxin, in addition to endogenous auxins, which continuously and uncontrollably activated the signaling pathway, leading to variations [62, 63].

Gene transfer via *Agrobacterium* is the most commonly used method in plant genetic transformation studies and has been successfully applied in many plant species and in highly complex genomes [96–99]. Cereal crops, and wheat in particular, have very large (≈17 Gbp) [100, 101]and highly complex genomes, which have caused them to lag behind dicotyledonous plants in *Agrobacterium*-mediated gene transfer studies. Therefore, to achieve efficient genetic transformation in monocotyledonous species such as wheat, an effective plant regeneration system and an appropriate transformation protocol are required. In this study, based on data obtained from in vitro regeneration optimization studies, the variety Kırik was identified as having the highest success rate, and MS4 was determined to be the most effective method; this combination was used throughout the gene transformation studies.

Just as it is important to develop an effective in vitro regeneration system for genetic transformation studies, it is also crucial to ensure the efficient integration of T-DNA into plant DNA using the *Agrobacterium*-mediated gene transfer method, thereby increasing transformation efficiency. Gene transfer into plants using the Agrobacterium method generally consists of the following stages: bacterial preparation, inoculation, co-cultivation, and selection. In this process, factors such as *Agrobacterium* density, acetosyringone (AS) concentration, inoculation and co-cultivation conditions, and the antibiotics, BBDs, and their concentrations used in the selection stage influence wheat gene transfer. In every plant species, optimizing each of these factors is a fundamental requirement for successful gene transfer

[102–104]. Just as it is important to develop an effective in vitro regeneration system for genetic transformation studies, it is also crucial to ensure the efficient integration of T-DNA into plant DNA using the Agrobacterium-mediated gene transfer method, thereby increasing transformation efficiency. Gene transfer into plants using the Agrobacterium method generally consists of the following stages: bacterial preparation, inoculation, co-cultivation, and selection. In this process, factors such as callus age, *Agrobacterium* density, acetosyringon (AS) concentration, inoculation and co-cultivation conditions, as well as the antibiotics and PGRs used in the selection stage, influence wheat gene transfer. In every plant species, the optimization of each of these factors is a fundamental requirement for successful gene transfer [102–104].

In this study, 10-day-old calli were used. Similarly, studies have reported that young calli possess higher transformation and regeneration capacities [105–107]. *Agrobacterium tumefaciens* is activated by signals such as acetosyringone (AS) secreted by wounded plants, which induces the *vir* genes and transfers the T-DNA into the plant cell [108]. Since cereals are not natural hosts, 200 µM AS was used in inoculation and co-cultivation media for the induction of *vir* genes in this study (Table). IAA (5.75 µM) was used in the selection medium to enhance plant formation. It was hypothesized that this was due to the rapid induction of Aux/IAA genes [109]. We obtained 12 T_0 plants from the selection medium and determined transformation efficiency using *HPTII* and *GUS* (Fig). Gene expression was increased in all 12 plants compared to control plants.

5. Conclusion

In this study, the effects of six different MS media (MS1–MS6) on in vitro responses, gene expression patterns (*WOX4*, *BBM1*, *LEC1*, and the *TaSERK* family), and somaclonal variation in the wheat varieties Kırik and Palandöken-97 were comprehensively evaluated. The results revealed that the composition of culture media significantly influenced callus induction, regeneration capacity, and gene expression profiles. Among the tested media and varieties, Kırik and MS4 exhibited favorable effects on both morphogenic responses and gene responses associated with embryogenesis.

Based on these findings, the MS4 medium was selected to facilitate Agrobacterium-mediated rapid transformation of the Kırık variety. Successful integration of the transformation was confirmed using primers specific to the NPTII and GUS genes, thereby validating the efficiency of the transformation system.

Overall, this study highlights the critical role of optimizing in vitro culture media in enhancing regeneration efficiency and transformation success. The combined evaluation of morphogenic, molecular, and genetic stability parameters provides a robust framework for improving genetic transformation protocols in wheat.

Declarations

Acknowledgements

The authors thank BAP for its support. Additionally, the first author was supported by the Council of Higher Education (YÖK) 100/2000 and BİDEB-2211A doctoral fellowship programs.

Funding

This study was supported by Atatürk University Scientific Research Projects (BAP) under the Grant Number 13483.

Author Contributions

S.U., E.İ., and M.A. proposed the idea and designed the study; S.U., A.G.K., and S.M. conducted the experiments; S.U. and E.İ. analyzed the data; E.İ., E.Y., and S.U. reviewed the manuscript together with S.U. All authors read and approved the final manuscript.

Availability of data

The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Clinical Trial Number

Not applicable.

Code availability

Not applicable.

Declarations

Conflict of interest

The authors declare no conflicts of interest.

Consent for publication

The authors give the journal consent to publish the paper.

Ethical standards

Human Ethics and Consent to Participate declarations: Not applicable.

References

1. Gupta P, Balyan H, Sharma S, Kumar R. Genetics of yield, abiotic stress tolerance and biofortification in wheat (Triticum aestivum L.). Theoretical and Applied Genetics. 2020;133(5):1569-602. doi: 10.1007/s00122-020-03583-3.
2. Abedi T, Mojiri A. Cadmium Uptake by Wheat (Triticum aestivum L.): An Overview. Plants-Basel. 2020;9(4). doi: 10.3390/plants9040500.
3. Iqbal M, Rahim J, Naeem W, Hassan S, Khattab Y, El-Sabagh A. RAINFED WINTER WHEAT (TRITICUM AESTIVUM L.) CULTIVARS RESPOND DIFFERENTLY TO INTEGRATED FERTILIZATION IN PAKISTAN. Fresenius Environmental Bulletin. 2021;30(4):3115-21.
4. Zhu P, Burney J, Chang J, Jin Z, Mueller N, Xin Q, et al. Warming reduces global agricultural production by decreasing cropping frequency and yields. Nature Climate Change. 2022;12(11):1016-+. doi: 10.1038/s41558-022-01492-5.
5. Basu P, Mandal M, Jana S, Biswas A, Ray S. Impact of anthropogenic activities on global warming and energy demand. The Intersection of Global Energy Politics and Climate Change: A Comprehensive Analysis of Energy Markets and Economics. 2025:63-81.
6. Toomer O, Oviedo E, Ali M, Patino D, Joseph M, Frinsko M, et al. Current Agronomic Practices, Harvest & Post-Harvest Processing of Soybeans (Glycine max)-A Review. Agronomy-Basel. 2023;13(2). doi: 10.3390/agronomy13020427.
7. Alexandratos N, Bruinsma J. World agriculture towards 2030/2050: the 2012 revision. 2012.
8. Kaur Sarao LK, Srivastava P, Singh Bakala HS, Mandahal KS. Breeding Wheat for Biotic Stress Resistance: Achievements, Challenges and Prospects. In: Ansari M-u-R, editor. Current Trends in Wheat Research. London: IntechOpen; 2021.
9. Singh C, Yadav S, Khare V, Gupta V, Patial M, Kumar S, et al. Wheat Drought Tolerance: Unveiling a Synergistic Future with Conventional and Molecular Breeding Strategies. Plants-Basel. 2025;14(7). doi: 10.3390/plants14071053.
10. Aydın M, Haliloğlu K, Tosun M. Buğday Doku Kültüründe Alternatif Eksplant Kaynağı: Olgun Embriyo. Ziraat Fakültesi Dergisi. 2009;4(2):34-45.
11. Bie X, Wang K, Liu C, Liu Y, Du L, Mao X, et al. EFFECTS OF SOIL DROUGHT STRESS ON PLANT REGENERATION EFFICIENCY AND ENDOGENOUS HORMONE LEVELS OF IMMATURE EMBRYOS IN WHEAT (&ITTRITICUM AESTIVUM&IT L.). Pakistan Journal of Botany. 2017;49(5):1673-9.
12. Zhao B, Wu T, Ma S, Jiang D, Bie X, Sui N, et al. TaD27-B gene controls the tiller number in hexaploid wheat. Plant Biotechnology Journal. 2020;18(2):513-25. doi: 10.1111/pbi.13220.
13. Malik S, Rashid H, Yasmin T, Minhas N. Plant regeneration by somatic embryogenesis from callus of mature seed explants of bread wheat (Triticum aestivum L.). Pakistan Journal of Botany. 2004;36(3):629-34.
14. Zale J, Agarwal S, Loar S, Steber C. Evidence for stable transformation of wheat by floral dip in Agrobacterium tumefaciens. Plant Cell Reports. 2009;28(6):903-13. doi: 10.1007/s00299-009-0696-0.

15. Chatha WA, Rashid U, Malik MF. Optimization of Agrobacterium tumefaciens-mediated transformation in wheat using mature embryos.
16. Kereša S, Barić M, Šarčević H, Marchetti S, Drezner G. Callus induction and plant regeneration from immature and mature embryos of winter wheat (Triticum aestivum L.) genotypes. Book of Abstracts of XVIth EUCARPIA Congress" Plant Breeding: Sustaining the Future": Edinburgh:; 2001. p. 41-.
17. Moyo M, Finnie J, Van Staden J. In vitro morphogenesis of organogenic nodules derived from Sclerocarya birrea subsp caffra leaf explants. Plant Cell Tissue and Organ Culture. 2009;98(3):273-80. doi: 10.1007/s11240-009-9559-1.
18. Bhatia S, Sharma K, Dahiya R, Bera T. Modern applications of plant biotechnology in pharmaceutical sciences. Academic press; 2015.
19. Wojcik A, Krypczyk K, Buchcik W, Gaj M. From root to embryogenic transition: WOX5 reprograms plant somatic cells via auxin-mediated pathways. Bmc Plant Biology. 2025;25(1). doi: 10.1186/s12870-025-06687-4.
20. Koehler AD, Irsigler AST, Carneiro VTC, Cabral GB, Rodrigues JCM, Gomes ACMM, et al. SERK genes identification and expression analysis during somatic embryogenesis and sporogenesis of sexual and apomictic Brachiaria brizantha (Syn. Urochloa brizantha). Planta. 2020;252(3):39. doi: 10.1007/s00425-020-03443-w.
21. Mahdavi-Darvari F, Noor N, Ismanizan I. Epigenetic regulation and gene markers as signals of early somatic embryogenesis. Plant Cell Tissue and Organ Culture. 2015;120(2):407-22. doi: 10.1007/s11240-014-0615-0.
22. Ramakrishnan M, Zhou M, Ceasar SA, Ali DJ, Maharajan T, Vinod KK, et al. Epigenetic modifications and miRNAs determine the transition of somatic cells into somatic embryos. Plant Cell Rep. 2023;42(12):1845-73. doi: 10.1007/s00299-023-03071-0.
23. Desai P, Desai S, Rafaliya R, Patil G. Chapter 5 - Plant tissue culture: Somatic embryogenesis and organogenesis. In: Chandra Rai A, Kumar A, Modi A, Singh M, editors. Advances in Plant Tissue Culture. Academic Press; 2022. p. 109-30.
24. Phillips G, Garda M. Plant tissue culture media and practices: an overview. In Vitro Cellular & Developmental Biology-Plant. 2019;55(3):242-57. doi: 10.1007/s11627-019-09983-5.
25. Kumar R, Mamrutha H, Kaur A, Venkatesh K, Grewal A, Tiwari V. Development of an efficient and reproducible regeneration system in wheat (Triticum aestivum L.). Physiology and Molecular Biology of Plants. 2017;23(4):945-54. doi: 10.1007/s12298-017-0463-6.
26. Phogat S, Poudel A, Bhurta R, Kalwan G, Madhavan J, Padaria J, et al. An improved in-vitro regeneration protocol using scutellum of mature and immature embryos of wheat (Triticum aestivum L.). Indian Journal of Genetics and Plant Breeding. 2023;83(2):195-204. doi: 10.31742/ISGPB.83.2.5.
27. Su Y, McGrath S, Zhao F. Rice is more efficient in arsenite uptake and translocation than wheat and barley. Plant and Soil. 2010;328(1-2):27-34. doi: 10.1007/s11104-009-0074-2.

28. Orlowska R. Barley somatic embryogenesis-an attempt to modify variation induced in tissue culture. Journal of Biological Research-Thessaloniki. 2021;28(1). doi: 10.1186/s40709-021-00138-5.
29. Leva A, Petruccelli R, Rinaldi LMR. Somaclonal Variation in Tissue Culture: A Case Study with Olive. In: Leva A, Rinaldi L, editors. Recent Advances in Plant in vitro Culture. London: IntechOpen; 2012.
30. Aydin M, Taspinar M, Arslan E, Sigmaz B, Agar G. AUXIN EFFECTS ON SOMACLONAL VARIATION AND PLANT REGENERATION FROM MATURE EMBRYO OF BARLEY (HORDEUM VULGARE L.). Pakistan Journal of Botany. 2015;47(5):1749-57.
31. Ucar S, Aldaif M, Yaprak E, Yigider E, Aydin M, Ilhan E, et al. Optimizing In Vitro Regeneration of Wheat via Somatic Embryogenesis Using Endosperm-Supported Mature Embryos. Phyton-International Journal of Experimental Botany. 2025;94(8):2461-77. doi: 10.32604/phyton.2025.068383.
32. Kudikala H, Jogam P, Sirikonda A, Mood K, Allini VR. In vitro micropropagation and genetic fidelity studies using SCoT and ISSR primers in Annona reticulata L.: an important medicinal plant. Vegetos. 2020;33(3):446-57. doi: 10.1007/s42535-020-00128-3.
33. Tikendra L, Potshangbam A, Dey A, Devi T, Sahoo M, Nongdam P. RAPD, ISSR, and SCoT markers based genetic stability assessment of micropropagated Dendrobium fimbriatum Lindl. var. oculatum Hk. f.- an important endangered orchid. Physiology and Molecular Biology of Plants. 2021;27(2):341-57. doi: 10.1007/s12298-021-00939-x.
34. Etminan A, Pour-Aboughadareh A, Mohammadi R, Ahmadi-Rad A, Noori A, Mahdavian Z, et al. Applicability of start codon targeted (SCoT) and inter-simple sequence repeat (ISSR) markers for genetic diversity analysis in durum wheat genotypes. Biotechnology & Biotechnological Equipment. 2016;30(6):1075-81. doi: 10.1080/13102818.2016.1228478.
35. Güngör H, Ilhan E, Kasapoglu A, Filiz E, Hossein-Pour A, Valchev D, et al. Genetic Diversity and Population Structure of Barley Cultivars Released in Türkiye and Bulgaria using iPBS-retrotransposon and SCoT markers. Journal of Agricultural Sciences-Tarim Bilimleri Dergisi. 2022;28(2):239-50. doi: 10.15832/ankutbd.886221.
36. Aldaif M, Ucar S, Yigider E, Aydin M, Kasapoglu A, Ilhan E. Micropropagation and genetic fidelity of Rheum ribes L.: a threatened and endemic medicinal herb from the Turkish Eastern Anatolia region. Plant Cell Tissue and Organ Culture. 2025;162(1). doi: 10.1007/s11240-025-03111-5.
37. Aydın M, Tosun M, Haliloğlu K. Plant regeneration in wheat mature embryo culture. African Journal of Biotechnology. 2011;10(70).
38. Murashige T, Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia plantarum. 1962;15(3).
39. Sharma N, Khurana P. Genome-Wide Identification and Analysis of the TaSERK Gene Family in Bread Wheat Triticum aestivum L. and TaSERK8 Overexpression Study in Rice. Journal of Plant Growth Regulation. 2023;42(7):4131-49. doi: 10.1007/s00344-022-10877-x.
40. Singh A, Khurana P. Ectopic expression of Triticum aestivum SERK genes (TaSERKs) control plant growth and development in Arabidopsis. Scientific Reports. 2017;7. doi: 10.1038/s41598-017-

10038-1.

41. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25(4):402-8. doi: 10.1006/meth.2001.1262.
42. Cota-Sánchez J, Remarchuk K, Ubayasena K. Ready-to-use DNA extracted with a CTAB method adapted for herbarium specimens and mucilaginous plant tissue. Plant Molecular Biology Reporter. 2006;24(2):161-7. doi: 10.1007/BF02914055.
43. Collard B, Mackill D. Start Codon Targeted (SCoT) Polymorphism: A Simple, Novel DNA Marker Technique for Generating Gene-Targeted Markers in Plants. Plant Molecular Biology Reporter. 2009;27(1):86-93. doi: 10.1007/s11105-008-0060-5.
44. Prosekov AY, Ivanova SA. Food security: The challenge of the present. Geoforum. 2018;91:73-7. doi: https://doi.org/10.1016/j.geoforum.2018.02.030.
45. Saleem A, Anwar S, Nawaz T, Fahad S, Saud S, Ur Rahman T, et al. Securing a sustainable future: the climate change threat to agriculture, food security, and sustainable development goals. Journal of Umm Al-Qura University for Applied Sciences. 2025;11(3):595-611. doi: 10.1007/s43994-024-00177-3.
46. Khare V, Khare A. Modern approach in animal breeding by use of advanced molecular genetic techniques. Int J Livest Res. 2017;7(1).
47. Das DN, Paul D, Mondal S. Chapter Thirteen - Role of biotechnology on animal breeding and genetic improvement. In: Mondal S, Singh RL, editors. Emerging Issues in Climate Smart Livestock Production. Academic Press; 2022. p. 317-37.
48. Zulkarnain Z, Tapingkae T, Taji A. Applications of In Vitro Techniques in Plant Breeding. In: Al-Khayri JM, Jain SM, Johnson DV, editors. Advances in Plant Breeding Strategies: Breeding, Biotechnology and Molecular Tools. Cham: Springer International Publishing; 2015. p. 293-328.
49. Wijerathna-Yapa A, Ramtekey V, Ranawaka B, Basnet B. Applications of In Vitro Tissue Culture Technologies in Breeding and Genetic Improvement of Wheat. Plants-Basel. 2022;11(17). doi: 10.3390/plants11172273.
50. Koti P, Bill T. Plant tissue culture and genetic transformation in crop improvement. J Bacteriol Mycol Open Access. 2025;13(1):61-9.
51. Kruglova NN, Zinatullina AE. In Vitro Culture of Autonomous Embryos as a Model System for the Study of Plant Stress Tolerance to Abiotic Factors (on the Example of Cereals). Biology Bulletin Reviews. 2022;12(2):201-11. doi: 10.1134/S2079086422020050.
52. Alshaharni M, Tahir M, Abro A, Farooq M. EMPOWERING WHEAT GROWTH: ESTABLISHING A PROTOCOL FOR EFFICIENT PLANT REGENERATION. Applied Ecology and Environmental Research. 2024;22(5):4867-75. doi: 10.15666/aeer/2205_48674875.
53. Nowak K, Wójcikowska B, Gajecka M, Elzbieciak A, Moronczyk J, Wójcik A, et al. The improvement of the in vitro plant regeneration in barley with the epigenetic modifier of histone acetylation, trichostatin A. Journal of Applied Genetics. 2024;65(1):13-30. doi: 10.1007/s13353-023-00800-9.

54. Jayusman, Hakim L, Dalimunthe A. Season, basal media and plant growth regulators effect in wood plant in vitro propagation: a comprehensive review. IOP Conference Series: Earth and Environmental Science. 2022;1115(1):012051. doi: 10.1088/1755-1315/1115/1/012051.
55. Akter K, Tasmin S, Yeasmin S, Islam M, Alam M, Mehedi M. Optimization of a basic and resource efficient regeneration protocol of tomatoes. Bangladesh Journal of Nuclear Agriculture. 2025;39(1):61-75.
56. Ahansal K, Abdelwahd R, Udupa S, Aadel H, Gaboun F, Ibriz M, et al. EFFECT OF TYPE OF MATURE EMBRYO EXPLANTS AND ACETOSYRINGONE ON AGROBACTERIUM-MEDIATED TRANSFORMATION OF MOROCCAN DURUM WHEAT. Bioscience Journal. 2022;38. doi: 10.14393/BJ-v38n0a2022-54513.
57. Liu H, Ma H, Zhang W, Wang W, Wu J, Wang K, et al. Identification of three wheat near isogenic lines originated from CB037 on tissue culture and transformation capacities. Plant Cell Tissue and Organ Culture. 2023;152(1):67-79. doi: 10.1007/s11240-022-02389-z.
58. Sundararajan S, Sivakumar H, Nayeem S, Rajendran V, Subiramani S, Ramalingam S. Influence of exogenous polyamines on somatic embryogenesis and regeneration of fresh and long-term cultures of three elite indica rice cultivars. Cereal Research Communications. 2021;49(2):245-53. doi: 10.1007/s42976-020-00098-x.
59. Vondráková Z, Krajňáková J, Fischerová L, Vágner M, Eliášová K. Physiology and role of plant growth regulators in somatic embryogenesis. Vegetative propagation of forest trees. 2016:123-69.
60. Takamori L, Neto N, Vieira L, Ribas A. Optimization of somatic embryogenesis and in vitro plant regeneration of Urochloa species using picloram. In Vitro Cellular & Developmental Biology-Plant. 2015;51(5):554-63. doi: 10.1007/s11627-015-9701-1.
61. Deo PC, Tyagi AP, Taylor M, Harding R, Becker D. Factors affecting somatic embryogenesis and transformation in modern plant breeding. The South Pacific Journal of Natural and Applied Sciences. 2011;28(1):27-40.
62. Prigge M, Greenham K, Zhang Y, Santner A, Castillejo C, Mutka A, et al. The Arabidopsis Auxin Receptor F-Box Proteins AFB4 and AFB5 Are Required for Response to the Synthetic Auxin Picloram. G3-Genes Genomes Genetics. 2016;6(5):1383-90. doi: 10.1534/g3.115.025585.
63. Delgado-Aceves L, Corona S, Castañeda-Nava J, Rodríguez-Domínguez J, Gutiérrez-Mora A. Indirect somatic embryogenesis of Agave maximiliana Baker. Frontiers in Plant Science. 2025;16. doi: 10.3389/fpls.2025.1648362.
64. Bell J, Schmitzer P, Walsh T, Jeschke P, Witschel M, Kramer W, et al. The Molecular Mode of Action of Auxin Herbicides. Modern Crop Protection Compounds, Vols 1-3, 3rd Edition. 2019:303-18.
65. Wahyuni FD, Fathoni A, Sukma D, Ardie SW, Zainuddin IM, Qi B, et al. Optimizing somatic embryogenesis in Indonesian cassava genotypes: Effects of 2, 4-D or Picloram supplementation and explant type. Plant Biotechnology. 2026;43(1):27-36.
66. Steinmacher D, Cangahuala-Inocente G, Clement C, Guerra M. Somatic embryogenesis from peach palm zygotic embryos. In Vitro Cellular & Developmental Biology-Plant. 2007;43(2):124-32. doi:

10.1007/s11627-007-9032-y.

67. Sener O, Can E, Arslan M, Çeliktas N. EFFECTS OF GENOTYPE AND PICLORAM CONCENTRATIONS ON CALLUS INDUCTION AND PLANT REGENERATION FROM IMMATURE INFLORESCENCE OF SPRING BARLEY CULTIVARS (HORDEUM VULGARE L.). Biotechnology & Biotechnological Equipment. 2008;22(4):915-20.
68. Barro F, Martin A, Lazzeri P, Barceló P. Medium optimization for efficient somatic embryogenesis and plant regeneration from immature inflorescences and immature scutella of elite cultivars of wheat, barley and tritordeum. Euphytica. 1999;108(3):161-7. doi: 10.1023/A:1003676830857.
69. Mahmood I, Razzaq A, Khan Z, Hafız I, Kaleem S. EVALUATION OF TISSUE CULTURE RESPONSES OF PROMISING WHEAT (TRITICUM AESTIVUM L.) CULTIVARS AND DEVELOPMENT OF EFFICIENT REGENERATION SYSTEM. Pakistan Journal of Botany. 2012;44:277-84.
70. Guo F, Liu C, Xia H, Bi Y, Zhao C, Zhao S, et al. Induced Expression of AtLEC1 and AtLEC2 Differentially Promotes Somatic Embryogenesis in Transgenic Tobacco Plants. Plos One. 2013;8(8). doi: 10.1371/journal.pone.0071714.
71. Wójcikowska B, Jaskóla K, Gasiorek P, Meus M, Nowak K, Gaj M. LEAFY COTYLEDON2 (LEC2) promotes embryogenic induction in somatic tissues of Arabidopsis, via YUCCA-mediated auxin biosynthesis. Planta. 2013;238(3):425-40. doi: 10.1007/s00425-013-1892-2.
72. Boulard C, Fatihi A, Lepiniec L, Dubreucq B. Regulation and evolution of the interaction of the seed B3 transcription factors with NF-Y subunits. Biochimica Et Biophysica Acta-Gene Regulatory Mechanisms. 2017;1860(10):1069-78. doi: 10.1016/j.bbagrm.2017.08.008.
73. Gambino G, Minuto M, Boccacci P, Perrone I, Vallania R, Gribaudo I. Characterization of expression dynamics of WOX homeodomain transcription factors during somatic embryogenesis in Vitis vinifera. Journal of Experimental Botany. 2011;62(3):1089-101. doi: 10.1093/jxb/erq349.
74. Rupps A, Raschke J, Rümmler M, Linke B, Zoglauer K. Identification of putative homologs of Larix decidua to BABYBOOM (BBM), LEAFY COTYLEDON1 (LEC1), WUSCHEL-related HOMEOBOX2 (WOX2) and SOMATIC EMBRYOGENESIS RECEPTOR-like KINASE (SERK) during somatic embryogenesis. Planta. 2016;243(2):473-88. doi: 10.1007/s00425-015-2409-y.
75. Chen B, Maas L, Figueiredo D, Zhong Y, Reis R, Li M, et al. BABY BOOM regulates early embryo and endosperm development. Proceedings of the National Academy of Sciences of the United States of America. 2022;119(25). doi: 10.1073/pnas.2201761119.
76. Nic-Can G, López-Torres A, Barredo-Pool F, Wrobel K, Loyola-Vargas V, Rojas-Herrera R, et al. New Insights into Somatic Embryogenesis: LEAFY COTYLEDON1, BABY BOOM1 and WUSCHEL-RELATED HOMEOBOX4 Are Epigenetically Regulated in Coffea canephora. Plos One. 2013;8(8). doi: 10.1371/journal.pone.0072160.
77. Haecker A, Gross-Hardt R, Geiges B, Sarkar A, Breuninger H, Herrmann M, et al. Expression dynamics of WOX genes mark cell fate decisions during early embryonic patterning in Arabidopsis thaliana. Development. 2004;131(3):657-68. doi: 10.1242/dev.00963.

78. Ucar S, Aydin M. Effects of Epigenetic Inhibitors on Somatic Embryogenesis in Wheat (Triticum aestivum L.). Iranian Journal of Science. 2025;49(1):1-10. doi: 10.1007/s40995-024-01679-9.
79. Somleva M, Schmidt E, de Vries S. Embryogenic cells in Dactylis glomerata L. (Poaceae) explants identified by cell tracking and by SERK expression. Plant Cell Reports. 2000;19(7):718-26. doi: 10.1007/s002999900169.
80. Hecht V, Vielle-Calzada J, Hartog M, Schmidt E, Boutilier K, Grossniklaus U, et al. The Arabidopsis SOMATIC EMBRYOGENESIS RECEPTOR KINASE 1 gene is expressed in developing ovules and embryos and enhances embryogenic competence in culture. (vol 127, pg 803, 2001). Plant Physiology. 2002;128(1):314-.
81. Pérez-Núñez M, Souza R, Sáenz L, Chan J, Zúñiga-Aguilar J, Oropeza C. Detection of a SERK-like gene in coconut and analysis of its expression during the formation of embryogenic callus and somatic embryos. Plant Cell Reports. 2009;28(1):11-9. doi: 10.1007/s00299-008-0616-8.
82. Orlowska A, Igielski R, Lagowska K, Kepczynska E. Identification of LEC1, L1L and Polycomb Repressive Complex 2 genes and their expression during the induction phase of Medicago truncatula Gaertn. somatic embryogenesis. Plant Cell Tissue and Organ Culture. 2017;129(1):119-32. doi: 10.1007/s11240-016-1161-8.
83. Baudino S, Hansen S, Brettschneider R, Hecht V, Dresselhaus T, Lörz H, et al. Molecular characterisation of two novel maize LRR receptor-like kinases, which belong to the SERK gene family. Planta. 2001;213(1):1-10. doi: 10.1007/s004250000471.
84. Thomas C, Meyer D, Himber C, Steinmetz A. Spatial expression of a sunflower SERK gene during induction of somatic embryogenesis and shoot organogenesis. Plant Physiology and Biochemistry. 2004;42(1):35-42. doi: 10.1016/j.plaphy.2003.10.008.
85. Singla B, Khurana JP, Khurana P. Structural Characterization and Expression Analysis of the SERK/SERL Gene Family in Rice (Oryza sativa). International Journal of Plant Genomics. 2009;2009(1):539402. doi: https://doi.org/10.1155/2009/539402.
86. Kwaaitaal M, de Vries S. The SERK1 gene is expressed in procambium and immature vascular cells. Journal of Experimental Botany. 2007;58(11):2887-96. doi: 10.1093/jxb/erm103.
87. Verdeil J, Alemanno L, Niemenak N, Tranbarger T. Pluripotent versus totipotent plant stem cells: dependence versus autonomy? Trends in Plant Science. 2007;12(6):245-52. doi: 10.1016/j.tplants.2007.04.002.
88. Ćosić T, Raspor M. The Role of Auxin and Cytokinin Signaling Components in de novo Shoot Organogenesis. In: Aftab T, editor. Auxins, Cytokinins and Gibberellins Signaling in Plants. Cham: Springer International Publishing; 2022. p. 47-75.
89. Bennur P, O'Brien M, Fernando S, Doblin M. Improving transformation and regeneration efficiency in medicinal plants: insights from other recalcitrant species. Journal of Experimental Botany. 2024;76(1):52-75. doi: 10.1093/jxb/erae189.
90. Dyduch-Sieminska M, Gawronski J. In Vitro Regeneration of Stevia rebaudiana Bertoni Using Somaclonal Variation as a Tool for Genetic Diversification. Genes. 2025;16(10). doi:

10.3390/genes16101203.

91. Gawronski J, Dyduch-Sieminska M. Potential of In Vitro Culture of Scutellaria baicalensis in the Formation of Genetic Variation Confirmed by ScoT Markers. Genes. 2022;13(11). doi: 10.3390/genes13112114.
92. Faroda P, Gautam N, Gupta A, Shekhawat N. An efficient micropropagation protocol with anatomy and genetic homogeneity assessment of Calligonum polygonoides Linn.: an endangered and woody species of Rajasthan. Plant Cell Tissue and Organ Culture. 2025;160(3). doi: 10.1007/s11240-025-02963-1.
93. Joyce SM, Cassells AC, Mohan Jain S. Stress and aberrant phenotypes in vitro culture. Plant Cell, Tissue and Organ Culture. 2003;74(2):103-21. doi: 10.1023/A:1023911927116.
94. Wani K, Naeem M, Castroverde C, Kalaji H, Albaqami M, Aftab T. Molecular Mechanisms of Nitric Oxide (NO) Signaling and Reactive Oxygen Species (ROS) Homeostasis during Abiotic Stresses in Plants. International Journal of Molecular Sciences. 2021;22(17). doi: 10.3390/ijms22179656.
95. Dutta S, Mitra M, Agarwal P, Mahapatra K, De S, Sett U, et al. Oxidative and genotoxic damages in plants in response to heavy metal stress and maintenance of genome stability. Plant Signaling & Behavior. 2018;13(8). doi: 10.1080/15592324.2018.1460048.
96. Wan L, Wang Z, Zhang X, Zeng H, Ren J, Zhang N, et al. Optimised Agrobacterium-Mediated Transformation and Application of Developmental Regulators Improve Regeneration Efficiency in Melons. Genes. 2023;14(7). doi: 10.3390/genes14071432.
97. Ali I, Salah K, Sher H, Ali H, Ullah Z, Ali A, et al. Drought stress enhances the efficiency of floral dip method of Agrobacterium-mediated transformation in Arabidopsis thaliana. Brazilian Journal of Biology. 2024;84:e259326.
98. Liu M, Yang Y, Liang T, Hou F, Zhang M, He S, et al. Dynamic transcriptome and GWAS uncover a hydroxyproline-rich glycoprotein that suppresses Agrobacterium-mediated transformation in maize. Molecular Plant. 2025;18(5):747-64. doi: https://doi.org/10.1016/j.molp.2025.03.011.
99. Park EJ, Sim J-R, Yang Y-J, Lee SB, Kim B-G, Kim S, et al. Agrobacterium-mediated transformation of wheat. Korean Society of Breeding Science. 2022;54(4):358-68.
100. Feldman M, Levy AA. Genome Structure of Triticeae Species. Wheat Evolution and Domestication. Cham: Springer International Publishing; 2023. p. 43-70.
101. Begum A, Kiruthika K, Rajamani K. Genome-wide in-silico identification of putative small chloroplast open reading frames (ORFs) in wheat with potential roles in stress adaptation. Genetic Resources and Crop Evolution. 2025;73(1). doi: 10.1007/s10722-025-02670-y.
102. Jones H, Doherty A, Wu H. Review of methodologies and a protocol for the Agrobacterium-mediated transformation of wheat. Plant Methods. 2005;1. doi: 10.1186/1746-4811-1-5.
103. Hayta S, Smedley M, Demir S, Blundell R, Hinchliffe A, Atkinson N, et al. An efficient and reproducible Agrobacterium- mediated transformation method for hexaploid wheat (Triticum aestivum L.) (vol 15, 121, 2019). Plant Methods. 2019;15(1). doi: 10.1186/s13007-019-0540-7.

104. Azizi-Dargahlou S, Pouresmaeil M. Agrobacterium tumefaciens-Mediated Plant Transformation: A Review. Molecular Biotechnology. 2024;66(7):1497-+. doi: 10.1007/s12033-023-00788-x.
105. Rahman Z, Seman Z, Basirun N, Julkifle A, Zainal Z, Subramaniam S. Preliminary investigations of Agrobacterium-mediated transformation in indica rice MR219 embryogenic callus using gusA gene. African Journal of Biotechnology. 2011;10(40):7805-13.
106. Jan N, Hussain S, Naseer B, Bhat T. Amaranth and quinoa as potential nutraceuticals: A review of anti-nutritional factors, health benefits and their applications in food, medicinal and cosmetic sectors. Food Chemistry-X. 2023;18. doi: 10.1016/j.fochx.2023.100687.
107. Kan J, Cai Y, Cheng C, Chen S, Jiang C, He Z, et al. CRISPR/Cas9-guided knockout of eIF4E improves Wheat yellow mosaic virus resistance without yield penalty. Plant Biotechnol J. 2023;21(5):893-5. doi: 10.1111/pbi.14002.
108. Xi J, Patel M, Dong S, Que Q, Qu R. Acetosyringone treatment duration affects large T-DNA molecule transfer to rice callus. Bmc Biotechnology. 2018;18. doi: 10.1186/s12896-018-0459-5.
109. Reed J. Roles and activities of Aux/IAA proteins in Arabidopsis. Trends in Plant Science. 2001;6(9):420-5. doi: 10.1016/S1360-1385(01)02042-8.

Figures

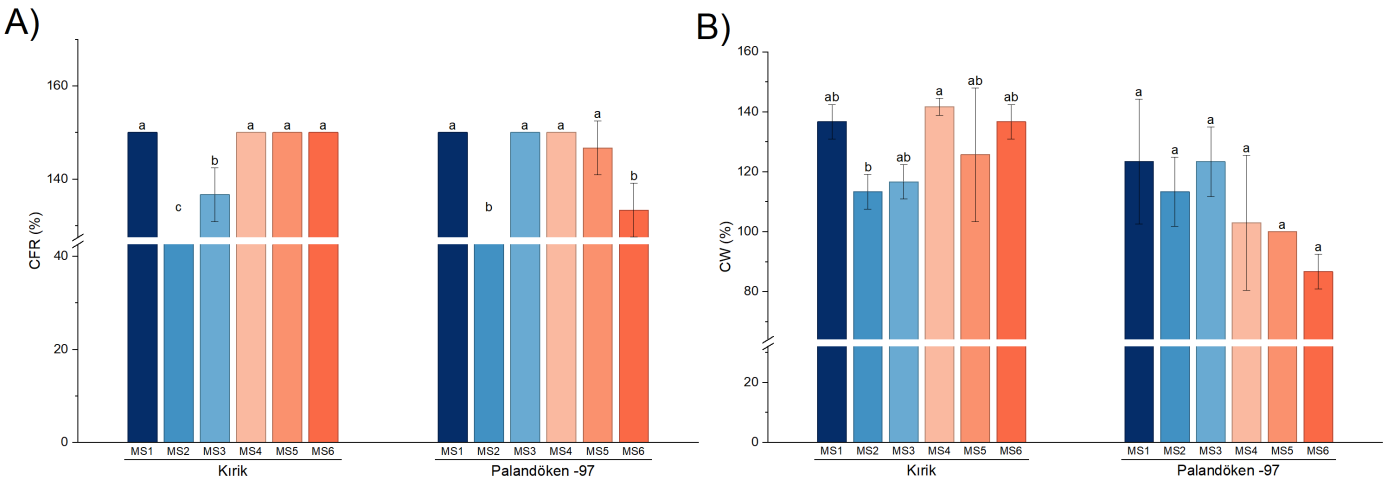


Figure 1

The effect of media conditions on A) CFR (%) and B) CW (%) at Kırık and Palandöken-97.

CFR: Callus formation rate, CW: Callus formation rate

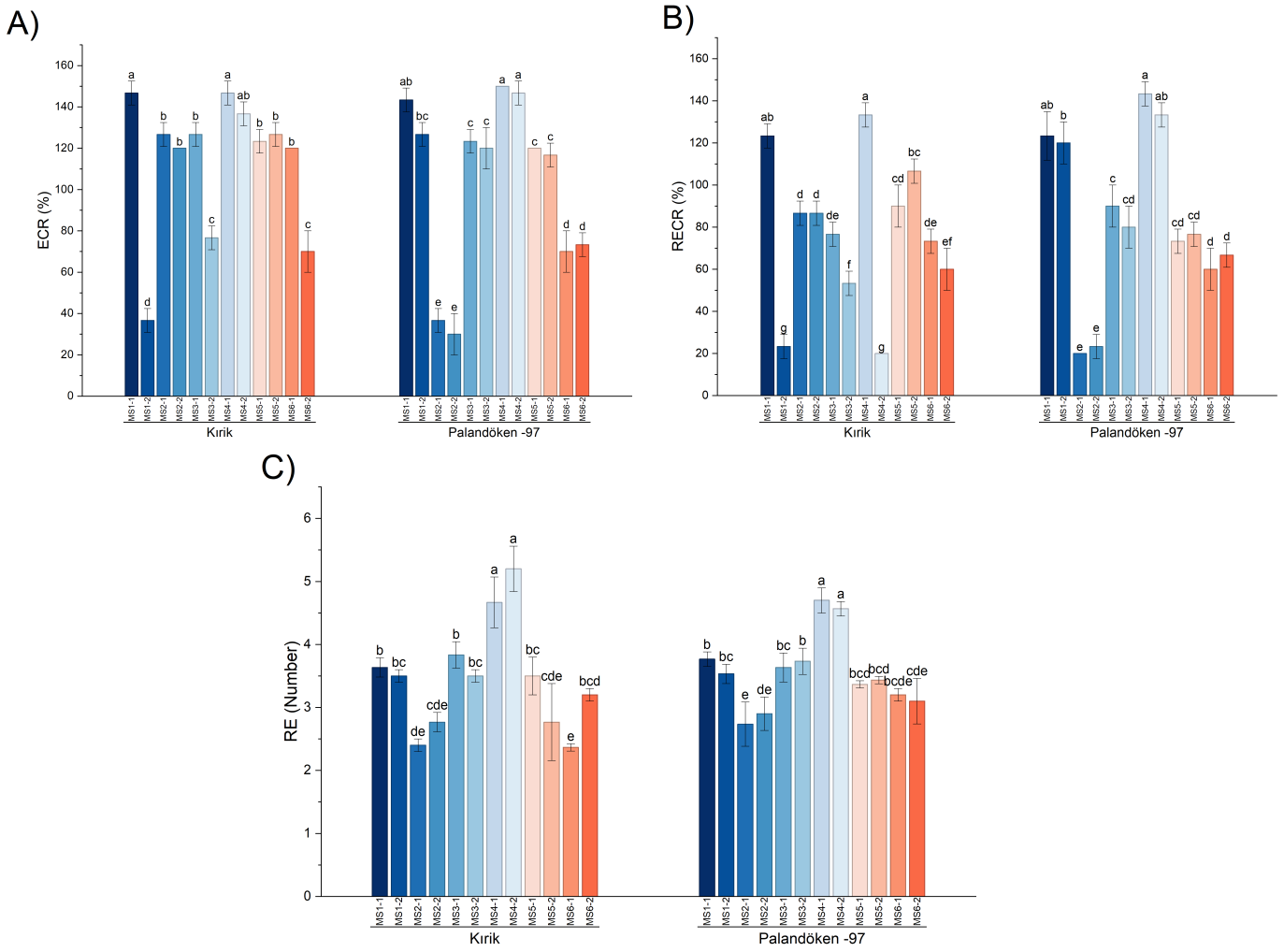


Figure 2

The effect of media conditions on A) ECR (%), B) RECR (%), and C) RE (number) at Kırık and Palandöken-97.

ECR: Embryogenic callus ratio, RECR: responding embryogenic callus ratio, RE: regeneration efficiency

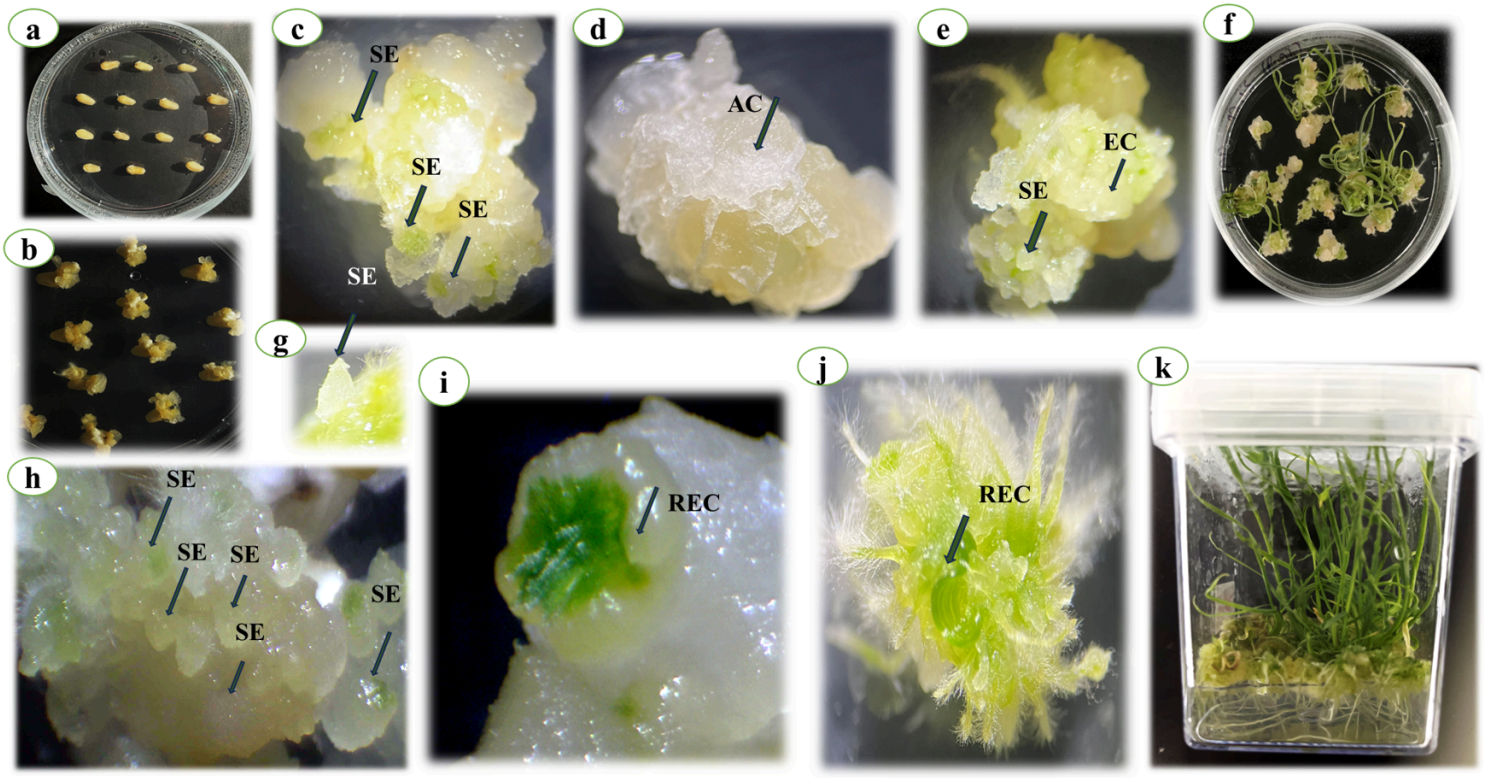


Figure 3

Schematic representation of the effect of different culture media on the regeneration process in Triticum aestivum: (a) initial culture stage, (b) callus induction, (c), (e), (g), and (h) somatic embryos (SE) and embryonic calli (EC) (arrows indicate SE and EC; MS1, MS4, and MS5 media), (d) amorphous (non-regenerating) callus (AC) (arrows indicate AC; MS2 medium), (f), (i), and (j) responsive embryogenic callus (REC) (arrows indicate REC), (k) regenerated plants.

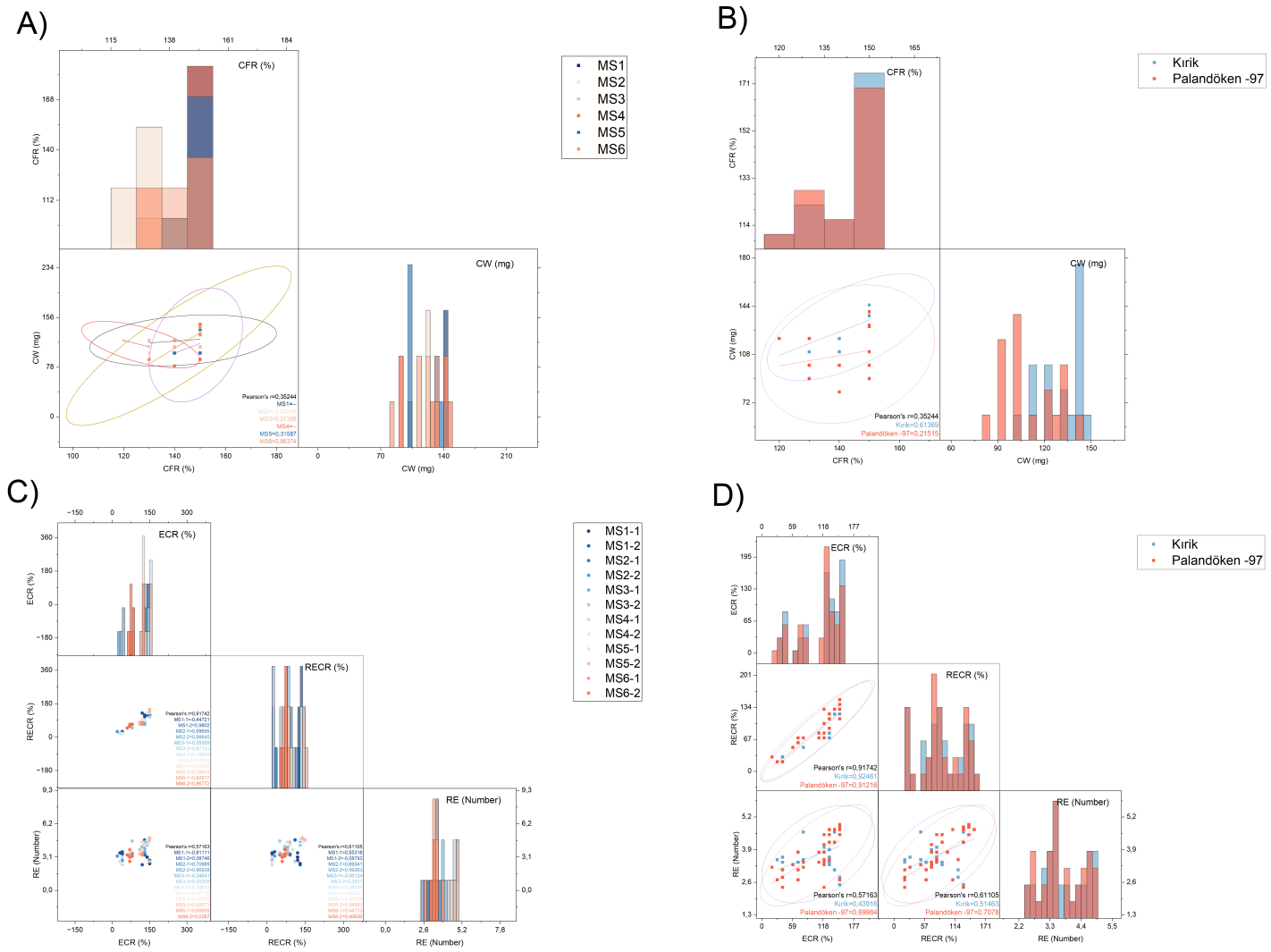


Figure 4

Scatter matrix among tissue culture parameters under different MS media and varieties. A) Correlation between callus formation rate (CFR) (%) and callus weight (CW) (%) across MS media (MS1–MS6). B) Genotype-based comparison (Kırık and Palandöken-97) of CFR and CW. C) Relationships among embryogenic callus rate (ECR) (%), regeneration capacity (RECR) (%), and regeneration efficiency (RE) (number) under different media conditions. D) Genotype-based correlations among ECR, RECR, and RE. Ellipses represent the distribution and strength of correlations, and Pearson correlation coefficients are indicated within the plots.

Kırik

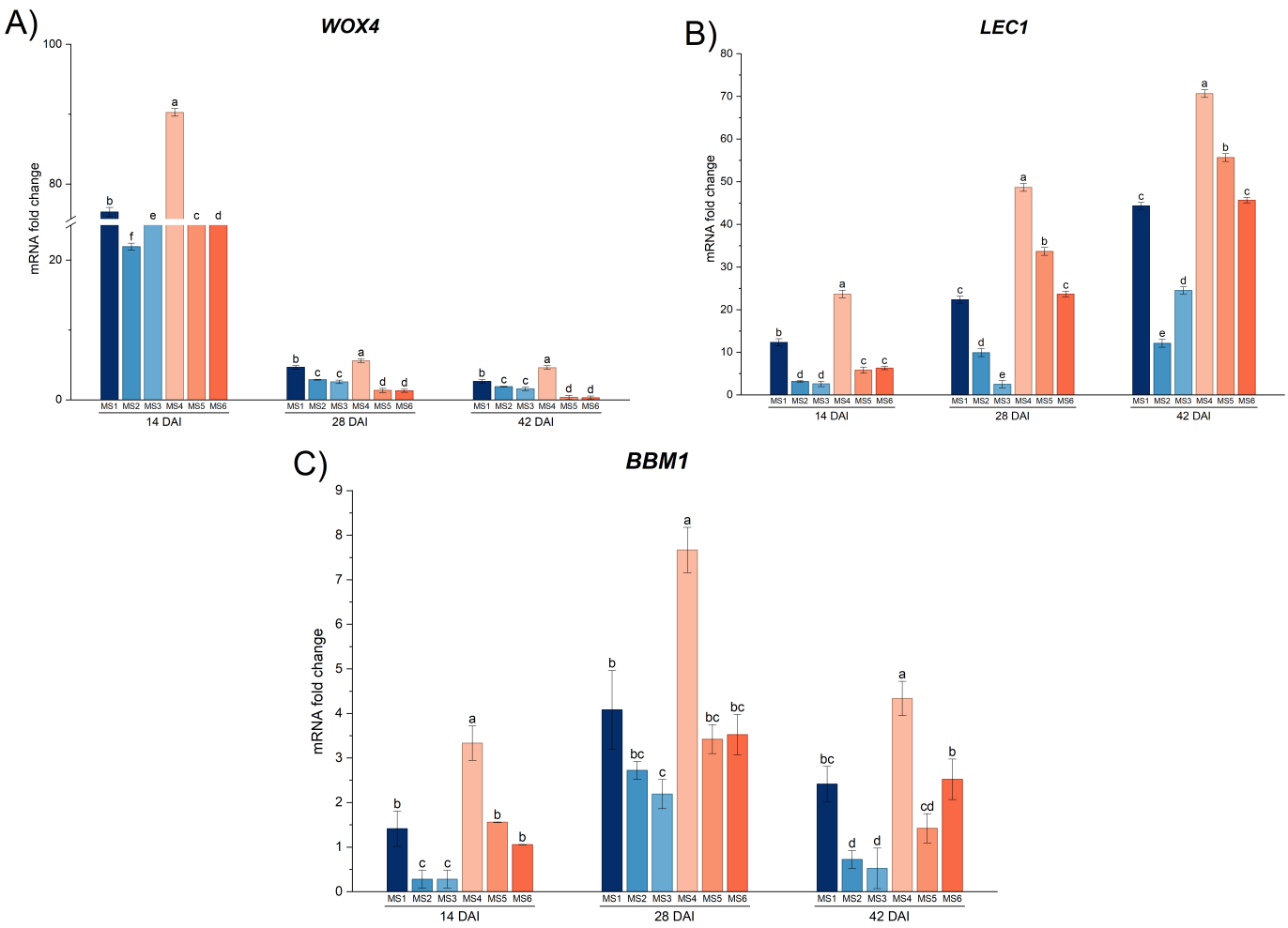


Figure 5

RT-qPCR analysis of WOX4, LEC1, and BBM1 genes in the Kırik under different MS media (MS1–MS6) at 14, 28, and 42 DAI. A) WOX4, B) LEC1, and C) BBM1

Palandöken-97

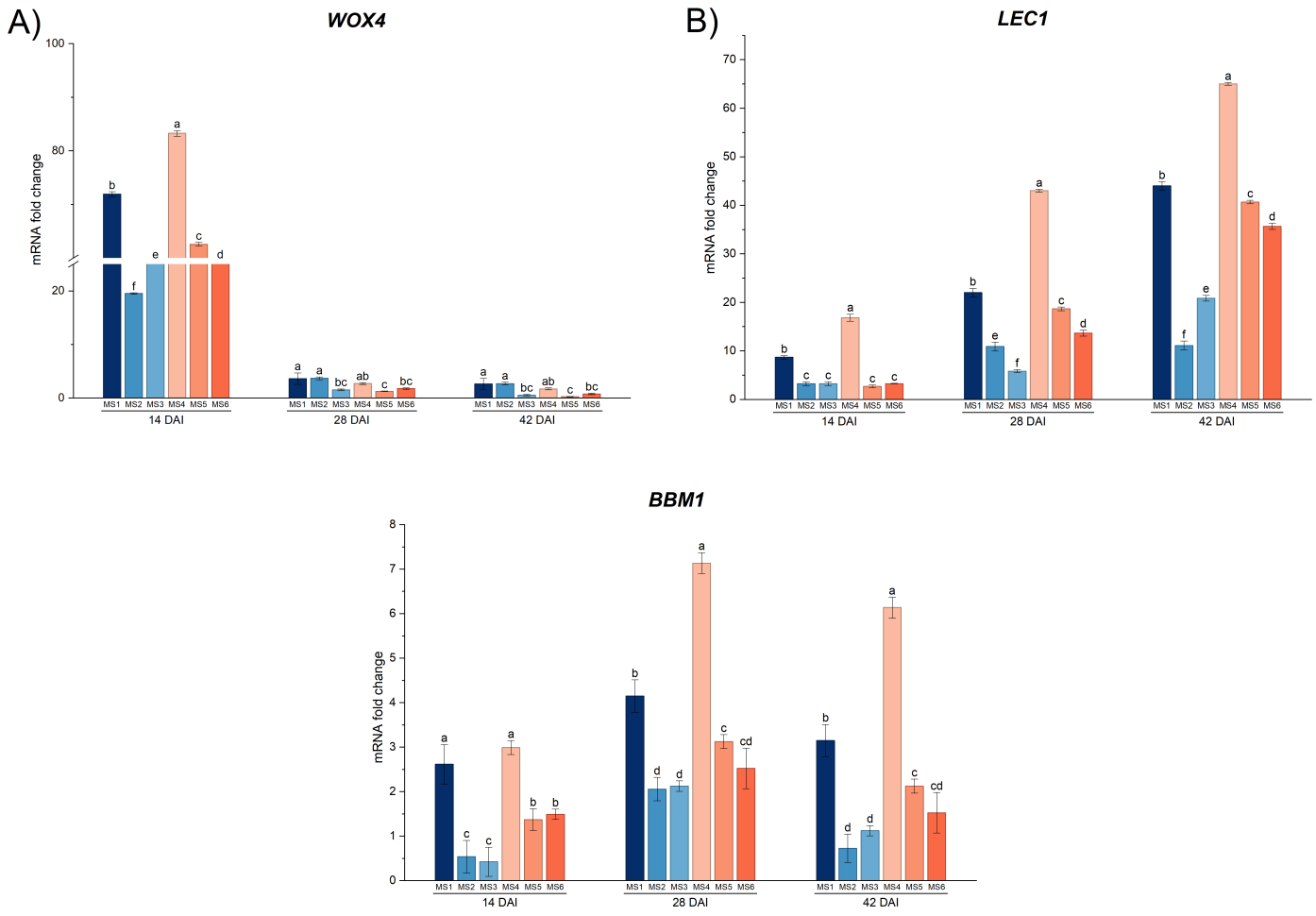


Figure 6

RT-qPCR analysis of WOX4, LEC1, and BBM1 genes in the Palandöken-97 under different MS media (MS1–MS6) at 14, 28, and 42 DAI. A) WOX4, B) LEC1, and C) BBM1

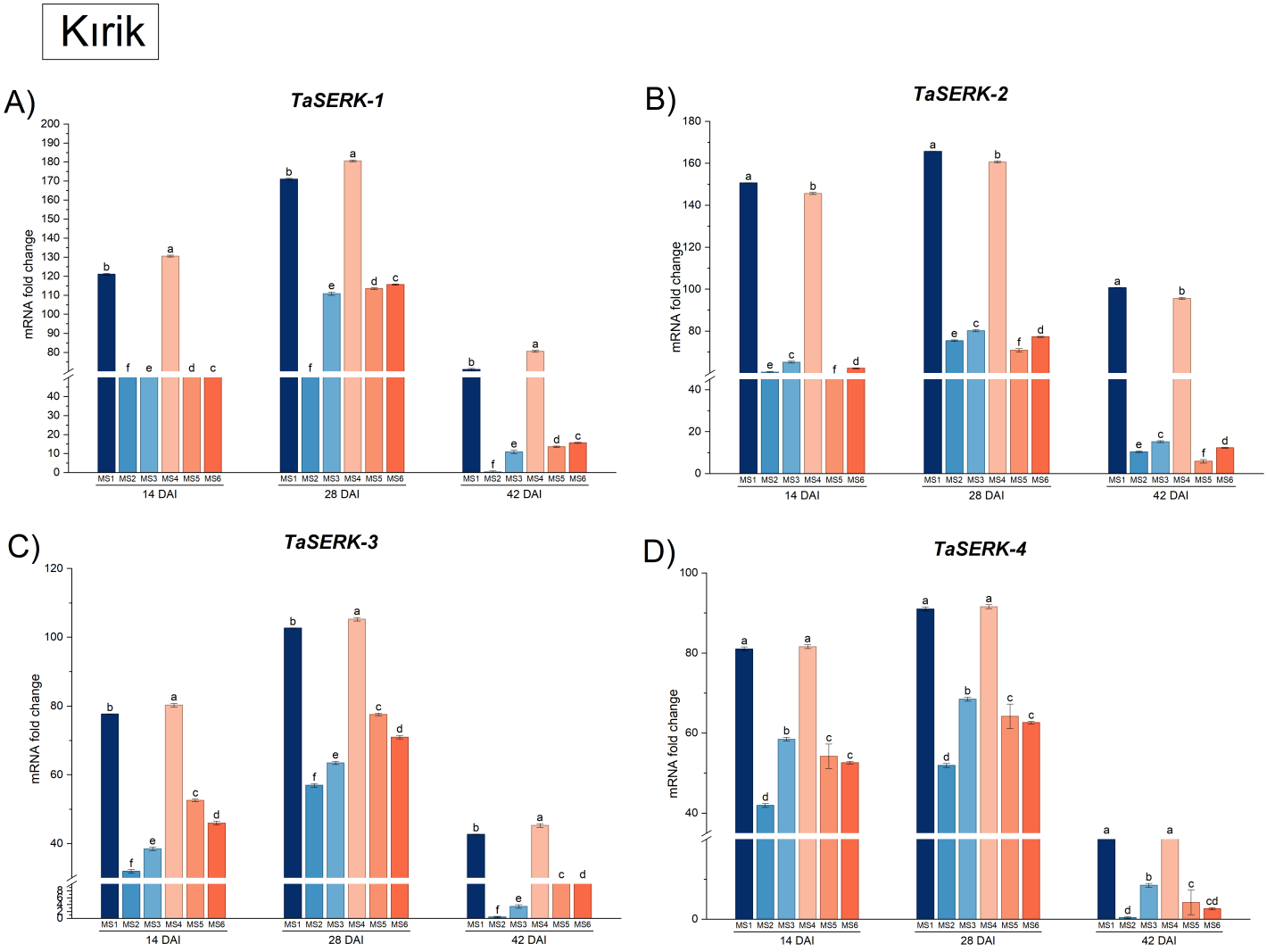


Figure 7

RT-qPCR analysis of TaSERK-1, -2, -4, -3 genes in the Kırik under different MS media (MS1–MS6) at 14, 28, and 42 days DAI. A) TaSERK-1, B) TaSERK-2, and C) TaSERK-3, D) TaSERK-4

Palandöken-97

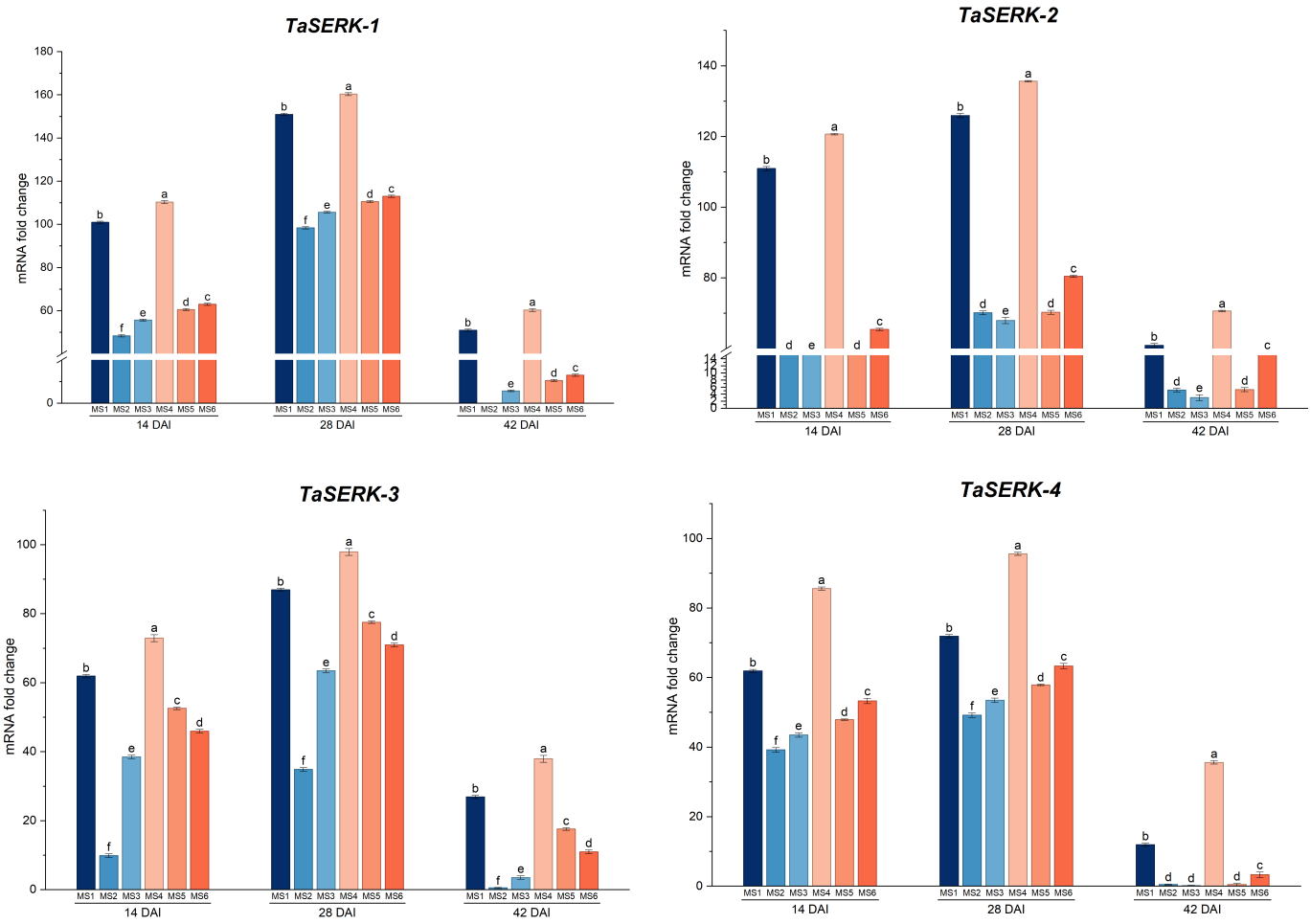


Figure 8

RT-qPCR analysis of TaSERK-1, -2, -4, -3 genes in the Palandöken-97 under different MS media (MS1–MS6) at 14, 28, and 42 DAI. A) TaSERK-1, B) TaSERK-2, and C) TaSERK-3, D) TaSERK-4

Kırik

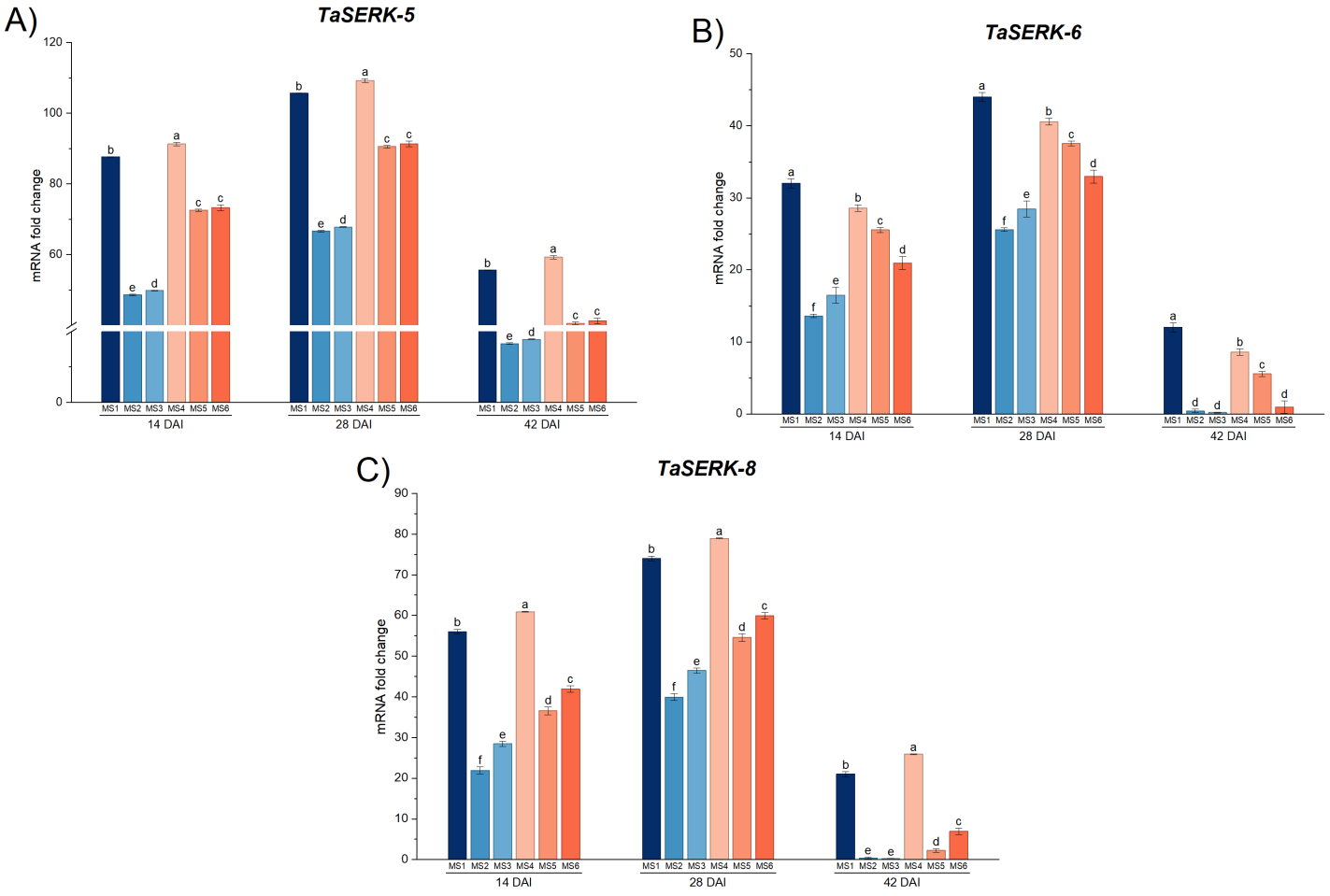


Figure 9

RT-qPCR analysis of TaSERK -5, -6, -8 genes in the Kırik under different MS media (MS1–MS6) at 14, 28, and 42 DAI. A) TaSERK-5, B) TaSERK-6, and C) TaSERK-8.

Palandöken-97

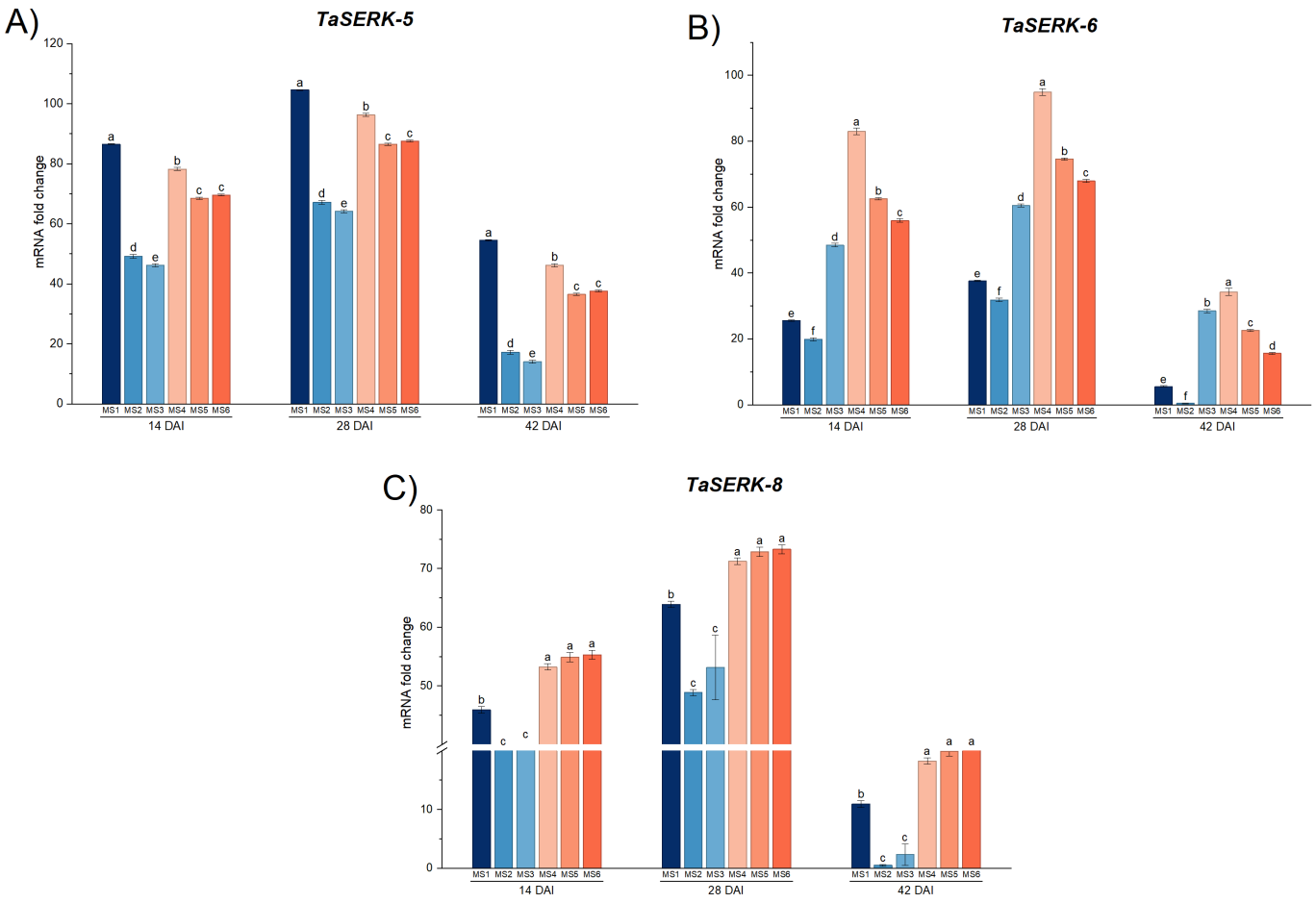


Figure 10

RT-qPCR analysis of TaSERK -5, -6, -8 genes in the Palandöken-97 under different MS media (MS1–MS6) at 14, 28, and 42 DAI. A) TaSERK-10, B) TaSERK-11, and C) TaSERK-12.

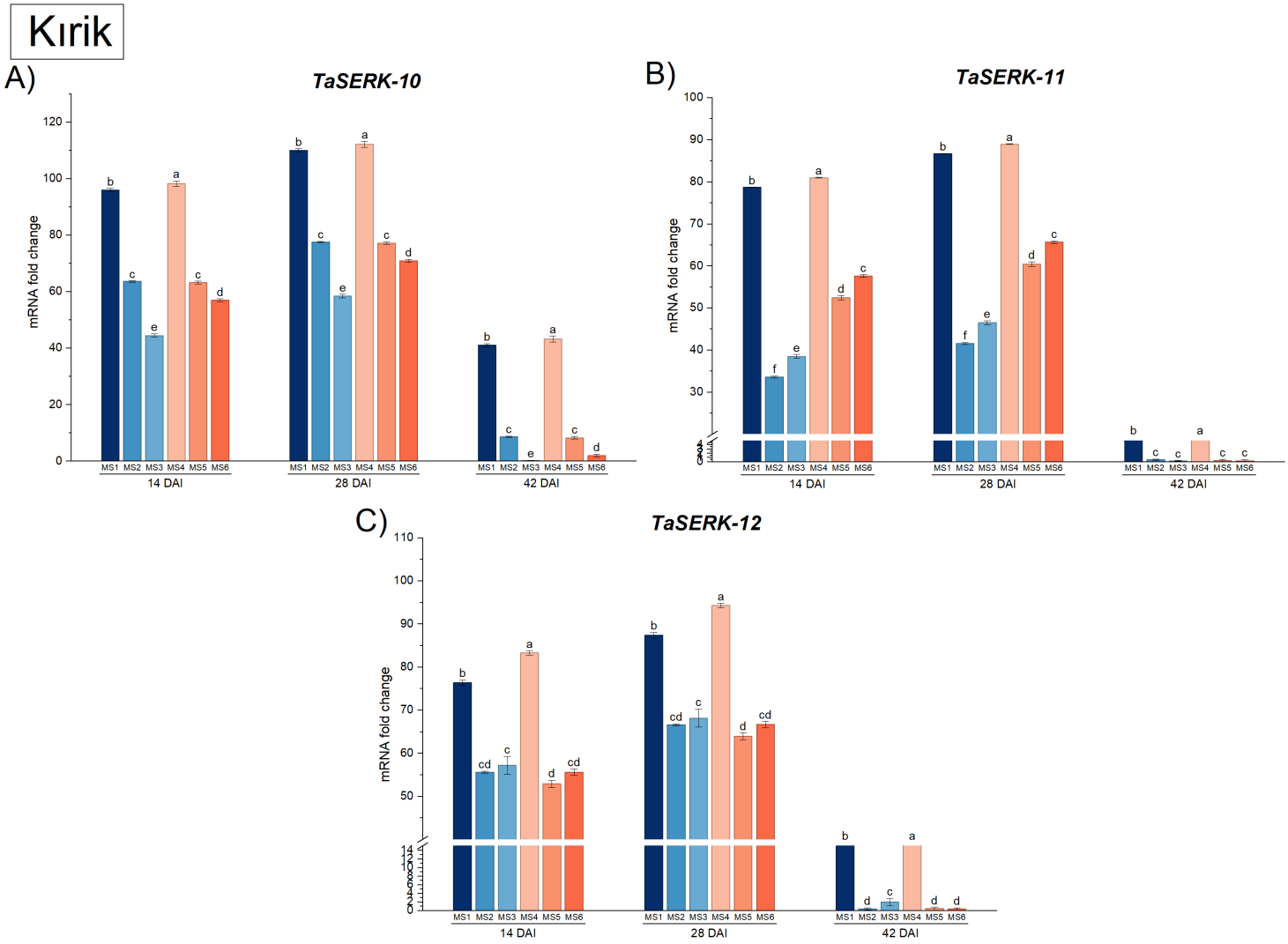


Figure 11

RT-qPCR analysis of TaSERK -10, -11, -12 genes in the Kırik under different MS media (MS1–MS6) at 14, 28, and 42 DAI. A) TaSERK-10, B) TaSERK-11, and C) TaSERK-12.

Palandöken-97

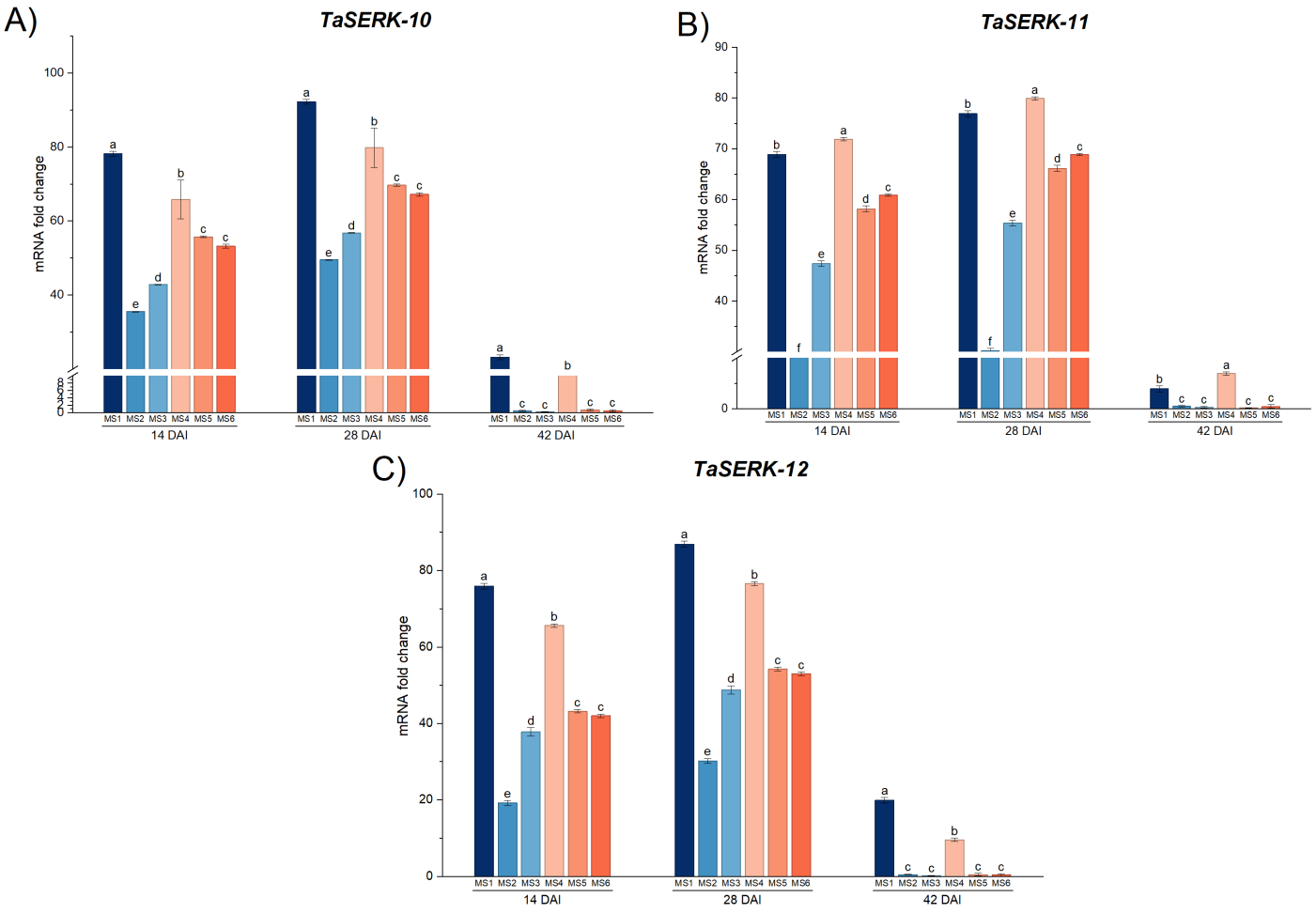


Figure 12

RT-qPCR analysis of TaSERK -10, -11, -12 genes in the Kırık under different MS media (MS1–MS6) at 14, 28, and 42 DAI. A) TaSERK-10, B) TaSERK-11, and C) TaSERK-12.

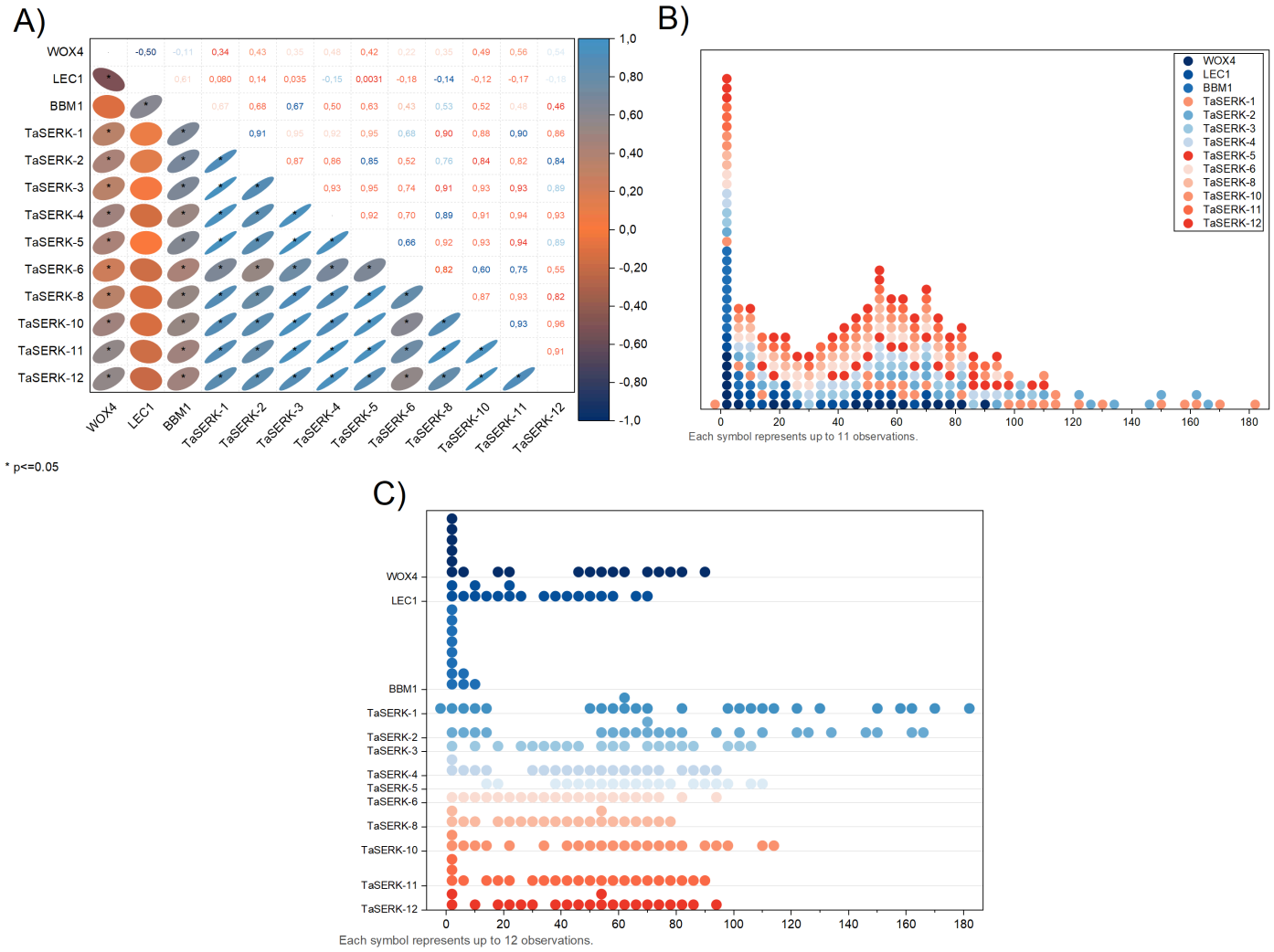


Figure 13

Gene plots: A) Correlation, B) Stacked dot plot, and C) Dot plot.

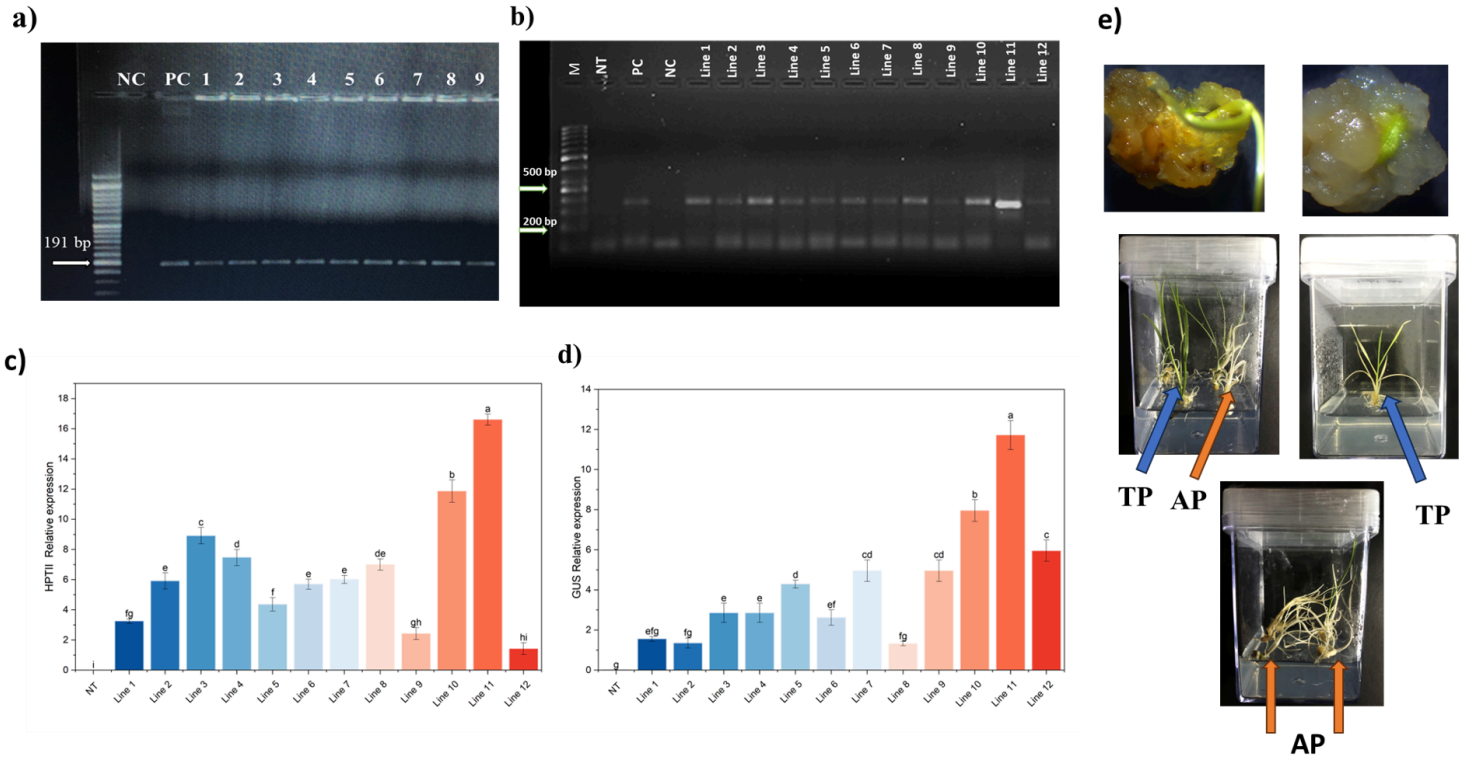


Figure 14

Schematic representation of gene transfer to Kırik using Agrocabterium. a) Confirmation of positive colonies using the NPTII primer b) Confirmation of positive plants using the HPTII primer c) HPTII d) Relative expression of GUS genes in transgenic plants e) Transgenic plants obtained in the selection medium.

M: marker, NT: non-transgenic, PC: positive control, NC: negative control, TP: transgenic plant, AP: albino plant

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFiles.zip