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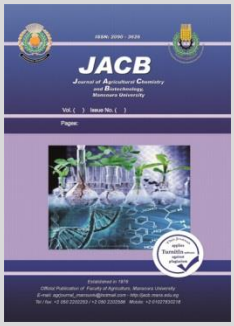
Expression of some Epigenetic-Related Genes in Regenerated Shoots of *Prunus persica*

Roba M. Ismail[1*]; Rania M. Abou Ali[2]; M. A. Nagaty[3] and M. Abdelsattar[4]

[1] Plant Genetic Transformation Department, Agricultural Genetic Engineering Research institute (AGERI), Agricultural Research Center (ARC), Giza, Egypt
[2] Nucleic Acid and Protein Structure Department, Agricultural Genetic Engineering Research institute (AGERI), Agricultural Research Center (ARC), Giza, Egypt
[3] Plant Production Department, Faculty of Environmental Agricultural Sciences, Arish University (AU), El-Arish, North Sinai, Egypt
[4] Plant Molecular Biology Department, Agricultural Genetic Engineering Research institute (AGERI), Agricultural Research Center (ARC), Giza, Egypt



ABSTRACT

Establishment of an efficient adventitious shoot regeneration system for mature embryo of *Prunus persica via* shoot organogenesis has been achieved. Three different combinations of plant growth regulators (PGRs) have been applied in the current study; 6-Benzyl adenine (BA) or thidiazuron (TDZ) with Indole acetic acid (IAA) or Indole butyric acid (IBA). The high regeneration frequency was illustrated by 1.5 mg/l BA with 0.5 mg/l IAA combinations. Quantitative expression of eight epigenetic regulatory genes; Chromomethylase 3 (*CMT3*), Domains Rearranged Methyltransferase2 (*DRM2*), Fertilization Independent Endosperm (*FIE2*) and Chromodomain Protein Like Heterochromatin Protein1 (*LHP1*), Pickle (*PKL*), Proliferating Cell Nuclear Antigen (*PCNA*), Shoot Meristemless (*STM*) and DICER-LIKE1 (*DCL1*) have been estimated in the peach regenerated shoots. We found that the auxins and cytokinins in different combinations especially BA effect on the expression of some epigenetic genes in relation to the mature embryo.

Keywords: *Prunus persica*, peach, adventitious shoot, DNA methylation, epigenetics, histone acetylation, histone methylation, qRT-PCR

INTRODUCTION

Nemaguard (Prunus persica L. Batsch× Prunus davidiana Carriere) peach rootstock resistant or tolerant to root-Knot nematode. Otherwise, it is poor tolerance to calcareous soil conditions. Farm's soil in North Sinai is calcareous with high soil's salt. So, we need to adapt the Nemaguard rootstock with soil conditions in the North Sinai, through using the genetic engineering tools, through transfer the salt tolerance genes to Nemaguard plants. But, at the first time we need to establish high regeneration efficiency system for Nemaguard plants. Plant regeneration from a tissue culture system is often the most critical step in the success of various biotechnological techniques of any plant improvement program (Pooler and Scorza, 1995; Yan and Zhou, 2002; Wu *et al.* 2006; Nagaty *et al.* 2007; Nagaty, 2012). Trees grafted on Nemaguard are fairly tolerant of waterlogged soils but tolerant to cold (https:// onegreen world. com/ product/ nemaguard- rootstock/).

Techniques of traditional breeding and modern genetics have been used to enhance Prunus species. However, these techniques are difficult and consuming time. Instead of these techniques, an adventitious shoot regeneration system was applied in peach regeneration using different explants: leaves (Ricci *et al.*, 2020); mature embryos (Pérez-Clemente *et al.*, 2004); immature embryos (Hammerschlag *et al.*, 1985); cotyledons of mature seed explant (Abdelsattar *et al.*, 2020), meristematic bulks (Sabbadini *et al.*, 2019) and flower, petiole and stem calyx (Pérez-Jiménez *et al.*, 2013).

In tissue culture and regeneration systems, plant growth regulators (PGRs) might cause stress and epigenetic variations in regenerated plants (Chinnusamy and Zhu, 2009). To overcome this stress, DNA methylation can be utilized for alterations of epigenetic and gene regulations (Taskin *et al.*, 2015). Some epigenetic changes (methylation of DNA, methylation & acetylation of histones) take place for gene expression regulations (Zhang & Reinberg, 2001; Wójcikowska, *et al.*, 2020).

The epigenetic modifications can be categorized into two main groups' chromatin modifications (histone ubiquitination, histone acetylation, histone methylation, and DNA methylation) and mRNA regulation.

Epigenetic modifications

Chromomethylase3 (*CMT3*) gene adds a methyl group to the CHG site (where H is A, C, T). It also initiates *de nono* methylation while Domains Rearranged Methyltransferase 2 (*DRM2*) gene adds a methyl group to the CHH site. *CMT3* and *DRM2* have major roles in modifying, maintaining, and establishing patterns of DNA methylation (Law and Jacobsen, 2010).

Trithorax-group (*TrxG*) and polycomb group (PcG) play an important role in regulating the development, growth, and cell proliferation (de la Paz Sanchez *et al.*, 2015; Duarte-Aké *et al.*, 2019). They regulate plastid development with the reprogramming of and responses of the plant to signals of the environment (de la Paz Sanchez *et al.*, 2015; Wójcikowska, *et al.*, 2020). They have histone methyltransferase activity (*HMTase*). They are responsible for lysine methylation in proteins in histone. Polycomb Repressive Complexes (PRCs) are formed of PcGs. Fertilization independent endosperm (*FIE2*) and chromodomain protein like heterochromatin protein1 (*LHP1*) genes are *PcG* genes. *LHP1* played a role in euchromatic genes silencing which

* **Corresponding author.**
E-mail address: r.medhat@yahoo.com
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belong to *PRC1* family (Hennig and Derkacheva, 2009). *FIE2* gene is one of PRC2 family which involves in the regulations of plant development. Pickle (PKL) is a chromatin remodeling factor and belongs to the *TrxG* group that is involved in the response of DNA damage (Desvoyes *et al*., 2010). Also, *PKL* represses embryonic traits (Joshi and Kumar, 2013) and restricts the meristematic genes expression. *PKL* gene is involved in *KNOX1* gene repression and regulates the identity of the cell (Aichinger *et al*., 2009). Homeobox genes have a major role in the formation of controlling patterns of regulatory genes. They are the main controlling genes regulating morphology differentiation and the formation of the pattern. They encoded for transcription factors which have activity for DNA binding (Joshi and Kumar, 2013). The factor proliferating cell nuclear antigen (*PCNA*) acts for the DNA polymerases as a processivity factor. It assists the DNA polymerases at the replication fork in DNA replication (Shibahara and Stillman, 1999), which provides a connection between both strands of DNA. Also, it links the inheritance of epigenetic marks with the synthesis of DNA. It might create a local connection between some factors involved in the deacetylation of histone, methylation of DNA, assembly of the nucleosome, remodeling of the nucleosome (Jasencakova and Groth, 2010). Shoot meristemless (*STM*) gene is *KNOX* related gene family. It is involved in *de novo* shoot apical meristem (SAM) formation and its functions (Scofield *et al*., 2014). DICER - LIKE1 (*DCL1)* gene has a major role in biogenesis of miRNA (Wójcikowska *et al*., 2020).

The current work describes a protocol for *in vitro* regeneration of peach *via* organogenesis using mature embryos. In addition, the expression levels of some epigenetic genes during the regeneration system have been quantified. The current study focuses on selected epigenetic regulatory genes which switch between phases of regeneration.

MATERIALS AND METHODS

Sterilization of peach Seeds, Explants preparation and callus induction

In this experiment, peach cv. Nemaguard was regenerated using embryo explant. The peach seeds sterilization and shell crack were applied as described by Abdelsattar *et al.* (2020). After cracking the shells by hand, the seeds were removed from the shells and pre-sterilized by immersion into 3.75% (v/v) sodium hypochlorite solution containing two to three drops of Tween 20 for 25 min. Then, they were washed by rinsing several times with sterile distilled water. Sterilized seeds were incubated in sterile distilled water for 60 hours under chaking condition at 17°C. For easy removal of testa water was replaced every 12 hours. Turgid seeds were sterilized again. Embryos were isolated from cotyledons, cut into small pieces and used as explants. For callus induction, explants were cultured on three different Woody Plant Medium (WPM) with 3 % (w/v) sucrose and different growth regulators: 1) 1.6 mg/l TDZ; 0.5 mg/l IBA (medium 1); 2) 1.6 mg/l TDZ; 0.5 mg/l IAA (medium 2) and 3)1.5 mg/l BA; 0.5 mg/l IAA (medium 3) were screened. Hormone free medium was used as control. The plates containing embryos were incubated for one month at dark at 25°C

Induction of adventitious shoots

For producing adventitious shoot regeneration, induced calli were transferred to the same media twice and incubated at light for three week. Frequency of regeneration was estimated as the number of differentiated calli divided by total number of obtained calli. Shoots were elongated on medium WPM containing 1.5 mg/l GA3.and rooted on 2.5 mg/l IBA WPM medium as pervious described by Abdelsattar *et al*. (2020). The plantlets were then acclimatized on pots in greenhouse with an 1:1 (v/v) Perlite: Petmos.

Isolation of total RNA and synthesis of cDNA

Total RNA was isolated from regenerated shoots derived from peach embryos induced in three different PGR combinations. RNA isolation and cDNA synthesis were carried out according to Abdelsattar *et al.* (2020).

In this investigation eight epigenetic regulatory genes were chosen: *LHP1* & *FIE2* (polycomb genes); *DRM2* & *CMT3* (methyltransferases); *PKL* & *PCNA* (Trithorax group genes); *DCL1* gene (Dicer-Like 1) and *STM* class1 KNOX 1 (knotted 1 like homeobox). These were investigated in *in vitro* tissues during the shoot regeneration process.

Quantitative Real-Time PCR for the selected genes

MX3005P Stratagene Real-Time System (Agilent, United States) was used to carry out the reactions of quantitative PCR (qPCR). According to Abdelsattar *et al*. (2020), primers design and reactions of qRT-PCR were performed. The used reference gene was 18s for expression normalization of genes of interest (Kondo *et al*., 2018). The calculations of the levels of gene expression quantification were done by the method of 2^-ΔΔCT (Schmittgen and Livak, 2008). Duncan's test was used to analyze qRT-PCR results by SPSS (20.0, IBM). The results were presented as means ± SE.

RESULTS AND DISCUSSION

Results

Adventitious shoot regeneration

A regeneration system has been established for *Prunus persica*, Nemaguard peach using mature embryo explant. Three WPM media plus control, with different growth regulators combinations have been examined.

The results showed that medium 3 revealed high regeneration frequency (3.47) followed by medium 1 (0.55) and medium 2 (0.17) (Figure 1 & 2).

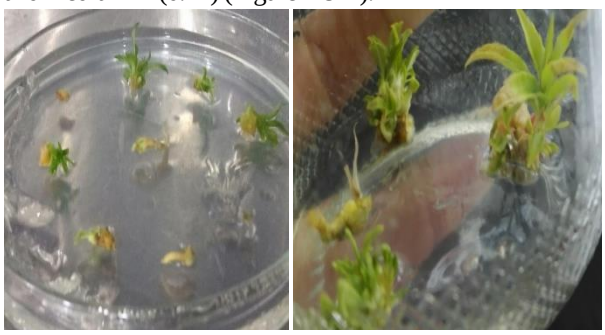

Figure 1. Adventitious shoot regeneration of *Prunuspersica* from mature embryos after incubation on medium 3 (containing 1.5 mg/l BA and 0.5 mg/l IAA).

qRT-PCR results

The gene expression of eight genes *CMT3*, *DRM2*, *FIE2*, *LHP1*, *PKL*, *PCNA*, *STM*, and *DCL1* have been estimated using qPCR during *Prunus persica* shoot formation from mature embryos on three different media.

The epigenetic modifications can be categorized into two main groups' chromatin modifications (histone ubiquitination, histone acetylation, histone methylation and DNA methylation) and mRNA regulation.

DNA methylation

DNA methyltransferases, *CMT3*, and *DRM2* had been quantified by qPCR during shoot formation on medium 1, medium 2 and medium 3. *CMT3* shows slight differences in folds between control, medium 1 and medium 2 (0.5, 0.6, and 0.8 folds, respectively), while it illustrates that there was no

increase in the fold on medium 3 (0.0004 folds) (Figure 3 a). For *DRM2* gene expression, it was observed that there were no remarkable changes between control, medium 2 and medium 3 (0.4, 0.5, and 0.5 folds, respectively). However, the expression pattern changed on medium 1 (0.9 folds) (Figure 3 b).

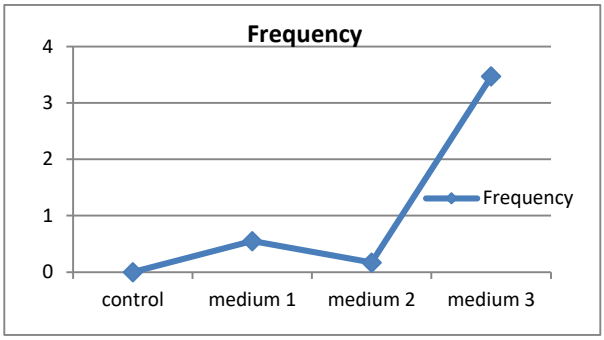

Figure 2. Regeneration frequencies of the three different regeneration media.

Histone methylation and ubiquitination *FIE2, LHP1,* and *PKL* genes expressions

The *FIE2* gene expression was found to be changed through control, medium 1, medium 2 and mediaum 3 as follows 1.1, 0.07, 0.7, and 0.3 folds, respectively (Figure 3 c). There was a remarkable difference in the *FIE2* expression between the three media. The expression pattern was increased by ten times from medium 1 and medium 2 while it increased four times between medium1 and medium 3. On the other hand, the *LHP1* expression pattern almost unchanged with medium 1, medium 2, and medium 3 (2.3, 2.1, and 2.1 folds, respectively) while it gave 0.3 folds with control medium (Figure 3 d).

For the *PKL* gene expression pattern, it shows a significant difference throughout control and different media. The expression was almost the same in control and medium 1 (4.3 and 4.9 folds, respectively) while it significantly increased in medium 2 (6.5 fold) and a moderate increase in medium 3 (5.3 fold).

The expression of the *PCNA* gene was the same in control and medium 3 (0.3 fold) however it was observed that it has moderate changes between medium 1 and medium 2 (1.0 and 0.6) (Figure 3 g). The expression pattern of the *DCL1* gene shows slight changes between the control, medium 1 and medium 2 (0.2, 0.3and 0.2 folds, respectively) while it decreased by half in medium 3 (0.1 fold) (Figure 3 f). *STM* gene expression was very low in control and medium 3 (0.002 and 0.0006 folds, respectively) while its expression was increased ten times in medium 1 and medium 2 (0.014 and 0.013 folds, respectively) (Figure 3 h).

The folds changes in expressions of the eight genes were illustrated in Table 1 and figure 3.

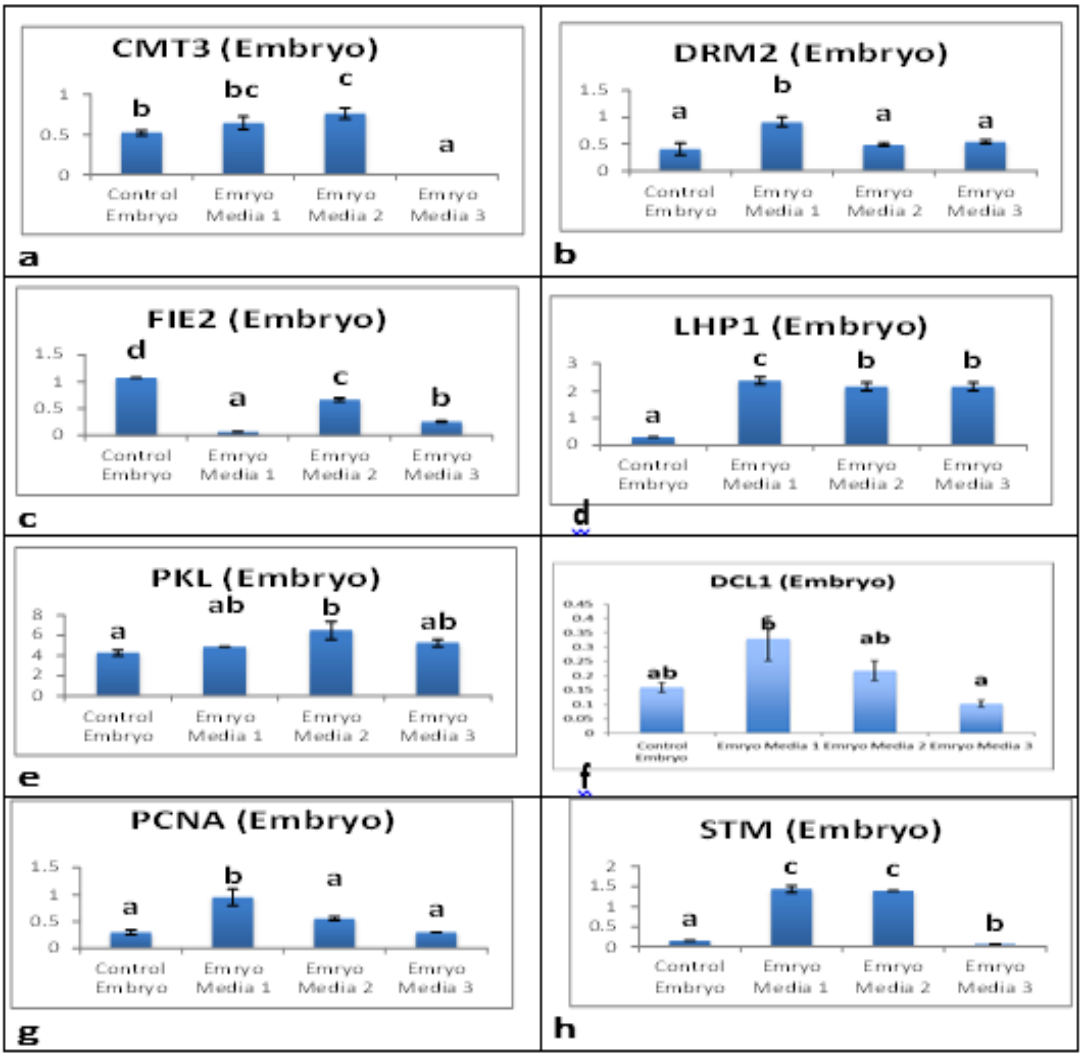

Figure 3. Quantitative Real Time PCR (qPCR) for eight (*CMT2, DRM2, FIE2, LHP1, PKL, DCL1, PCNA,* and *STM*) genes estimated in regenerated shoots from mature embryos of peach.

Table 1. The expression patterns of *CMT3, DRM2, FIE2, LHP1, PKL, PCNA, STM,* and *DCL1* genes.

	Folds changes			
Gene Name	**Control Medium**	**Medium 1**	**Medium 2**	**Medium 3**
CMT3	0.5	0.6	0.8	0.0004
DRM2	0.4	0.9	0.5	0.5
FIE2	1.1	0.07	0.7	0.3
LHP1	0.3	2.4	2.2	2.2
PKL	4.3	4.9	6.5	5.3
PCNA	0.3	1.0	0.6	0.3
STM	0.002	0.014	0.013	0.0006
DCL1	0.2	0.3	0.2	0.1

Discussion

Adventitious shoot regeneration in plants

Organogenesis establishes the biological characters' dynamic *in vitro* plant regeneration in which, plant hormones play a vital role. They modulate the occurrence of signaling events during the organogenesis where cytokinins and auxins are the main players. They worked in a harmony to lead the organogenesis process (Shin *et al*., 2019). García-Pérez *et al*. (2020) characterized the combination of cytokinin BA and auxin IAA on *in vitro* organogenesis using the machine learning approach. They showed that the BA affected several organogenesis responses such as direct organogenesis of shoots while IAA displayed an inhibitory effect. Regeneration of plant and *in vitro* organogenesis protocols are constructed on the explant transfer on culture media with different PGRS which lead to adventitious shoots formation and whole plant at the end (George *et al*., 2008). Induction of somaclonal variations during organogenesis might cause some plant genetic changes (Pasqual *et al*., 2014). Exogenous PRGs play a critical role in accomplishing a suitable phytohormonal balance to obtain efficient protocols for tissue culture (Thorat *et al*., 2018). During the organogenesis, the epigenetic modifications might be caused due to the interaction between cytoplasmic and nuclear genes in the PGRs existence under the differentiation-related genes control (Zhao *et al*., 2008). The genetic control of PGRs, which includes a complex interaction, may give rise to changes during organogenesis.

The balance between auxins and cytokinins regulates the organogenesis process (García-Pérez *et al*. 2020) where they pointed that BA cytokinin promoted shoot elongation.

Peach regeneration

It was recommended to used mature tissues from peach as explants for plant propagation to keep the desirable features especially in commercial propagation of peach (Sabbadini *et al*., 2019). In this study mature embryo of peach cv. Nemagurd has been used as explants for regeneration. The mature embryos were successfully applied by Pérez-Clemente *et al*. (2004). Three media with different growth regulators, (TDZ & IBA), (TDZ & IAA), and (BA & IAA), have been used in this investigation. The combination of BA with IAA showed the highest regeneration frequency among the three different combinations used in peach regeneration. Our result showed that medium (BA & IAA) gave the highest regeneration frequency in mature embryo explants while Abdelsattar *et al*. (2020), reported that the highest regeneration frequency was medium (TDZ & IAA) when using cotyledon as an explant. From these results regeneration frequency might change with different explant types from the peach on the same media compositions.

Auxins are essential phytohormones that participate in several processes during plant regeneration. Using auxin in high concentrations in maize induces embryos (Joshi and Kumar, 2013). To improve regeneration, cytokinin BA was applied. Cytokinins are promoting shoots regeneration in peach (Pérez-Jiménez *et al*., 2012; Pérez-Jiménez *et al*., 2013; Pérez-Jiménez, *et al*., 2014; Sabbadini *et al*., 2019).

Differences in response to organogenesis depend on the type of explant and different PGRs combinations. The basal media enriched with cytokinin BA with combinations of IBA or NAA shows the highest regeneration frequencies (Sabbadini *et al*., 2019; Ricci *et al*., 2020). BA has a positive influence on peach shoot regeneration (Gentile *et al*., 2002; Pérez-Jiménez *et al*., 2012; Pérez-Jiménez *et al*., 2013; Sabbadini *et al*., 2019; Ricci *et al*., 2020). The addition of BA causes callus induction and elongation of the axis in peach using embryos as explant (Bhansali *et al*., 1990) and increase the number of shoot per explant in *Boechera oleracea* (Cheng *et al*., 2001).

Chromatin modifications

Epigenetic genes have an important role in changing the action in phases of regeneration. It has been suggested that regenerated plants have different DNA methylation and histone modifications profiles. It can be regarded as epigenetic changes which might be connected with somatic variations (Taskin *et al*., 2015).

The concentration of plant growth regulators has an important influence on somaclonal variation (Huang *et al*., 2012). Studying the existence and reasons for somaclonal variation in plants is essential for a better understanding of epigenetic phenomena. The somaclonal variations occurrence and degree in tissue culture might vary between different plant species and even among genotype of the same sp. The epigenetic variations are usually complemented with DNA methylation-induced gene silencing (Huang *et al*., 2012).

Some epigenetic changes (methylation of DNA, methylation, ubiquitination & acetylation of histones, and mRNA regulation) take place for gene expression regulations (Zhang & Reinberg, 2001; Wójcikowska, *et al*., 2020).

DNA Methylation

The conditions of tissue culture cause changes in epigenetic modification through methylation of DNA (Taskin *et al*., 2015). The levels of DNA methylation genes are highly influenced by applied PGRs in tissue culture (Huang *et al*., 2012; Taskin *et al*., 2015).

In plants, *CMT3* preserves the presence of the methylation patterns and transposons silences (Tompa *et al*., 2002). In our experiment, *CMT3* expression changes in relation to PGRs combination. The control medium and medium 1 (TDZ & IBA) were almost the same expression (0.5 and 0.6 folds, respectively) while medium 2 (TDZ & IAA) has a minor increase (0.8 fold). However, *CMT3* has very small expression in medium 3 (BA & IAA) (0.0004 fold). This expression pattern changes might be related to the different combinations of PRGs used. These results were in contrast with Taskin and his co-workers' results who

indicated that the expression of *CMT3* was high in *Boechera divarica*. *CMT3* played the main part in challenging the stress conditions through shoot regeneration (Taskin *et al*., 2015). *DRM2* expression was unchanged in the three media (0.4, 0.5, and 0.5 folds, respectively except with medium 1 (TDZ & IBA) (0.9 fold) which has a slight increase than the other media (control, medium 2, and medium 3). This result was in agreement with Taskin *et al*. (2015), who indicated that the expression level of *DRM2* unchanged in the shoot regeneration of *Boechera divarica*. *DRM2* is involved in *de novo* methylation in Arabidopsis (Cao & Jacobsen, 2002). DNA methyltransferases were either low or unchanged with different PGRS combinations during shoot regeneration except with medium 2 (TDZ & IAA) where *CMT3* expression was increased and *DRM2* was increased on medium 1 (TDZ & IBA). The two media might activate the two DNA methyltransferases which produce some epigenetic variations. Those results were countering to Abdelsattar *et al*. (2020) who found that DNA methyltransferases were highly accumulated during shoot regeneration from peach mature cotyledons. Our results were opposing those obtained by Taskin *et al*. (2015) with all applied media with different combinations except medium 1 and medium 2 for *CMT3* and *DRM2* expressions, respectively. It was concluded that cytokinins are vital for cell division and epigenetic genes.

Huang *et al*. (2012) indicated that DNA methylation showed a remarkable decrease when cytokinin BA was increased in tissue culture media. They found that *DRM2* concentration in the regenerated shoots was negatively linked with BA concentration.

Methylations of DNA are well-maintained epigenetic modifications which are essential for the stability of the genome and regulations of genes. The plant irregularities in development could be caused by DNA methylations abnormalities (Zhang *et al*., 2018). The DNA *de novo* methylation is catalyzed by a family of DRM.

Methylations of DNA influence the induction and differentiation of callus, the development of a plant, and the differentiation of organs (Miguel and Marum, 2011). The lowest DNA methylation level and *CMT3* concentration were noticed in culture media with cytokinin BA (0.1 mg/l) (Ran *et al*., 2016) whose results agree with our findings. DNA Methylation was found to be increased in *Daucuscarota* embryonic cell cultures treated with auxins (LoSchiavo *et al*., 1989).

Histone methylation and ubiquitination

Remodeling of chromatin is regulating the expression of genes that participate in several biological processes. Also, it is essential for the development and growth of plants.

The methylations of histone have an essential part in the expression of genes through epigenetic regulation during environmental and developmental changes in plants (Cheng *et al*., 2019). It is essential for the growth and development of plants. *LHP1*and *FIE2* belong to PcG proteins which play a role in remodeling of chromatin as repressor factors during the division of the cells. The expression of *LHP1* was significantly increased in the three media (2.4, 2.2 and 2.2 folds, respectively) compared with the control one (0.3 fold) while the expression of *FIE2* was increased in control (1.1 fold) in comparison with the three media (0.07, 0.7 and 0.3 folds, respectively). *FIE2* expression in medium 2 and medium 3 was ten and four times the media 1, respectively which might be related to IAA and BA hormones in the media.

PKL expression was changed in the control and three PGRs combinations. The expression was unchanged from control and medium 1 (4.3 and 4.9 folds, respectively). On the other hand, its expression was remarkably changed in medium 2 (6.5 folds) compared to moderate ones in medium 3 (5.3 folds). It is essential for vegetative growth by promoting H3K27me3 (H3 histone trimethylation at lysine 27) (Cheng *et al*., 2019). The methylations of histone have an essential part in the expression of genes through epigenetic regulation during environmental and developmental changes in plants (Cheng *et al*., 2019).

PCNA expression was unchanged throughout the control and medium 3 (0.3 folds) while it was duplicated in medium 1 in comparison with medium 2, (1.0 and 0.6 folds, respectively).

For the *STM* gene was expressed in very small amounts in control and medium 3 (0.002 and 0.0006 folds, respectively) while it was significantly increased about ten folds in medium 1 and medium 2 (0.014 and 0.013, folds respectively). Similar to Abdelsattar *et al*. (2020), *STM* expression in media containing TDZ + IAA with high regeneration frequency (0.6 fold) was less than the two other media, (TDZ + IBA, BA+ IAA) (0.9 and 1.2 folds, respectively), *STM* expression in medium 3 (highest regeneration frequency) has the smallest expression in our results compared with the other two media (mediaum 1 and medium 2).

KNOX genes were activated in *PKL* mutants in Arabidopsis (Hay and Tsiantis, 2010). *STM* is playing an essential role in stopping the differentiation of meristem cells through cytokinins production. It maintains the shoot apical meristem development and function (Xin *et al*., 2019). *PRC2* gene (*FIE*) is involved in the development of shoot through controlling *STM* expression and other class 1 *KNOX* genes. *LHP1* represses the genes belong to class 1 *KNOX* (Li *et al*., 2016). The *LHP1*/ Terminal Flower 2 (*TFL2*) accompanied with H3K27me3-rich chromatin which reacted with the Arabidopsis E3 ubiquitin ligase RING domain proteins (AtRING1a and AtRING1b). These two proteins are essential for the silencing of the KNOTTED1-like homeobox class 1 (*KNOX*) genes. Those genes are the target for *PCR2* mediated suppression which acts with *PRC1 LHP1-Ring* in plants to repress several targets of PcG genes (Thorstensen *et al*., 2011).

MicroRNA regulation

DCL1 expression was slightly increased in medium 1 (TDZ & IBA) (0.3 fold) while it was the same in control and medium 2 (0.2 and 0.2 folds, respectively). On the contrary, it was decreased by half in medium 3 (0.1 folds). This change might be the presence of auxin IBA and in this media. This result was opposing the Abdelsattar *et al*. (2020) results that showed a significant increase in *DCL1* expression on peach regenerated shoots using cotyledons as explants. We can conclude that the type of mature embryo explants, cytokinin, and the auxin used affect the *DCL1* expression.

Small RNAs (siRNAs) cause transgenes, transposons, and viruses post-transcriptional gene silencing.

Also, they are essential for maintenance and establishing the DNA methylation of cytosine. *DCL1* has an unknown function in the siRNAs production, this besides that it plays an important role for miRNAs processing as the key enzyme (Henderson *et al*., 2006). In the same context, the remarkable decrease in DNA methyltransferases (*CMT3 and DRM2*) expression might be in relation to the decrease in *DCL1* expression medium 3.

DCL1 protein is essential in the shoot apical meristem (SAM) to stop uncontrolled meristematic cell proliferation. *DCL1* regulates the amount of SAM cell division. *DCL1* was found in the meristemic tissue (Schauer *et al*., 2013). When a defect in the DICER -LIKE1 (*DCL1*) gene has happened (*dcl1* mutant), the induction of somatic embryogenesis was corrupted (Wójcik and Gaj, 2016). DCL1 protein is found in meristematic cells of the shoot. It is essential for SAM to avoid uncontrolled meristematic cell proliferation (Schauer *et al*., 2013). Bai and his coworkers have speculated that the variance in shoot and leaves mature miRNAs accumulation might be a consequence of changed amounts of miRNA biosynthesis-related proteins such as *DCL1* (Bai *et al*., 2014).

CONCLUSION

Auxins cooperate with several hormones especially cytokinins. cytokinins are recognized to act in homology with auxins. They have a major part in maintenance in meristems of shoots and root through a well alteration of the equilibrium between cytokinin and auxin activities (Taylor-Teeples *et al*., 2016).

Methylation of DNA and chromatin modifications (including acetylation of histone and methylation) are controlling explant cells transcriptome in response to treatment of auxins (Wójcikowska *et al*., 2020). The complexes of epigenetic regulating transcriptome include *LHP1*, *FIE2*, *CMT3*, and *DRM2* repressors and TrxG (*PKL*) activators.

During tissue culture, *PCNA*, *PKL*, *FIE2*, *DRM2* & *CMT3* genes (chromatin modifiers genes) and *LHP1*, *DCL1* & *STM* genes (interacting genes) have essential roles in the division and differentiation of the cells of plants. In spite of the identification of those main regulators, it remains unclear to know how they work at the molecular bases (Miguel and Marum, 2011). Those genes in addition to more regulatory ones and must be under more investigations in the future works for a fully understanding of their roles in tissue culture specifically and plant development in general.

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التعبير الجيني لبعض الجينات المرتبطة بالوراثة اللاجينية في الأفرع المتمايزة لنبات الخوخ

ربى مدحت اسماعيل[1]*، رانيا محمود أبوعلي[2] ، محمد نجاتي[3] و محمد عبد الستار[4]

[1] قسم التحول الوراثي للنبات ، معهد بحوث الهندسة الوراثية الزراعية (AGERI) ، مركز البحوث الزراعية (ARC) ، الجيزة ، مصر

[2] قسم كيمياء الأحماض النووية والبروتين ، معهد بحوث الهندسة الوراثية الزراعية (AGERI) ، مركز البحوث الزراعية (ARC) ، الجيزة ، مصر

[3] قسم الإنتاج النباتي ، كلية العلوم الزراعية البيئية ، جامعة العريش ، العريش ، شمال سيناء ، مصر

[4] قسم البيولوجيا الجزيئية النباتية ، معهد بحوث الهندسة الوراثية الزراعية (AGERI) ، مركز البحوث الزراعية (ARC) ، الجيزة ، مصر

تم في هذه الدراسة تأسيس نظام اعادة التميز للأفرع الخضرية للجنين الناضج من بذور الخوخ باستخدام ثلاث بيئات بتركيبات مختلفة من منظمات النمو, بنزيل أدينين (BA) و ثيديازرون (TDZ) واندول اسيتك اسيد (IAA) و اندول بيوتريك اسيد (IBA) . تم الحصول على اعلى نسبة اعادة التميز عند استخدام 1.5 مليجرام/ليتر BA مع 0.5 مليجرام/ليتر IAA. وتم دراسة التعبير الكمي لثمانية جينات Chromomethylase 3 (*CMT3*), Domains Rearranged Methyltransferase2 (*DRM2*), Fertilization Independent Endosperm (*FIE2*) and Chromodomain Protein Like Heterochromatin Protein1 (*LHP1*), Pickle (*PKL*), Proliferating Cell Nuclear Antigen (*PCNA*), Shoot Meristemless (*STM*) and DICER-LIKE1 (*DCL1*) في الافرع الخضرية لنبات الخوخ. و قد وجد أن الاكسينات و السيتوكاينينات وخاصة الـ BA مؤثرة علي التعبير الجيني للجينات و متأثرة بالجنين كجزء نباتي.