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RESEARCH ARTICLE

Regeneration of pinwheel phenotype and evaluation of anthocyanin in African violet (*Saintpaulia ionantha* Wendl.) periclinal chimera

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ABSTRACT: The mutation in the meristem layers creates different genetic backgrounds (chimera) in the plant tissue. The mutation in L1 layer of shoot apical meristem generates a periclinal chimera. UF3GT is an effective enzyme in floral coloration, inducing anthocyanin accumulation in petals. This study investigates direct and indirect regeneration systems and different explants to propagate two cultivars of periclinal chimera (*Saintpaulia ionantha*), namely Taro taraneh and Aghaz, using *in vitro* culture. The evaluation of UF3GT gene expression pattern by Real-Time PCR revealed the role of anthocyanin accumulation in the petal coloration of chimera plants. Results pertaining to both cultivars showed that inflorescence and leaf explant had the highest and lowest percentage of pinwheel phenotype, respectively. In addition, mutant characteristics were faded in the leaf regeneration of periclinal chimera. Furthermore, the highest percentage of periclinal chimera was generated in direct regeneration. Gene expression analysis revealed that UF3GT was expressed in the colorful part of chimera petal, while UF3GT expression was significantly reduced in the muted part. HPLC chromatogram also detected that cyanidin and delphinidin components were not present in the white part of either cultivar. The anthocyanin biosynthesis pathway appears to be blocked and anthocyanin accumulation does not occur in the petals. Inflorescence is likely to induce a pinwheel pattern in regenerated plants, probably owing to its lateral bud. It seems that different meristem layers are associated with the formation of epidermis and induce pinwheel phenotype.

KEYWORDS: Cyanidin, Delphinidin, *In vitro* culture, Ornamental plants, UF3GT gene

INTRODUCTION

Saintpaulia ionantha Wendl. (African violet) is an ornamental, herbaceous and perennial flowering plant belonging to the Gesneriaceae family [8]. In *Saintpaulia*, the flowers are located on peduncle with a cyme inflorescence. Sympetalous petals are available in different colors (purple, red, blue, pink and white) [8]. Monochromatic and bicolor phenotypes with different forms (1. different margin color, 2. pinwheel) and various shapes have been further reported in this plant [8]. The *Saintpaulia* fruit is of capsule type with seeds located inside the capsule. *Saintpaulia* is a type of day-neutral plant which flowers in all seasons [15]. African violet is widely propagated commercially, and its seeds are cultivated in early spring, germinating after 2 to 3 weeks [15]. Sexual propagation by seed is uncommon in African violet, while asexual propagation (leaf cuttings) is widespread [34]. Each leaf cut creates a new plant in an appropriate condition [34]. Other processes of *Saintpaulia* propagation include *in vitro* culture. More than a thousand African violet cultivars are produced by advanced techniques, where several new cultivars are annually added to improve the growth and flowering time of the

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new cultivars [20]. Many plant cultivars are the result of mutation and chimera phenomena [38]. The chance of mutation formation is around one in a million, with only one-thousandth of them becoming mutant cultivar and chimera tissue [38]. Many, on the other hand, return to the primary state or remain in neutral mutations [38]. Some mutations are random and not usually passed on to the next generation through seed culture [7]. In plants, certain variegation patterns are of genetic origin, yet quite natural and related to plant cultivars [38]. Occasionally, the tissue coloration is associated with plant stress that is caused by unfavorable conditions (light or temperature) [7]. Cell division occurs rapidly in shoot apical meristem (SAM) tissues [17]. SAM is included of 3 layers (L1-L3) in the angiosperm plants, and cell division is under genetic control in this tissue [17]. The epidermis of leaves and petals commonly originate from the outer layer of meristem, whereas subepidermal tissues emanate from the inner one [17].

Depending on the type of mutation and its location in SAM, different types of chimeras are formed and categorized into periclinal, mericlinal and sectional types [41]. In the periclinal type, L1 layer is mutated in the meristem and the mutant layer surrounds other meristem layers, hence the genetic difference between the L1 layer and the rest of the meristem layers [6]. Periclinal chimera is stable when mutant tissue is used in the propagated plants. In the mericlinal type, the epidermis contains half the mutant cells, and if originated from the mutant part, the bud will be able to display mutant characteristics [7]. In sectional chimera, all the three meristem layers are mutated [6]. Although most chimeras occur naturally, they can also be generated by grafting [6]. Propagation of chimera plants can determine the difference between the bud origin and the number of cell layers regarding the formation of adventitious shoots [30]. The genotypic modification in the initial meristem cells is able to affect the cells resulting from division and completely change the phenotype [36]; however, the changes in the flank region of the meristem rearrange different parts of the organ phenotypes [9]. Pierik and Steegmans (1983) observed that green branches were also formed in the leaf chimera regeneration. Consequently, different layers are implicated in the regeneration of leaf chimera plants [26]. Plants produce more than 200,000 different colors, including many pigments [13]. The biosynthesis of anthocyanins is derived from phenylpropanoid and phenylalanine [5]. Genetic engineering has used flavonoids/ anthocyanin biosynthesis to generate cultivars with new colors in flowers [5]. The early stage is the production of tetrahydroxy chalcone (THC) compound by Chalcone Synthase enzyme [5]. THC is catalyzed to naringenin component by Chalcone Isomerase (CHI) enzyme [42]. Dihydromyricetin is converted into anthocyanidins by the activities of Dihydroflavonol 4-Reductase (DFR) and Anthocyanidin Synthase (ANS) enzymes. UDP glucose flavonoid 3-glucosyltransferase (UF3GT) enzyme is glycosylates anthocyanin [42].

Ornamental plant propagation has become a global industry that has made a great progress over the years [1]. The importance of chimera plant studies is obvious with regards to breeding and increasing flower beauty and diversity in different African violet cultivars owing to their high economic and ornamental values. Therefore, it is important to investigate different methods of conservation and propagation pertaining to chimera cultivars for a higher productivity and more thorough research. The research purpose was to study the direct and indirect regeneration of Taro taraneh and Aghaz cultivars as an effective step in the propagation of new cultivars using the *in vitro* culture of African violet. In addition, the expression pattern of *UF3GT* gene was evaluated as an effective factor concerning the stability of anthocyanin components in the late stage of anthocyanin biosynthesis pathway. Quantification of anthocyanin was further assessed by HPLC in both chimera cultivars. The accumulation study of anthocyanin compounds and *UF3GT* expression in two African violet cultivars plays a significant role in understanding the petal coloration and attractiveness of chimera plants (pinwheel phenotype).

MATERIALS AND METHODS

Plant collection and *in vitro* culture

The Taro taraneh and Aghaz cultivars were obtained from commercial providers. *In vitro* culture of plants was performed as a factorial experiment in a completely randomized design with three replicates. 30 Inflorescence, petal, pedicel and leaf explants (1cm) were used for *in vitro* propagation (from generative stage). The explants were washed, separated using a sterile scalpel, and sterilized by 70% ethanol (1min) and 0.5% hypochlorite sodium solution (7min) in the laminar hood, respectively. MS media containing different concentrations of plant growth regulators were prepared according to Table 1 [21]. The explants were cultured in different media. In indirect regeneration, plantlets were regenerated in M2 after the callus induction. One month after the last subculture of disinfected plantlets, their roots

Table1. Concentration of plant growth regulators in each culture medium.

Media	Concentration (mgL^{-1})
M1	MS+ 1BAP
M2	MS+ 1IBA+ 1BAP
M3	MS+ 0.1 TDZ
M4	MS+ 0.5 2,4-D

were washed with water, transferred to the pots containing perlite-peat moss (1: 2). After a month, the plantlets were incubated under acclimatization conditions (16:8 h of light: darkness at 26–27°C). About six months after planting, flowering was occurred. Callus induction percentage, direct regeneration percentage, number of adventitious shoots and percentage of pinwheel phenotype were evaluated 25, 40, 41 and 150 days following cultivation, respectively. The percentage of total chimera plants was calculated based on the results of direct and indirect regeneration systems. To determine the percentage of total chimera plants, we enumerated populations of regeneration plants in both regeneration systems.

Gene expression analysis

Petal samples were collected in three biological replicates, and their white and colored sections were separated. Total RNA was isolated and cDNA was synthesized with Qiagen kit, Germany. To evaluate the gene expression of *UF3GT* (MN128019.1), petal samples were analyzed by qPCR method [32]. The sequence of *UF3GT* gene primers was F-5'gtaactacgccaccggaaaa3' and R-5'caggagacaaccttcccaaa3' and the sequence of *ACTIN* gene primer as housekeeping gene was F-5'ttgattctggtgacggggtg3' and R-5'agcaagatccaaccgcagaa3'.

HPLC analysis

Primarily, different parts of both pinwheel petals were separately powdered and 0.5g of samples mixed with 30ml of methanol. Extractions were performed by ultra-sonication (Branson 3510, USA) [11]. The extracts were filtered with 0.45 µm syringe filter and 10 µl was injected into HPLC column. Anthocyanin was evaluated at wavelength of 530nm. Compounds were separated by C18 column (250mm × 0.5mm × 0.5m). Solution A (pH: 2.8) included 3% acetic acid / 7% methanol / 90% H_2O, and solution B (pH 3.4) contained 3% acetic acid / 90% methanol / 7% H_2O (flow rate: 0.35 ml/min). The calibration curves of delphinidin and cyanidin standards (Sigma-Aldrich, USA) were constructed, and anthocyanins were then quantified [11].

Data analysis

Statistical analysis was calculated using SPSS software (v23) and mean comparison was done using Tukey test at 5% level.

RESULTS

Regeneration of *S. ionantha* periclinal chimera

The callus induction and direct regeneration were investigated in Taro taraneh and Aghaz cultivars to compare the explants in terms of pinwheel phenotype formation. As expected, the explants were directly regenerated in M1, M2 and M3 media. M4 medium stimulated callus induction in all explants. Callus was formed in each explant approximately three weeks following *in vitro* culture (Fig. 1 c,d), and plantlets were regenerated in M2 around four weeks after the callus induction (Fig. 2).

Leaf explants and inflorescences showed the highest percentage of callus induction in both cultivars. The callus induction percentage of leaf and inflorescences was 62% in Taro taraneh and 51-52% in Aghaz. The lowest callus induction percentage (15%-20%) was observed in the pedicel explants of both cultivars.

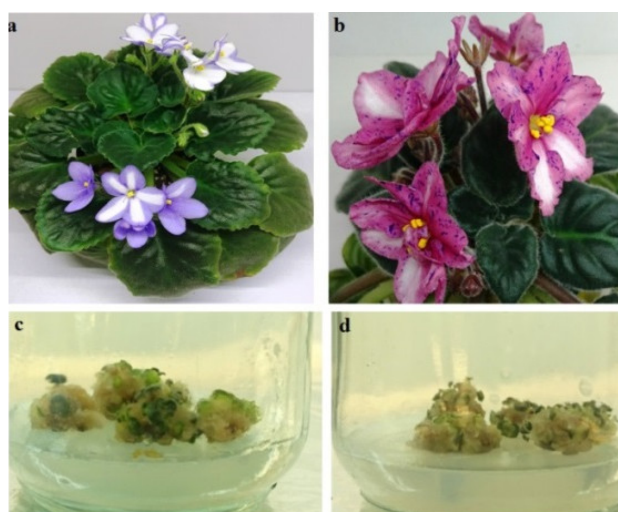

Figure 1. Callus induction in two cultivars, **a** Taro taraneh cultivar and **b** Aghaz cultivar. **c-d** the regeneration of Taro taraneh and Aghaz cultivars (pinwheel), respectively, after the callus induction stage on M2 medium.

Table 2. Effect of medium vs. explants in two chimera cultivars of African violet 3-4 weeks following cultivation.

Cultivar	Explant	Regeneration (%)			Callus induction (%)	No. of adventitious shoots
		M1	M2	M3	*M4	
Taro taraneh	Petal	70^{f}	81^{a}	78^{d}	35^{f}	61^{c}
	Inflorescence	80^{e}	90^{e}	80^{d}	62^{e}	71^{a}
	Pedicle	59^{a}	63^{d}	58^{a}	20^{b}	52^{b}
	Leaf	90^{d}	95^{e}	90^{c}	62^{e}	80^{d}
Aghaz	Petal	43^{c}	58^{d}	54^{a}	26^{d}	50^{b}
	Inflorescence	60^{a}	70^{c}	50^{a}	51^{c}	62^{c}
	Pedicle	31^{b}	35^{b}	31^{b}	15^{b}	51^{b}
	Leaf	58^{a}	82^{a}	56^{a}	52^{a}	70^{a}

Different letters in each column indicate significant difference at $P< 0.05$ level. Error bars represent standard deviation (SD). Data are the mean ± SD of three replicates. 30 Inflorescence, petal, pedicel and leaf explants (1cm) were used. * The highest percentage of regeneration was observed in MS + 1IBA + 1BAP (M2) treatment. Therefore, plantlets were regenerated in M2 after the callus induction.

Data comparison revealed a significant difference between explants concerning the percentage of regeneration and number of adventitious shoots (Table 2). The highest percentage of regeneration (95%) was observed in MS + 1IBA + 1BAP (M2) treatment and in the leaf, inflorescence and petal explants of both cultivars, respectively. In fact, there was a significant difference between these explants regarding both African violet cultivars. The mean comparison indicated that all explants in Taro taraneh cultivars showed better results than Aghaz cultivar (Table 2). Moreover, the number of adventitious shoots was significantly increased in the Taro taraneh cultivar compared to Aghaz cultivar. The highest number of adventitious shoots in both cultivars was observed in leaf, inflorescence, petal and pedicel explants, respectively (Table 2).

The results showed that in both cultivars, inflorescence explant propagated the highest percentage of chimera phenotype (Table 3). In fact, 93% of the regenerated plants showed a pinwheel phenotype in Taro taraneh cultivar whereas no chimera plant was generated in the leaf explants (Table 3). There was a difference ($p<0.05$) between reproductive and vegetative explants regarding the regeneration of Taro taraneh cultivar. In fact, the regeneration of petal explants in Taro taraneh cultivars showed that 74% of the regenerated plants were able to pass the pinwheel phenotype, while 20% revealed a purple phenotype. There was a difference ($p<0.05$) between the petal and pedicel explants of both cultivars in regeneration. The percentage of chimera plants in Taro taraneh cultivar was higher than Aghaz cultivar (Table 3). The regeneration of petal explants in Aghaz cultivar revealed that 67% of the regenerated plants had a pinwheel phenotype, while 33% showed a pink phenotype (Table 3).

The percentage of total chimera plants was calculated based on the results of direct and indirect regeneration systems. To determine the percentage of total chimera plants, we enumerated populations of regeneration plants in both regeneration systems. The data showed that the direct regeneration system propagated more chimera plants. In the direct regeneration, 93% of pinwheel phenotype was propagated, whereas only 7% of chimera plants were obtained by indirect regeneration method (Fig. 3).

UF3GT gene expression pattern and HPLC analysis

To evaluate the gene expression levels, white and colored petals were studied in two African violet periclinal chimera phenotypes. The *UF3GT* gene expression level in the colored section of Aghaz cultivar was significantly different from that of Taro taraneh cultivar. In fact, *UF3GT* gene had a higher expression in Aghaz cultivar. The expression of *UF3GT* gene was significantly increased in the colored section of petal in Taro taraneh

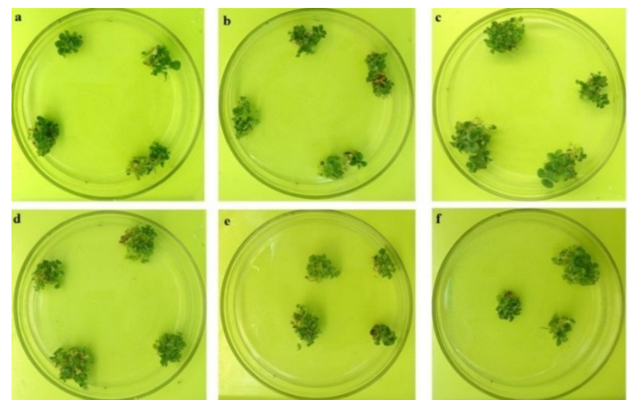

Figure 2. Direct regeneration of periclinal chimera. **a-c** regeneration of Taro taraneh cultivar in M1, M2, M3 medium, respectively. **d-f** regeneration of Aghaz cultivar in M1, M2, M3 medium, respectively.

Table 3. Phenotype of the regenerated plants.

Cultivar	Explant	Monochromatic Phenotype % (White)	Monochromatic Phenotype % (Purple/Pink)	Pinwheel Phenotype %
Taro traneh	Petal	6[a]	20[f]	74[f]
	Inflorescence	2[a]	5[c]	93[c]
	Pedicle	5[a]	35[d]	60[d]
	Leaf	0[a]	100[a]	0[a]
Aghaz	Petal	0[a]	33[d]	67[d]
	Inflorescence	0[a]	10[c]	90[c]
	Pedicle	0[a]	70[b]	30[b]
	Leaf	0[a]	100[a]	0[a]

White and purple colors belong to Taro taraneh cultivar, while the pink color is associated with Aghaz cultivar. Different letters in each column indicate significant difference at P< 0.05 level. Error bars represent standard deviation (SD). Data are the mean ± SD of three replicates.

cultivar compared to the control sample, and the *UF3GT* gene expression level was five times higher than the control (Fig. 4a). Whereas Real-Time PCR results indicated that the *UF3GT* expression was decreased in the white section of the petal in Taro taraneh cultivar, the expression level revealed a significant increase compared to the control sample. Furthermore, the *UF3GT* gene expression in white and red petal sections of Aghaz cultivar underwent significant changes (p <0.05). In the red sections, *UF3GT* gene expression was 7.5 times more than the control, while the *UF3GT* gene expression level in the white section was lower than the red section. The expression level of *UF3GT* gene in the red section of the Aghaz cultivar petal was approximately 2.5 times that of the white section in the petal. However, the expression level of *UF3GT* gene was significantly increased (p <0.05) compared to the control sample (Fig. 4a).

In the *UF3GT* gene product analysis, cyanidin and delphinidin components were identified in the pinwheel petals of both African violet cultivars. Fig. 4b-e demonstrates the separation of anthocyanin compounds in HPLC profile. The anthocyanin amount and retention time of each compound were further quantified. The retention time of delphinidin and cyanidin compounds was calculated at 18 and 12 min, respectively. Unlike the white petals, delphinidin component was identified in the HPLC chromatogram of the purple section (Fig. 4). HPLC analysis showed that, while undetectable in the white section, delphinidin component was 20.47 mg/g in the purple section. In contrast to the white section of the petals, a cyanidin component was detected in the HPLC chromatogram of the red section of petals in Aghaz cultivar (Fig. 4). The HPLC analysis showed that the cyanidin component in the red section was 15.37 mg/g, whereas it was undetectable in the white section of Aghaz cultivar. In fact, the results of gene expression are consistent with the HPLC analysis of anthocyanin compounds.

DISCUSSION

Cultivars derived from chimera tissues are highly valuable, and chimeras may occur between the plants of the same or different species [7]. The results showed that in both Taro taraneh and Aghaz cultivars, inflorescence explants the highest percentage of chimera phenotype. In fact, regeneration of petal and inflorescence explants in Taro taraneh and Aghaz cultivars showed that most regenerated plants succeeded in propagating the periclinal chimera phenotypes, whereas 20-33% showed pink and purple phenotype.

Different organs of flower originate from meristem layers. Epidermis commonly originates from the L_1 meristem layer, yet the $L_1 + L_2$ meristem layer sometimes participates in the formation of the epidermis [41]. Concerning the periclinal chimera of potato plants, Filippis et al. (2013) showed that the leaves, in which

Figure 3. Regenerated plants. a vegetative stage, b flowering stage of Taro taraneh cultivar, c-d flowering stage of Aghaz cultivar.

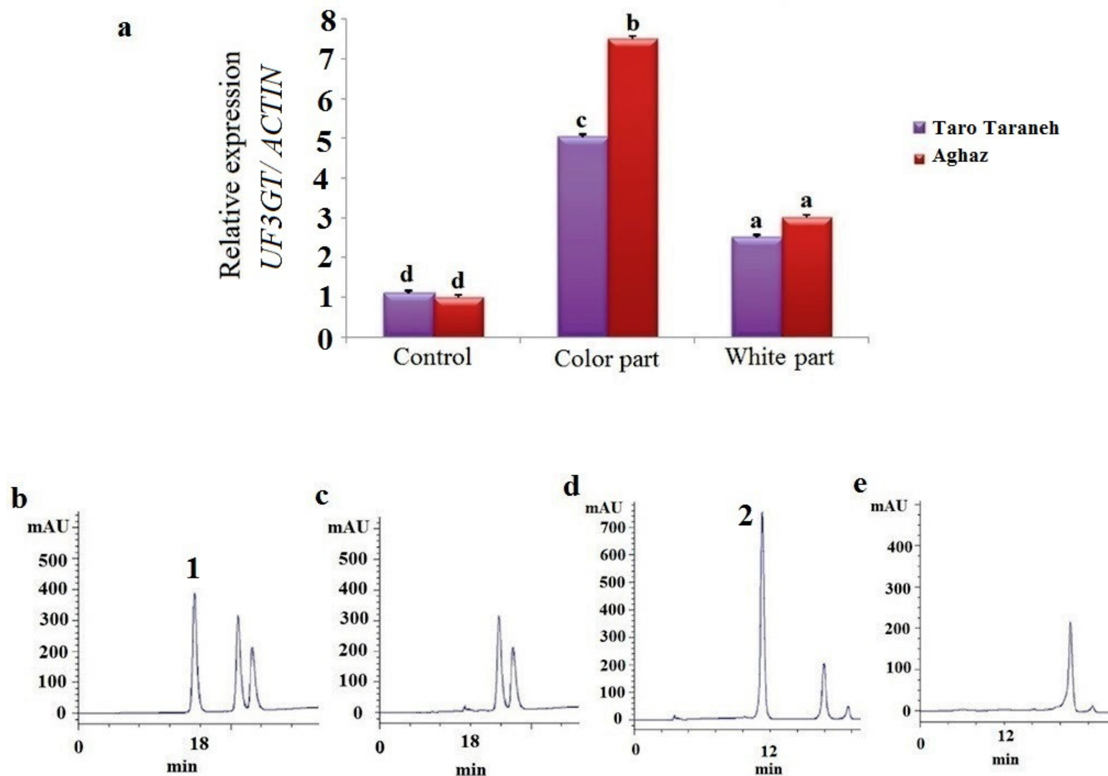

Figure 4. The changes in anthocyanin component in different sections of the pinwheel petals. **a** *UF3GT* gene expression level in the pinwheel petals of both cultivars. Purple and red monochromatic petals were considered as control samples in Taro taraneh and Aghaz cultivars, respectively. **b**, **c** HPLC chromatogram of delphinidin compound in the purple and white sections of Taro taraneh cultivar, respectively. **d, e** HPLC chromatogram of cyanidin compound in the red and white sections of Aghaz cultivar, respectively. Different letters in each column showed a significant difference at $p<0.05$. Error bars represent standard deviation (SD). Data are the mean ± SD of three replicates. 1: Delphinidin, 2: Cyanidin.

polymorphism was also detected, were less pale than normal leaves. Their results indicated that the expression of chloroplast-target proteins involved in chlorophyll biosynthesis was inhibited in L_1 layer, ultimately affecting the biosynthesis pathway of chlorophyll [6]. Teixeira et al. (2016) reported that in *Saintpaulia ionantha* priclinal chimera flowers, the petal margin (pink) was derived from L_1 layers, yet the center of petal (purple) was derived from $L_1 + L_2$ layers [37]. The type of explant and the time of sampling also influence the regeneration results in tissue culture [37]. The present study revealed that leaf and florescence explants had the highest percentage of callus induction in both cultivars. The lowest callus induction percentage (15-20%) was observed in the pedicel explants of both cultivars. The highest percentage of regeneration (95%) belonged to MS + 1IBA + 1BAP (M2) treatment. The data showed that all explants had better results in Taro taraneh cultivars compared with Aghaz cultivar. The highest number of adventitious shoots in both cultivars was observed in leaf, inflorescence, petal and pedicel explants, respectively. In a study on the African violet plant, Sunpui and Kanchanapoom (2002) observed that the most optimal callus induction medium, the highest number of regenerated adventitious shoots and the highest regeneration percentage were obtained in the media containing different concentrations of BA and IBA.

Sunpui and Kanchanapoom (2002) reported that leaf explants best suited the callus induction, with the type of tissue being also effective in callus formation. Yunqing et al. (2010) revealed that the medium containing 2mg/L 2,4-D had the highest rate of callus induction compared to other plant growth regulators. The stability of chimera phenotype is related to the absence of callus induction stage as it entails the re-differentiation of cells, and the pattern may not be propagated [31, 44]. Using different explants, Peary et al. (1988) reported the *in vitro* culture of Tommie Lou cultivar in African violet chimera to result in a very high stability of chimera pattern. However, different tissues of Valencia cultivars had at

least cells with two different genotypes [24, 25]. Gordienko (2002) investigated the *in vitro* culture of leaf chimera plants in African violet and observed thick and variegated leaves; however, the color of flower was reported to be constant in all the regenerated plants. In fact, Gordienko (2002) showed that the leaf tissue contains and retains the mutant cells. Periclinal chimera propagation is not passed by leaf cutting because mutant cells should be derived from the mutant layer of the meristem [7]. Therefore, in case of leaf cut, the buds will originate from the inner cells (L_2 and L_3). As a result, they will not be able to pass the genotype of L_1 layer [41]. Shajiee et al. (2006) investigated the phenotypic changes of African violet leaf chimera via *in vitro* culture and showed that only 33% of the plants were similar to parental plants, whereas 67% underwent different morphological changes. The percentage of changes in leaf color, leaf shape and flower color were 67%, 19% and 19%, respectively [30]. The results of propagation via leaf cutting in different cultivars showed a variegated pattern only with regards to the propagation of one cultivar [30]. During the developmental stages, the variegated pattern of these cultivars was severely affected by environmental conditions [30]. Although the propagation of variegated sections in one cultivar followed a regular pattern, this characteristic was non-existent in other cultivars regenerated from the leaf tissues [16].

In the present research, the propagation of African violet periclinal chimera cultivars with the help of vegetative organs did not produce the desired results. Regeneration of reproductive organs (inflorescences and petals) showed the highest number of chimera plants in the current study. The existence of different phenotypes in the regenerated plants seems to indicate the origin of each phenotype from different explant layers. In periclinal chimera propagation, the lateral bud plays a significant role owing to the existence of the genotype of all three meristematic layers [29]. The results also showed that explants containing mutant tissues were able to propagate the chimera phenotype. Owing to their lateral buds, inflorescences are likely to induce a pinwheel pattern in the regenerated plants [41]. It appears that the association of L_2 layer in the formation of epidermis causes the bicolor flower formation in *Saintpaulia* priclinal chimera. The pinwheel pattern illustrates the role of meristematic layers in the formation of the *Saintpaulia* petal epidermis [41]. The color of ornamental plants is considered as a qualitative characteristic which adds to the economic value of flowers [40]. Genetic mutations in flowers and vegetative organs induce changes in the number, shape, and color of leaves and petals [40]. The flower color is associated with structure, pigment type and pigment distribution, all controlled by exogenous and genetic factors [16]. Color variability in ornamental plants has been achieved through hybridization methods, mutation and somaclonal variation, some of which have been transformed into experimental models [28]. Many researchers make use of the knowledge of flavonoids biosynthesis pathway and genetic engineering to obtain unique flowers [23]. The genotype of meristem layers controls the variegation pattern of flowers in African violet [22]. The chimera phenotype may be caused by a number of processes, including mutation, grafting, and protoplast fusion [7]. Becraft (2013) stated that the insertion of active transposons generated a variety of chimeras. Calderwood et al. (2016) suggested that the movement of RNAs from cell to cell was involved in the formation of chimera. Warschefsky et al. (2016) showed that cell invasion from one meristem layer to another might cause the formation of periclinal chimera. In fact, the invasion of L_2 layer into L_1 layer affects the formation of pigmentation and the periclinal chimera phenotype [38].

Both African violet phenotypes indicated that in the white section of pinwheel petal, *UF3GT* gene expression was significantly reduced. However, the expression pattern of *UF3GT* gene in the colored section was significantly higher than the white part. HPLC analysis indicated that the cyanidin and delphinidin components were accumulated in the colored section of petals. However, the combination of cyanidin and delphinidin was undetectable in the white section. In fact, the results of gene expression are consistent with the HPLC analysis of anthocyanin compounds. The presence or absence of pigments is considered as a marker for tracking chimera plants [38]. Nabeshima et al. (2017) classified the periclinal chimera tissue into two types, one with fast L_1 layer divisions that eventually form monochromatic flowers (single genotypes), and the other with low L_1 layer divisions resulting in epidermis originating from different layers of meristem, hence the bicolor flower formation (different genotype backgrounds).

The color of African violet flower is formed during the anthocyanin biosynthesis pathway, which suppression blocks the anthocyanin biosynthesis and blench the petals [22]. Modification of anthocyanins is accomplished by the involvement of methyl transferase, acyl transferase and glycosyl transferase enzymes [18]. Anthocyanins

remain stable only in acidic conditions [39]. Glycosyl transfer to the 3-hydroxyl group of anthocyanidins is catalyzed by UF3GT enzyme (belonging to the glycosyl transferase family) in the cytosol, after which the anthocyanidins are transferred to the vacuole. In fact, UF3GT enzyme forms a stable compound by anthocyanin glycosylation [39]. Glycosylation further results in the solubility of anthocyanin and their recognition by receptors [12]. When *UF3GT* gene expression is inhibited, anthocyanin is not properly transferred to vacuoles, hence the fact that the accumulation of anthocyanin does not occur [4]. Kobayashi et al. (2002) revealed that the ectopic expression of *UF3GT* gene generated red spots on the grapes. They further showed that, unlike white cultivar, *UF3GT* mRNA was accumulated in the skin of red grape. Lo piero et al. (2005) demonstrated that the UF3GT enzyme was accumulated in blood oranges, but not in blonde oranges. In fact, when *UF3GT* is not expressed, anthocyanidins are degenerated. Harborne and Williams (2002) showed that cyanidin was modified by acylation and glycosylation in *Orchids* flowers. Sun et al. (2017) reported that the overexpression of *UF3GT* gene caused a white to pink discoloration and anthocyanin accumulation in *Petunia* flowers. *MYB* gene expression, which regulates the anthocyanin biosynthesis pathway, was also increased. In fact, the overexpression of *UF3GT* gene controls the expression of genes that are effective in anthocyanin biosynthesis [33]. Chen et al. (2011) revealed that *ANS* and *UF3GT* genes had a higher expression in *Phalaenopsis* red flowers in comparison with white flowers. Silencing and reducing the activity of *UF3GT* gene faded the color of certain flower parts and reduced the anthocyanin content [4]. Consequently, unstable *UF3GT* expression was not able to induce flower pigmentation [4].

Pourcel et al (2013) reported that the overexpression of *UF3GT* gene activated the *F3H* gene, resulting in the increased biosynthesis of flavonoids. In fact, changing *UF3GT* expression resulted in a feedback that increased the expression of anthocyanin biosynthetic pathway genes [33]. Nabeshima et al. (2017) investigated African violet chimera cultivars and demonstrated that in the L1 layer of these plants, *F3H* muted and *WDR1* gene were present and the anthocyanin biosynthesis pathway was blocked. The accumulation of anthocyanin was further reduced in the mutant tissue [22]. The epidermis layer in African violet had a different genotype background; therefore, two meristem layers contributed to the formation of periclinal chimera. In addition, expression of *F3'5'H* gene in the blue and pink sections of Kaname pinwheel petals showed that in each segment, the anthocyanin biosynthesis pathway functioned independently [41]. In fact, the expression of *F3'5'H* gene was higher in the blue part than in the pink part, and the malvidin component was only accumulated in the blue part. However, *F3'5'H* gene was mutated in the pink section, and the biosynthesis of anthocyanin was redirected to the pink color [41]. Additionally, unlike the blue part, the pelargonidin compound was detected in the pink part of the pinwheel petals [41].

CONCLUSION

The present study showed that leaf explants and inflorescences had the highest percentage of callus induction in both cultivars. It seems that the most optimal induction medium and the maximum regeneration rate occurred in MS 1IBA+ 1BAP (M2) treatment. The highest percentage of regeneration was observed in leaf, inflorescence and petal explants, respectively. Furthermore, the direct regeneration system propagated more chimera plants. Overall, the propagation efficiency of periclinal chimera showed better results in Taro taraneh cultivars compared with Aghaz cultivar. Gene expression evaluation further showed that *UF3GT* gene expression was not significantly different between the colored parts of chimera phenotype, while the *UF3GT* expression was decreased in the muted part of petals. The HPLC analysis of *UF3GT* gene product also indicated the presence of cyanidin and delphinidin components in the colored section of petals although these compounds were not detectable in the mutant section. The present study reveals the necessity of employing efficient methods to increase the number of chimeras and genetic abilities of African violet and develop novel traits.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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باززایی فنوتیپ pinwheel و ارزیابی آنتوسیانین در شیمر پریکلینال بنفشه آفریقایی

(*Saintpaulia ionantha* Wendl)

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چکیده

جهش در لایه‌های مریستمی سلول‌هایی با بیش از یک آرایش ژنتیکی (شیمر) در بافت ایجاد می‌کند. وقوع جهش در لایه L1 مریستم رأس ساقه موجب ایجاد شیمر پریکلینال (فنوتیپ pinwheel) می‌شود. رنگ گل بنفشه آفریقایی از رنگیزه آنتوسیانین منشا می‌گیرد. آنزیم UDP-گلوکز فلاونوئید ۳-گلیکوزیل ترانسفراز به عنوان عضو موثر در مسیر بیوسنتز آنتوسیانین و رنگ گل به شمار می‌رود که موجب تجمع آنتوسیانین در گلبرگ‌ها می‌شود. این تحقیق به مطالعه روش‌های باززایی مستقیم و غیر مستقیم و ریزنمونه‌های متفاوت به منظور تکثیر دو فنوتیپ pinwheel (ارقام "تار و ترانه" و "آغاز") گیاه شیمر پریکلینال بنفشه آفریقایی در شرایط *in vitro* پرداخته است. بیان ژن *UF3GT* با روش qPCR مقایسه شد. همچنین ارزیابی آنتوسیانین با روش HPLC صورت گرفت. نتایج در هر دو رقم مشخص کرد که ریزنمونه ساقه گل‌دهنده بیشترین درصد فنوتیپ pinwheel را تولید می‌نماید، درحالی که ریزنمونه برگ کمترین درصد فنوتیپ pinwheel را نشان می‌دهد. علاوه بر این، خصوصیت جهش‌یافته در نتایج حاصل از باززایی برگ ارقام شیمر محو گردید. همچنین مطابق نتایج در روش باززایی مستقیم بیشترین درصد شیمر پریکلینال ایجاد شد. بررسی بیان ژن مشخص کرد که در بخش رنگی گلبرگ pinwheel، ژن *UF3GT* بیان بالایی داشته در حالی که بیان ژن *UF3GT* در بخش سفید به طور معنی‌داری کاهش یافته است. همچنین آنالیز HPLC مشخص کرد محتوی دلفینیدین و سیانیدین در بخش سفید گلبرگ هر دو رقم حضور نداشته است. به نظر می‌رسد که مسیر بیوسنتز آنتوسیانین بلوکه شده و تجمع آنتوسیانین‌ها در گلبرگ رخ نداده است. گل‌آذین احتمالا به دلیل داشتن جوانه جانبی، می‌تواند الگوی pinwheel را در گیاهان باززایی‌شده القا کند. به نظر می‌رسد که لایه‌های L1+L2 در شکل‌گیری اپیدرم مشارکت داشته و موجب ایجاد فنوتیب pinwheel شده است.

کلمات کلیدی: ژن *UF3GT*، سیانیدین، دلفینیدین، کشت بافت، گیاهان زینتی.