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Araştırma Makalesi

Farklı sitokinin ve oksin kaynaklarının *Kalanchoe blossfeldiana* mikroçoğaltımı üzerine etkisi

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ÖZ

Mikroçoğaltım, aseptik koşullar altında birçok farklı süs ve tıbbi bitki türünün vejetatif olarak çoğaltılması amacıyla yaygın olarak kullanılmaktadr. Bu teknoloji, mevsim ve zaman-mekan kısıtlamalarına bağlı kalmaksızın yaygın pratik uygulamalara sahiptir. Bu çalışmada, *Kalanchoe blossfeldiana* yaprak sapına (petiol) yakın kısımlardan hazırlanan yaprak segmentleri eksplant olarak kullanılmış ve 30 g l^{-1} sakkarozla desteklenen 18 adet farklı BAP, NAA, TDZ ve GA_3 kombinasyonları in vitro çoğaltım amacıyla denemelerde yer almıştır. Kültürün 12. haftasında yaprak eksplantlarının kesilmiş yüzeyleri üzerindeki mikro kalluslarda, daha sonra sürgünlere dönüşen meristematik dokular meydana gelmiştir. 3, 8 ve 9 numaralı uygulamalar (BAP:NAA:GA3= 1.5:0.5:0.0, 1.0:0.5:0.2, 1.5:0.5:0.2 mgL^{-1}), eksplant başına en yüksek sürgün sayısını vermiştir (eksplant başına 5.03±1.21, 4.32±0.14 ve 4.28±0.18 sürgün). Yaprak eksplantlarının TDZ içeren ortamlarda kültüre alınması, kültürün 8. haftasından itibaren küme benzeri sürgün farklılaşmasına yol açmıştır. Proliferasyon aşamasında 0,3 mg L-1 GA3 + 0,3 mg L^{-1} BAP içeren MS ortamına aktarılan her sürgünden ortalama 84,52 ± 24,21 adet sürgün elde edilmiştir. Köklenme, 0,01-0,05 mg L^{-1} IBA içeren ½ MS ortamlarında %100 performansla elde edilmiştir.

Anahtar Kelimeler: *:* PGR, ticari çoğaltım, in vitro, mikroçoğaltım, sürgün rejenerasyonu, Kalanchoe

The effect of different cytokinin and auxin source on micropropagation of *Kalanchoe blossfeldiana*

ABSTRACT

Micropropagation is widely used for vegetative propagation of many different ornamental and medicinal plant species under aseptic conditions. This technology has widespread practical applications irrespective of seasonal and time-space constraints. In this study, leaf segments prepared from parts close to the petiole of *Kalanchoe blossfeldiana* were used as explants and 18 different combinations of BAP, NAA, TDZ and GA3 supplemented with 30 g l^{-1} sucrose were included in the experiments for in vitro propagation.The development of meristematic tissues was noted directly at the 12th week of culture on the micro callus formed on the cut surfaces of the leaf explants into shoots. Treatments 3, 8, and 9 (BAP:NAA:GA_3= 1.5:0.5:0.0, 1.0:0.5:0.2, 1.5:0.5:0.2 mgL^{-1}), yielded the highest shoot counts per explant (5.03±1.21, 4.32±0.14, and 4.28±0.18 shoots per explant). Culturing leaf explants in TDZ-containing culture treatments led to cluster-like shoot differentiation from the 8th week of culture. An average of 84.52 ± 24.21 adventitious shoots was obtained from each shoot transferred to the MS medium amended with 0.3 mg L^{-1} GA_3 + 0.3 mg L^{-1} BAP at the proliferation stage. Rooting was obtained in ½ MS media 0,01-0,05 mg L^{-1} IBA added with 100% performance.

Key Words: PGR, commercial multiplication, in vitro, micropropagation, shoot regeneration, Kalanchoe.

Introduction

The *Crassulaceae* family consists of around 1,400 species that range in structure, from small, and relatively nonsignificant annual herbaceous plants to perennial shrubs or trees, and are characterized by their striking and attractive flowers. Plants from the Kalanchoe genus, found in this family, are predominantly perennial succulent shrubs or bushes, and rarely small trees. *Kalanchoe blossfeldiana* is a hairless, succulent plant; with leaves and stems possessing a low surface-area-to-volume ratio (Mackenzie et al., 2018). The breeding of new varieties of vegetatively propagated *K. blossfeldiana* (2n=34) with limited variation began in late 1939, after interspecific hybridizations between *K. glaucescens* × *K. blossfeldiana* (Descoing, 2006; Mackenzie et al., 2018). Commercial Kalanchoe varieties are tetraploid in chromosome structure (2n=4×=68) with multiple number of varieties currently cultivated as pot and cut flower plants. All varieties traded in Türkiye have variable exotic origins. There are over 700 registered Kalanchoe varieties worldwide with a large proportion of them bred from *K. blossfeldiana* (Community Plant Variety Office - CPVO). The rest of them were bred from *K. marmorata, K. humilis, K. manginii, K. laciniata, K. uniflora,* and *K. thyrsiflora* etc. (CPVO, 2017). Although both annual and perennial forms are found in nature, potted outdoor perennials are more preferred (Kahraman and Boyaci, 2021). Several Kalanchoe taxa in Madagascar and India are also used as medicinal plants (Descoings, 2006).

The Kalanchoe plants can be propagated generatively and vegetatively using leaf cuttings (Love, 1980; Mackenzie et al., 2018*)*. Generative propagation is generally not desired in the Kalanchoe due to the risk of heterozygosity.Tissue cultures offer a significant advantage in terms of vegetative propagation, and facilitate in production of true-to-type plants especially when breeding is not the focus. Some breeding through *in vitro* mutation and embryo rescue techniques to produce interspecific Kalanchoe hybrids have also been reported in Türkiye with the production of many varieties (Kökpınar et al., 2021; Kahraman et al., 2022).

Numerous studies have been conducted on the propagation of Kalanchoe through tissue culture. Sanikhani et al. (2006) and Lütken et al. (2011) reported that shoot regeneration could be obtained on leaf and petiole sections of *K. blossfeldiana* using combinations of auxins (NAA or IAA) and cytokinin's (BAP or Kinetin), or from internodal explants using TDZ singly. Zheng et al. (2013) used leaf petioles as explants for *in vitro* propagation of *K. blossfeldiana* in a medium containing 1.0 mg L^{-1} BAP and 0.1 mg L^{-1} NAA. These induced rooting on 1/2 × MS medium amended with 0.2 mg L^{-1} NAA. Xue et al. (2019) also used the apical bud as the initial explant. Dinani et al. (2018), have mentioned indirect shoot regeneration from leaf explants with applications of low doses of IAA and Zeatin. Gümüs and Ellialtioglu (2018), have reviewed the tissue culture of Kalanchoe with a mention of 1×MS medium amended with different concentrations of NAA and BAP. Rooting (100%) was achieved on ½ ×or 1×MS medium amended with 0.01 – 0.5 (0.01-0.5 mg L^{-1} IBA with 95% rooting (Liu, 2010). The researchers were also successful in rooting the plants under *ex-vitro* conditions. Survival percentage had a general range of 85-100% after acclimatization, where the weak plants were eliminated (Pierik 1997; Liu, 2010; Bejaoui, 2022).

TDZ is a powerful plant growth regulator that has been used singly or in combination to promote and encourage cell division at very low concentrations (Giridhar et al., 2018). There are reports of using TDZ in combination with kinetin, 2iP, or auxins to regenerate new shoots on many plant species like *Portulaca pilosa*, *Neolamarkia cadamba*, lentil on MS or other regeneration media and increase the number of shoots (Gaba, 2000; Sevimay et al., 2005; Guo et al., 2011; Chen et al., 2020; Huang et al., 2020) to increase induction efficiency (Huetteman and Preece, 1993; Gaspar et al., 1996; Corredoira et al., 2008; Podwyszyńska et al., 2022; Aasim et al., 2023).

The aim of the study was to design *in vitro* multiplication studies for the breeding of local indigenous Kalanchoe varieties and optimize the regeneration and proliferation of shoots involving TDZ or BAP (cytokinin), NAA (auxin), and GA_3. Therefore, more studies are needed to encourage and find the related threshold levels of plant growth regulator concentrations for micropropagation. The rooting of *in vitro*-induced shoots under aseptic conditions and their acclimatization to external conditions was also ensured, to complete the entire propagation process.

MATERIALS AND METHODS

This study employed a white-flowered commercial variety of *K. blossfeldiana* as plant material. The experimental material was obtained from the Department of Horticulture of Ankara University. The 1 × MS (Murashige and Skoog, 1962) basal medium amended with various plant growth regulators was used as a culture medium (Table 1), it was supplemented with 30 g L^{-1} sucrose as a carbon source. Distilled water was used to prepare the cultures. The medium pH was adjusted to 5.7±0.1 before autoclaving.

The study used a leaf segment (explant) having 3-6 shoot tips of Kalanchoe and subjected them to surface sterilization by shaking them in 15% commercial bleach (1.5% sodium hypochlorite) for 15 minutes.

Subsequently, they were rinsed 3×5 min with sterilized distilled water. These explants were dried on sterile drying paper under aseptic conditions in a laminar flow cabinet before their culture.

Each treatment contained 45 explants, which were equally divided into 9 replications containing 5 explants. Shoot regeneration was carried out on 18 different combinations of BAP, NAA, GA_3, and TDZ as explained in Table 1:

(a) 0.5, 1, and 1.5 mg L^{-1} BAP + 0.5 or 0.1 mg L^{-1} NAA (3 combinations each).

(b) 0.5+ 0.5 and 0.1 mg L^{-1} NAA (two combinations), 1+ 0.5 and 0.1 mg L^{-1} NAA (two combinations), or 1.5 mg L^{-1} BAP + 0.5 and 0.1 mg L^{-1} NAA (two combinations) with or without 0.2 mg L^{-1} GA_3.

(c) 0.1, 0.2, 0.5 mg L^{-1} TDZ with and without 0.1 mg L^{-1} NAA (3 combinations each).

Table1. 18 different combinations of BAP, NAA, GA_3, and TDZ used to regenerate kalanchoe shoots

Treatments	**BAP (mg L^{-1})**	**NAA (mg L^{-1})**	**GA_3 (mg L^{-1})**
1	0.5	0.5	-
2	1.0	0.5	-
3	1.5	0.5	-
4	0.5	0.1	-
5	1.0	0.1	-
6	1.5	0.1	-
7	0.5	0.5	0.2
8	1.0	0.5	0.2
9	1.5	0.5	0.2
10	0.5	0.1	0.2
11	1.0	0.1	0.2
12	1.5	0.1	0.2
		Treatments	
	TDZ (mg L^{-1})	NAA (mg L^{-1})	GA_3 (mg L^{-1})
13	0.1	-	-
14	0.2	-	-
15	0.5	-	-
16	0.1	0.1	-
17	0.2	0.1	-
18	0.5	0.1	-

Hardening or vegetative maturity stage: Each treatment on MS medium amended with respective phytohormones or their combinations at this stage was compared with control treatment to find their source or carry-over effect, in terms of shoot thickness, and number of new regenerated shoots per mother shoot and their length. Morphological variations were noted on the regenerated plantlets using 18 different plant growth regulator combinations including TDZ after 4 weeks, when the shoot growth stopped. Thereafter, they were transferred to ½ × MS medium amended with 0.2 mg L^{-1} GA_3 medium to harden or vegetatively mature them before proceeding to the rooting. Non rooted shoots were kept as stock for later use to multiply them or use in micropropagation studies (not given here). All other incubation conditions remained the same during the hardening stage. They were acclimatized in the greenhouseusing high quality transparent plastic cell trays with transparent vented covers (mini greenhouse) to maintain a condition of controlled humidity for 4 days. Subsequently, the mini greenhouse covers were gradually removed in agreement with Bejaoui et al. (2023 b). This treatment was of a crucial and significant importance for early vegetative maturity to complete the acclimatization and rooting.

The developing shoots were rinsed under tap water after removing agar from the nutrient medium. The first method used planting in soil mix containing equal proportions of (i) sand, (ii) vermiculite, and (iii) their mixture (sand and vermiculite (1:1 v:v) in 1/4 L of cell cubes (Bauhaus Türkiye – a commercial vendor), to acclimatize them in mini greenhouse.

The vents of mini-greenhouse were opened twice daily to maintain the humidity. This process was continued until the relative humidity reached 50% in corroboration with the external environmental conditions after one week. During carrying out of this process the room temperature was maintained at 20±2 °C using a fixed 16 h light 8 h dark day length. Thereafter, acclimatization and hardening; the plants were transferred to 10×10×10 cm pots, until flowering in an unheated greenhouse under ambient conditions of temperature and humidity until the end of February.

Statistical analysis

The experiments were carried out in randomized complete block design in split plots. Three replications were repeated thrice. Measurements and counts were made on 15 explants in each replication. The data were subjected to variance analysis using the "SPSS 25.0 for Windows". Where appropriate LSD or Duncan's test was applied to compare the treatment means (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

Some level of contamination was encountered in the study at the initial culture stage. No infection was observed in the first week of culture, fungal or bacterial growth occurred within the following two weeks. Petri dishes exhibiting contamination were discarded by autoclaving them and were replaced new healthy cultures. The explants induced callus after cell divisions at the cut edges obtained after 10-12 days of culture with the induction of whitish green calli at peripheral edges of leaves (Fig 1a), which allowed variable regeneration of healthy shoots and leaves after 16-18 days of culture and incubation (Fig 1 b) depending on the concentration and combination of plant growth regulators. The explants induced direct somatic embryogenesis by showing profuse rooting. However, this type of regeneration was limited to petiole regions of the leaves. These explants inhibited regeneration on the other parts of the explants due to gradual development of necrosis that engulfed the whole explants with the passage of time. Therefore, these explants were not encouraged for micropropagation (Fig 1 c).

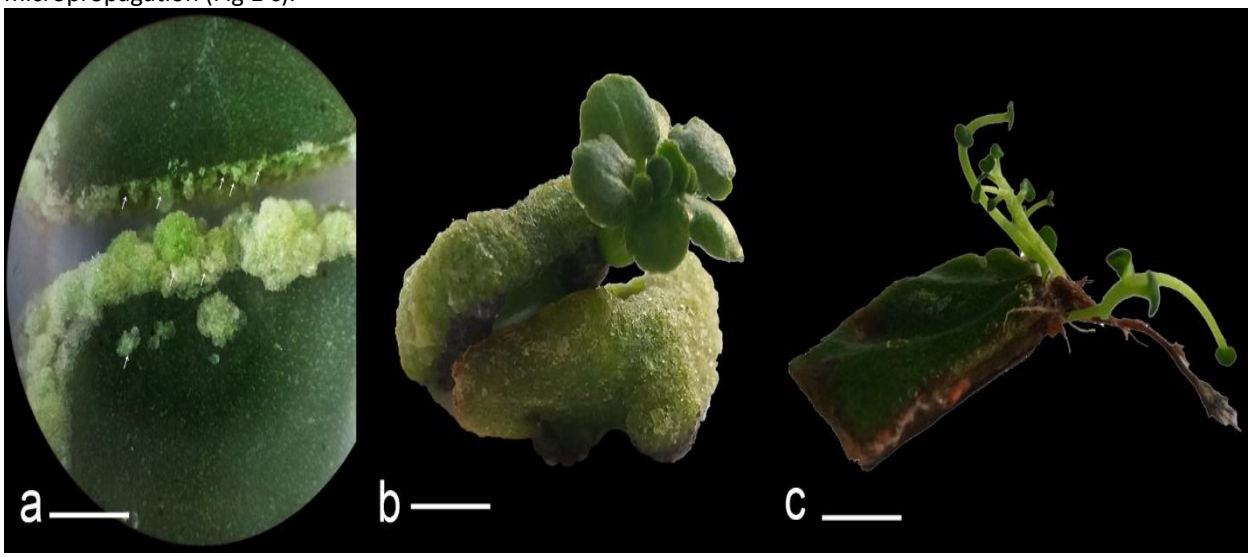

Figure 1. Micropropagation of K. blossfeldiana (a) The disc is green and slightly curled at the edges. The leaf explant starts to form a white callus at the periphery (arrows) on a portion of the leaf with many micrsomatic embryo induction on the calli induced on 1.5 mg L^{-1} BAP- 0.5 mg L^{-1} NAA and 0.2 mg L^{-1} NAA after 13-15 days of culture at the cut edges of two leaf discs with different stages of somatic embryo induction (b) At 7 weeks in culture the callus has increased considerably in size with a microshoot under microscope (c) direct shoot regeneration with rooting on the leaf cut edges after 25-27 days of culture with adventitous roots arising from the epidermis on cut edges with necrosis (bar slide 1 a= 250 µm, slide 1 b= 0.4 mm, slide 1c=1 cm).

All explants induced more than 80% somatic embryo induction on explants with statistically significant differences among them depending on the concentration of the TDZ. Increased concentrations of TDZ inhibited the induction of roots and their percentage. The first regenerating explant (with roots and shoots) in a petri dish had a high depressive effect on the regeneration of other explants compared to the rest of the explants (Fig 2). It was not difficult to separate and isolate induced plantlets through somatic embryogenesis on the explants.

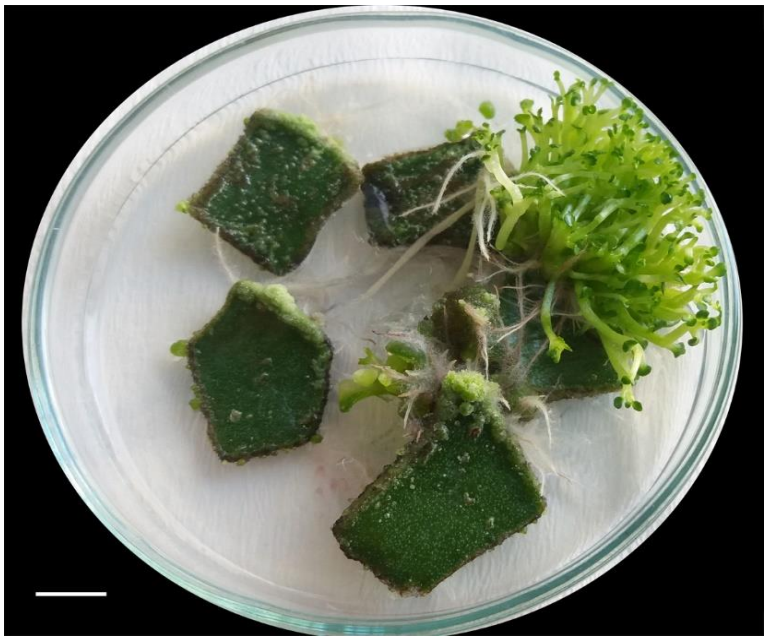

Figure 2. Somatic embryogenesis on TDZ containing medium showing dominance of first regenerant over the other explants rectangular leaf disc explants bar=0.75 cm.

It was further noted that rooting occurred in range of 10-56% explants depending on the amount of TDZ growth regulator combinations in the nutrient medium. Culture treatments 3, 10, and 11 yielded the highest percentage of roots. It was concluded that the interaction of genetic structure of mother explant with different intrinsic auxin levels within the explant and exterior treatments with plant growth regulators affected the physiologic growth and development of the induced shoots. It was assumed that regardless of the presence of GA_3, the source effect of BAP and NAA affected the rooting percentage (Fig 3 a, b, c, d, e).

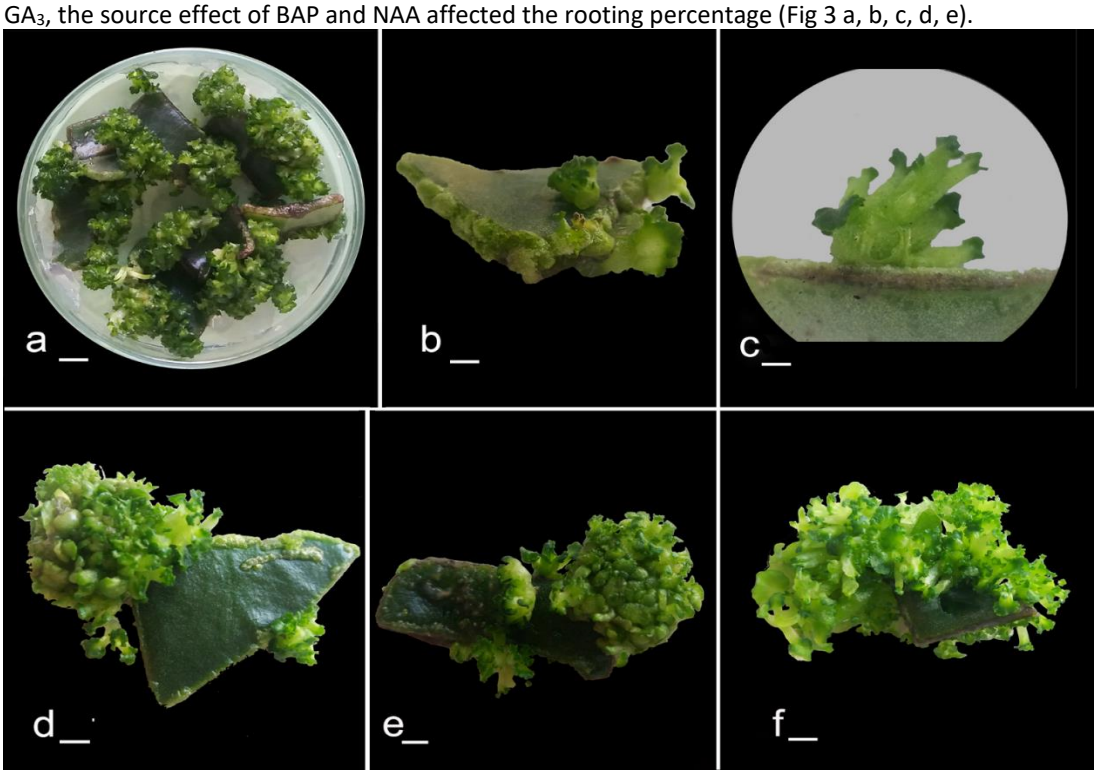

Figure 3. Different stages of shoot regeneration on triangular leaf disc of *K. blossfeldiana* after week 8 of culture (a) Medium No. 13 (0.1 mg L^{-1} TDZ), (b) Medium No. 14 (0.2 mg L^{-1} TDZ), (c) Medium No. 15 (0.5 mg L^{-1} TDZ), (d)) Medium No. 16 (0.1 mg L^{-1} TDZ + 0.1 mg L^{-1} NAA), (e) Medium No. 17 (0.2 mg L^{-1} TDZ + 0.1 mg L^{-1} NAA), (f) No. 18 (0.5 mg L^{-1} TDZ + 0.1 mg L^{-1} NAA) showing organogenesis (Bar fig 1a 0.3 cm, 1b=0.4 cm, 1c, 1d, e, 1f=0.6 cm).

Culture treatment 10 and 11, (where 0.5 and 1.0 mg L^{-1} BAP + 0.5 and 0.1 mg L^{-1} NAA and 0.1, and 0.2 mg L^{-1} GA_3) were used 54% and 56% rooting was noted in the same order. The fact that no callus, root, or shoot differentiation occurred in cultures without hormone additives in the initial experiments for 12 weeks, suggests that the additions of PGRs promoted indirect organogenesis. It was assumed that differences in rooting percentages could also be related to age of the leaves on the plants, their source and their position close to the leaf tips or stems. It was observed that the shoots regenerated on explants prepared from the lower leaves on the stem or parts of the leaf closer to the stem tended to induce a greater number of roots.

Bejaoui et al. (2023a), used GA_3 added to BAP and NAA and induced the lowest numbers of shoots on 0.5 mg L^{-1} BAP + 0.1 mg L^{-1} NAA after the 8th week of culture (culture treatment with 0.34±0.04 - 0.65±0.05 shoots/explant. The addition of 0.2 mg L^{-1} GA_3 to the medium slightly increased their counts (culture treatment No 10-12) statistically similar to culture treatments 1-3 where NAA at 0.5 mg L^{-1} were used (Table 2). The culture treatments 9 and 8 (which contained all the growth regulators mentioned in this study) provided the highest shoot induction at the end of the 8th week (3.40±0.40 and 2.54±0.16 shoots per explant). Culture treatment 3 (BAP: 1.5 and NAA: 0.5 mg L^{-1}) was statistically similar and remained within the same Duncan group and stood out with 2.41±0.27 shoots per explant. The addition of GA_3 had a positive effect. Deng et al. (2005), indicated role of NAA and BAP combinations more effective as a starter treatment; however, the number of shoots in culture treatments where GA_3 was added was 9.3 folds higher when they added 0.1 mg L^{-1} GA_3 to a 10 BAP: 1 NAA to cultures. Xinzheng et al. (2006) reported that the addition of 2 or 1.5 mg L^{-1} GA_3 to culture treatments containing 10 BAP: 0.5 IBA was successful for *in vitro* shoot induction in kalanchoe genotypes with white flowers. GA_3 is reported to have positive influence on promoting cell elongation in compact clusters for shoot inductions, as mentioned by Liu (2010). It has been recorded that GA_3 had a very positive effect on the transformation of clustered adventitious shoot initials into shoots regenerated on young leaves and stems into shoots and the highest adventitious shoot induction on MS medium+ 0.5 mg L^{-1} BA + 0.1 mg L^{-1} NAA + 0.1 mg L^{-1} GA_3 culture treatment. Luo et al. (2009) also reported the highest shoot induction in the kalanchoe genotype they worked on using MS medium amended with 0.30 mg L^{-1} IAA + 2.1 mg L^{-1} BA + 0.6 mg L^{-1} GA_3, and highlighted the positive effects of GA_3 on propagation under *in vitro* conditions.

Table 2. Effect of different doses of BAP, NAA, and GA_3 on induction of shoots and roots after 8 and 12 weeks of culture (n=45 explants).

Treatment	BAP (mg L^{-1})	NAA (mg L^{-1})	GA_3 (mg L^{-1})	Percentage (%) of shoot induction	Number of shoots per explant after 8 weeks	Number of shoots per explant after 12 weeks	Rooting percentage (%)
1	0.5	0.5	-	100.00	1.28±0.37 b-e	2.65±0.15 de	15±3.55 e
2	1.0	0.5	-	100.00	1.12±0.25 de	3.84± 0.27bc	35±3.56 c
3	1.5	0.5	-	100.00	2.41±0.27 a-c	5.03±1.21 a	52±3.74 a
4	0.5	0.1	-	100.00	0.34±0.04 e	1.65±0.19 f	15±2.94 e
5	1.0	0.1	-	100.00	0.65±0.05 e	2.18±0.09 de	10±2.16 f
6	1.5	0.1	-	100.00	0.52±0.04 e	1.82±0.19 ef	37±2.16 c
7	0.5	0.5	0.2	100.00	2.04±0.22 b-d	3.73±0.14 cd	24±3.74 d
8	1.0	0.5	0.2	100.00	2.54±0.16 ab	4.32±0.14 ab	28±2.94 d
9	1.5	0.5	0.2	100.00	3.40±0.40 a	4.28±0.18 ab	43±2.94 b
10	0.5	0.1	0.2	100.00	1.43±0.49 b-e	3.52±0.23 cd	54±3.56 a
11	1.0	0.1	0.2	100.00	1.56±0.06 b-e	3.03±0.17 cd	56±5.71 a
12	1.5	0.1	0.2	100.00	1.22±0.08 c-e	3.18±0.19 cd	37±3.56 c
	CV				13.14	100.00	9.99
		Treatments					
	TDZ (mg L^{-1})	**NAA (mg L^{-1})**					
13	0.1	-	-	100.00	>20	>50	0.00
14	0.2	-	-	100.00	>20	>50	0.00
15	0.5	-	-	100.00	>20	>50	0.00
16	0.1	0.1	-	100.00	>20	>50	0.00
17	0.2	0.1	-	100.00	>20	>50	0.00
18	0.5	0.1	-	100.00	>20	>50	0.00

The development of meristematic tissues was noted directly after 12th week of culture on leaf tissues or on the micro callus formed on the cut surfaces of the leaf explants into shoots. The results from the 8th week showed the same trend that continued until 12th week. Treatments 3, 8, and 9 yielded the highest shoot counts per explant values at this stage of the experiment (with induction of 5.03±1.21, 4.32±0.14, and 4.28±0.18 shoots per explant). Instead of intensive and compact mass shoot multiplication at high BAP doses, it was found preferable to use GA_3-supplemented and balance BAP: NAA ratios in terms of kalanchoe shoot induction. Kaviani et al. (2014) also reported that the MS medium amended with 1.0 mg L^{-1} BA + 1.0 mg L^{-1} NAA provided the highest shoot induction and healthy shoot development for *in vitro* propagation in *K. blossfeldiana* plants, achieving 7.012 cm in height, 4.516 internode counts, and 8.860 root counts. The highest shoot induction with 5.886 shoots was also recorded in this treatment. Figures 4a, b, and c present some views of regeneration from *K. blossfeldiana* explants in this study with BAP, NAA, and GA_3-amended MS medium.

Figure 4. Microscopic images of the development of left over leaf explants after the 8th week of culture on treatment 1 (a), 2 (b) and 3 (c) in which NAA using 0.5 mg L^{-1}. Bar = 4 a, c= 0.5 cm, Fig 4b=300 µm.

Culturing leaf explants in TDZ-containing culture treatments led to cluster-like shoot differentiation from the 8th week of culture. No rooting occurred in 0.1, 0.2, and 0.5 mg L^{-1} TDZ amended medium with low-dose NAA.TDZ, exhibited a strong effect, inducing 20 fold shoots even at the lowest dose in the initial count and more compared to 50-fold shoots after the 12th-week counts. A significant portion of these shoots were noted at the meristem stage, many of them were smaller compared to 0.5 mm and were induced in clusters. Therefore, it was not possible to provide a precise count. Indeed, Frello et al. (2002), used 12 species, including different Kalanchoe species such as *K. beharensis, K. manginii, K. pumila, K. serrata, K. tomentosa*, and other plant species from the *Crassulaceae* family, inducing over 50 (≥50) shoots in their experiments and they could not precisely determine the number of induced shoots.

When considering tissue culture establishment from Kalanchoe leaf explants, the use of TDZ in terms of tissue response and shoot differentiation was undeniably more effective compared to the use of BAP and NAA. However, the decision should be made according to preference for selection at the subculturing stage is desired. Sanikhani et al. (2006) used different TDZ dosages, in addition to BAP and NAA combinations, for the purpose of regeneration from Kalanchoe explants. Among the TDZ quantities tested at doses ranging 0 to 15 mg L^{-1}, it has been observed that a dose of 0.1 mg L^{-1} TDZ provided sufficient shoot differentiation on leaf and nodal explants,

TDZ facilitated higher meristematic tissue formation compared to BAP and NAA in combination. These findings are in line with our study. Nieves et al. (2016) also examined the use of BAP and TDZ, either singly or in combination with paclobutrazol, in nodal explants of the white-flowering Kalanchoe variety. Bhuiyan et al. (2006) achieved a 100% shoot induction percentage and a count of 9.5 shoots per explant on MS medium amended with 1.0 mg L^{-1} BAP + 0.4 mg L^{-1} IAA. The researchers reported that, they induced high number of short and clustered shoots on TDZ-containing cultures. Shoots below 5 mm were too small to count, and thus were expressed with approximate evaluations, such as "over 50". These researchers have also indicated that TDZ is a more effective regeneration stimulant compared to BAP (Mok et al., 1987; Huetteman and Preece, 1993). Dense, bushy shoot regeneration from explants of various plant species with the addition of TDZ has been reported in other studies (Bates et al., 1992; Murthy et al., 1998; Frello et al., 2002, Majumder et al. 2023).

An average of 84.52 ± 24.21 adventitious shoots was obtained from each shoot transferred to the MS medium amended with + 0.3 mg L^{-1} GA_3 + 0.3 mg L^{-1} BAP. These shoots were transferred to a maturation medium to ensure their transformation into a healthier and stronger plants before the rooting stage. Figure 5 shows the transfer of explants to an MS nutrient medium containing 0.2 mg L^{-1} GA_3 at the proliferation stage. Elongation and maturation of the shoots was noted during incubation on 1/2 ×MS medium amended with 0.2 mg L^{-1} GA_3. This facilitated induction of healthy, robust root on plants with formation of 4.62 ± 0.39 shoots per explant. These shoots were further transferred to glass tubes to boost their growth (Figure 6) and plastic pots to prepare them for trade.

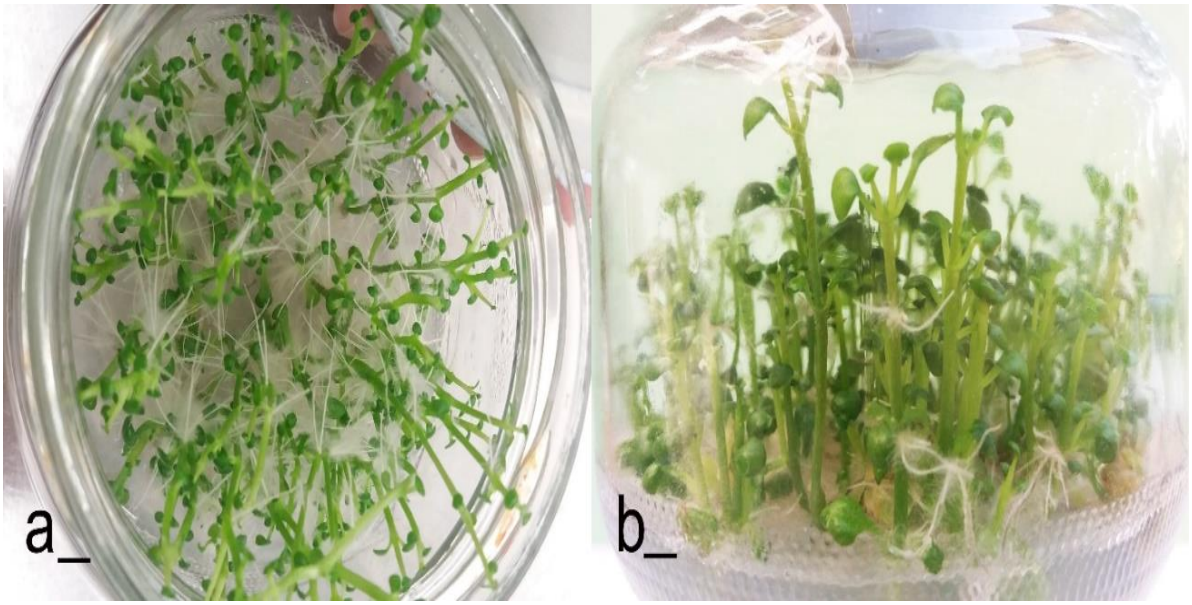

Figure 5. *K. blossfeldiana* shoots transferred to the (a) curing and (b) vegetative maturity stage on ½ Ms medium amended with 0.2 mg L^{-1} GA_3 induced shoots.

Figure 6. *K. blossfeldiana* plantlets transferred to the glass tubes for the curing. Bar = 1 cm.

CONCLUSION

The experiments were carried out to find the effects of different growth regulator combinations in half and full-strength MS medium to micro-propagate and root *K. blossfeldiana*. Leaf explants are a suitable explant source for *in vitro* propagation. Adding GA_3 to the medium created a significant difference in the number of shoots. The use of TDZ singly (0.1, 0.2, 0.5 mg L^{-1}) or in combination with NAA (0.1 mg L^{-1}) resulted in immeasurable levels of meristematic shoot tip differentiation from leaf explants shown in clusters depending on the objective of the study (regeneration for genetic transformation, mutation regeneration, etc.).

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Author's contributions

All authors contributed significantly to this work. **RB:** Methodology, writing—original draft preparation; **ŞŞE** and **RY:** Conceptualization, review and editing, supervision.

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