In vitro propagation of *Kalanchoerhombopilosa* (Crassulaceae)

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ABSTRACT

In vitro propagation of *Kalanchoe rhombopilosa* Mannoni & Boiteau was studied. To examine the effect of explants type and orientation on shoot and root induction, stem explants placed vertically and horizontally, shoot tips, leaf blade attached with ventral and dorsal side were cultured on semi-solid MS medium for 9 weeks. The highest shoot and root number could be obtained when stem placed horizontally were used as explants. In addition, the optimum concentration of TDZ and NAA on shoot induction was investigated for 8 weeks. The result found that the highest percentage of shoot induction as well as shoot and root number and their length was obtained when the horizontally placed stem segments were cultured on MS medium that supplemented with 0.1 mg/l NAA; whereas the callus formation was observed in all concentration of TDZ added medium. Furthermore, optimal types and concentration of organic supplements on shoot regeneration efficiency was also investigated. The result revealed that the highest percentage of shoot induction and shoot number could be obtained when the horizontally placed stem segments were cultured on MS medium augmented with 5% v/v coconut water for 8 weeks. Influence of the types of planting materials and their ratio on the survival percentage of the plantlets after planting for 4 weeks was studied. The result indicated that the use of sand: coconut husk: volcanic rock (1:1:1) as the planting materials provided the highest survival percentage.

Keywords: Kalanchoe rhombopilosa, Growth regulators, Organic supplements

INTRODUCTION

Kalanchoe rhombopilosa Mannoni & Boiteau was classified in the family Crassulaceae which many species are succulent plants comprising 1,410 species of 34 genera (Saleh et al., 2014). There are 130 species of *Kalanchoe* distributing through sub-desert area in Africa, Madagascar, Australia, Asia, and tropical America (Khan et al., 2006). *Kalanchoe* species is important as economical potting plant (Currey and Erwin, 2011). Moreover, some species have pharmacological property e.g. anti-oxidant and anti-fungus (Saleh et al., 2014). *K. rhombopilosa* is endemic to Madagascar and natural population trend to be decreasing (Hassler, 2017).

Ordinary, *Kalanchoe* species were propagated by seed which this method takes long time and varies in genetically characteristic (Sanikhani et al., 2006). Although *Kalanchoe* species can propagate via leaf and stem cutting, the methods cannot produce enough plant for marketing demand (Ioannou and Ioannou, 1992). Recently, plant tissue culture technique is widely used in plant propagation, including in *Kalanchoe* species (e.g. Sanikhani et al., 2006; Ioannou and Ioannou, 1992). However, there was no study on *in vitro* propagation of *K. rhombopilosa*. To achieve on *in vitro* plant tissue culture, explants selecting should be considered (George, 2008). Moreover, the examining for optimum concentration between auxin and cytokinin provided the best result in organogenesis (Van Staden et al., 2008). TDZ has more

efficiency on shoot induction than other cytokinins in *Kalanchoe* species (Sanikhani et al., 2006). NAA is typically used for inducing plant growth in plant tissue culture (Machakova et al., 2008). In addition, organic additives which supplemented in culture medium could promote growth and development in some plant species (Yonget al., 2009; Molnar et al.,2011).

The aims of this study are to investigate the effect of explants types and orientation on explants response, to optimize the combination of TDZ and NAA on explants growth, to examine the effect of organic source types and concentration on explants growth, and to screen the suitable planting materials for seedling transplantation. The results from this study will provide a beneficial way for *K. rhombopilosa* propagation.

MATERIALS AND METHOD

Plant material, media preparation, and culture condition

Aseptic plantlets of *Kalanchoe rhombopilosa* were used as plant materials in all *in vitro* experiments. The second to fourth 5×5 mm^2 leaf blades from shoot tip were used as leaf explants. The 5 mm stems which leaves were cut off were used as stem explants, while the 5 mm shoot tips were used as shoot tip explants. Semi-solid MS medium (Murashige and Skoog, 1962) was used as basal medium. Medium was adjusted pH to 5.8, and then boiled by mixing with 7 g/l agar. Coconut water was prepared by pouring liquid endosperm through sheet cloth. Potato extract was prepared by boiling the small pieces of peeling potato with 200 ml distil water. The liquid supernatant was deserved as potato extract. Banana homogenize was prepared by weighing banana pieces and then homogenizing them. The prepared media were autoclaved at 121 ^oC and 1.05 kg/cm pressure for 15 min. All explants treatments were kept in the room at 25 ± 2 ^oC with 3,000 lux light intensity and 16/8 h (L/D) photoperiod.

Effect of explants type and orientation

To examine the effect of explants type and orientation, explants of leaves, stem and shoot tip were chosen in this experiment. The five treatments are manipulated in the laminar air flow. The explants of leaves, stem, and shoot tip were aseptically cut by scalpel. The 5×5 mm^2 in size of leaf explants were cultured on the medium of which ventral or dorsal side was attached to medium surface. The 5 mm in length of stem explants were placed vertically or horizontally on medium. The 5 mm in length shoot tip explants were placed vertically on the medium. All treatments were cultured on semi-solid MS medium for 9 weeks. Each treatment was used 6 replications and repeated thrice.

Effect of TDZ and NAA on explants growth

To investigate the effect of TDZ and NAA on explants growth, the concentration of 0, 0.1, 0.5, 1.0, 2.0, or 4.0 mg/l TDZ combined with 0, 0.05, or 0.1 mg/l NAA were selected. The best response of explants from the former experiment was chosen as plant materials in this study. All 18 treatments were replicated 6 times and then repeated thrice.

Effect of organic supplements types and concentration on explants growth

The effect of organic supplements types and concentration on explants growth was examined by using 5, 10, 15, and 20% v/v coconut water, 5, 10, 15, and20% w/v potato extract, and 5, 10, 15, 20% w/v banana homogenate and MS medium without organic additives as experimental treatments. The best response of explants from the first experiment was chosen as plant materials in here. All treatments were replicated 6 times and then repeated thrice.

Planting materials on plantlet survival

To determine the effect of planting materials on plantlet survival, five different planting materials were chosen. The chosen planting materials were sand: soil: perlite (1:1:1), sand: soil: volcanic rock (1:1:1), sand: coconut husk: volcanic rock (1:1:1), sand: coconut husk: perlite (1:1:1), and sand: soil: coconut husk: volcanic rock: perlite (1:1:1:1:1). All treatments were replicated 5 times and then repeated thrice. The survival rates were recorded when plantlets were grown for 4 weeks.

Experimental design and statistical analysis

All experiments were completely randomized designed. All data were analyzed by ANOVA, following by Duncan's new multiple range test for multiple comparison at significant level $p < 0.05$.

RESULTS AND DISCUSSION

Effect of explants type and orientation

The explants of *Kalanchoe rhobopilosa* in each treatment respond differently after culturing for 9 weeks on MS medium (Data shown in Table 1 and Fig. 1). The explants of horizontally placed stem showed the best result with the highest percentage of shoot regeneration as well as number of shoot and root. In the present study, the stem explants showed the high percentage of shoot regeneration in both horizontal (94.4%) and vertical (72.2%) placing. The concordant results that stem explants gave the best result on shoot regeneration percentage were also found in the two species of Crassulaceae, *Kalanchoe blossfeldiana* (Sanikhani et al., 2006) and *Sedum spectabile* (Yang et al., 2012). The stem explants gave the high rate of shoot regeneration might be due to that new shoots regenerated from bud meristem. In many plants, this region contains numerous lateral buds that can develop into new shoot (George, 2008). Moreover, shoot regeneration of explants on plant-growth-regulator free medium could be induced by endogenous-cytokinins content in explants (Sanikhani et al., 2006). No significant difference of leaf length was found in this experiment. The highest percentage (77.8%) of root regeneration was observed on shoot tip explants in our study. A similar result was observed in the study in *Kalanchoe tomentosa* (Khan et al., 2006). Shoot tip explants showed high rate of root regeneration because shoot tip is generally known as the region producing auxins which control cell division and root stimulation (Ljung et al., 2001). On root production, there was no significant different between the explants of shoot tip (5.9 roots) and horizontally placed stem (5.1 roots) in our experiment. Root length did not depend on explants type and orientation.

In this study, horizontally placed stem treatment showed the highest new shoot induction number (4 shoots) significantly on shoot production. The difference of shoot number between the horizontal and vertical placing indicate that orientation affect to stem-explants response. This result concurs in the *in vitro* stem culture of *Hevea*

brasiliensis that the researchers suggested that the horizontal placing is more advantage on surface area than vertical placing resulting in nutrient absorption for growth (Kalawong and Te-chato, 2004). The effect of explants orientation is also supported by the difference of root number between horizontally and vertically placed stem treatments in this study. Nevertheless, the response did not rely on orientation in all explants types. Our study found that different orientation when using leaf blade as explants did not showed significant difference on shoot and root production. This result was also found in *Kalanchoe daigremontiana* (Bhuiyan et al., 2006).

Effect of TDZ and NAA on explants growth

The present study observed that the media without TDZ could induce *K. rhombopilosa* explants to develop new organ after cultured for 8 weeks. The organogenesis of explants cultured on TDZ free media, with different NAA concentration, was shown in Table 2. The best result was found on MS medium with 0.1 mg/l NAA which served 100% of shoot and root regeneration as well as 3.3 new shoots and 12.1 new roots. Our study showed that NAA did not affect shoot production, agreeing with the study in *K. blossfeldiana* (Sanikhani et al., 2006). The advantage of NAA content was that higher concentration trend to increase more root regeneration percentage and root number in the present study. However, an increasing of NAA concentration could reduce shoot and root length as well as leaf number. Therefore, the suitable concentration based on utilized purpose.

All media supplemented with TDZ in present study induced explants develop into callus after cultured for 8 weeks. The development of explants was shown in Table 3. The explants respond variously to the combination of TDZ and NAA. The highest fresh weight (5.78 g) of callus was found on MS medium supplemented with 0.1 mg/l TDZ and 0.1 mg/l NAA. The explants cultured on TDZ supplemented media had no significant different on callus diameter. In our observation, callus found on all TDZ supplemented media could be classified into 3 stages as follows; stage 1 is a compact callus forming in globular shape with greenish smooth surface; stage 2 is a friable callus with light greenish rough surface; stage 3 is a friable callus forming young shoot-like shape with green-yellowish color (Fig. 2). The present study discovered that higher concentration of TDZ and NAA seem to decrease the percentage of callus development into stage 3 (Table 3 and Fig. 3). The combination of 0.1 mg/l TDZ with no NAA could induce the highest percentage of explants that developed into stage 3. Our result that explants were induced to be callus by TDZ was common. It can be found in plant cell tissue culture of many species because TDZ usually stimulates cell division better than other cytokinins (Murthy et al., 1998). TDZ promote plant growth and cell metabolism, and control the synthesis and accumulation of endogenous hormones in plant cell (Mok et al., 1987). In our study, TDZ induced explants to be callus in all media. This result agrees with the study in *Sedum sarmentosum* (Yang et al., 2012). However, many studies in other *Kalanchoe* species found that TDZ induced numerous shoots (Sanikhani et al., 2006; U-kong et al., 2011). Although TDZ did not promote new shoot in this study, further study on indirect organogenesis could produce numerous plantlet. In addition, secondary metabolite from callus is interested because many *Kalanchoe* species had important pharmacological phytochemistry.

Effect of organic supplements types and concentration on explants growth

The explants response after culture for 8 weeks on MS medium supplemented with different types and concentration of organic supplements was shown in Table 4. Different types and concentration of organic source had no significant influence on shoot and root regeneration. Our study revealed that coconut water seem to be a suitable organic supplements which promoted shoot number, shoot length, leaf number and callus regeneration. The best result of those parameters depended on concentration level. At 5% coconut water, *K. rhombopilosa* explants showed slightly high of shoot production than other concentrations. At 10% coconut water, the explants showed the high percentage of callus regeneration. At 15% coconut water, the explants showed the best result in shoot length and leaf number. However, in present study, the best result in root production and root length were derived from MS medium with no organic supplements. Recently, the study on floral bud culture of *K. pinnata* on White's basal medium reported that the medium supplemented with coconut water and 2,4-D enhanced shoot regeneration (Ram and Wadhi, 1968). Effect of selected organic supplement sources in our study is the first reported on *Kalanchoe* species. In some plants, optimum concentration of coconut water promoted shoot regeneration and shoot production (Pakum et al., 2016). Coconut water contains beneficial nutrient and mineral for plant growth e.g. sucrose, vitamin, amino acid, and phytohormones (Yong et al., 2009). An important composition on coconut water is cytokinin that plays an essential role for cell division and plant growth (Arditti and Ernst, 1993). Potato was recognized as protein, carbohydrate, vitamin, and many types of polyamine (Mikitzel and Knowles, 1989); while banana support plant growth and adjust pH in culture medium (Thorpe et al., 2008). Contrarily, in this study, potato extract and banana homogenate could not promote the best result in any parameter.

Planting materials on plantlet survival

After 4 weeks, survival percentage of *K. rhombopilosa* plantlets grown in different planting materials were in range of 86.7-100%, but there was no significant different among treatments. The highest survival percentage (100%) were observed on sand: soil: volcanic rock (1:1:1), sand: coconut husk: volcanic rock (1:1:1), and sand: coconut husk: perlite (1:1:1). The plantlets grown in planting materials that had composition of soil showed the syndrome of fungal infection. The mortality percentage that found on sand: soil: volcanic rock (1:1:1), sand: soil: perlite (1:1:1) and sand: soil: coconut husk: volcanic rock: perlite (1:1:1:1:1) were 6.7, 20.0, and 26.7%, respectively. There was no significant effect of planting materials on plantlet growth. According to those results the proper planting materials were two candidates of sand: coconut husk: volcanic rock (1:1:1), and sand: coconut husk: perlite (1:1:1) which give 100% survival and no fungal infection. In comparison, the suggested planting materials for *K. rhombopilosa* plantlets was sand: coconut husk: volcanic rock (1:1:1) because of cheaper values. Some studies also achieved in plantlet exposure of *Kalanchoe* species to the greenhouse e.g. *K. tomentosa*, *K. daigremontiana*, and *K. pinnata* (Khan et al., 2006; Naz et al., 2009).The achievements are the same using sand as a composition of planting materials. It could be recommended that sand is necessary composition of planting materials for *Kalanchoe* species due to similarity condition in their natural habitat.

Table 1 Types and orientation of *Kalanchoe rhombopilosa* explants after cultured onMS medium for 9 weeks

Explants	% shoot regeneration	No. of shoots	Shoot length (cm)	% rooting	No. of roots	Root length (cm)
Leaf ventral attached	16.7 ± 0.0 ^{c}	1.0 ± 0.0^{b}	0.40 ± 0.06	16.7 ± 9.6 ^{c}	1.3 ± 0.9 ^{c}	0.23 ± 0.12
Leaf dorsal attached	27.8 ± 11.1 ^{c}	1.3 ± 0.3 ^{b}	0.31 ± 0.05	33.3 ± 0.0 ^{bc}	3.3 ± 0.4 ^{bc}	0.34 ± 0.07
Stem vertical placed	72.2 ± 9.6^{ab}	1.8 ± 0.3 ^{b}	0.32 ± 0.01	44.4 ± 5.6 ^{b}	2.2 ± 0.5 ^{c}	0.33 ± 0.03
Stem horizontal placed	94.4 ± 5.6 ^{a}	4.3 ± 0.8 ^{a}	0.44 ± 0.07	50.0 ± 0.0 ^{b}	5.9 ± 1.1 ^{a}	0.47 ± 0.03
Shoot tip	66.7 ± 9.6^{b}	2.4 ± 0.7 ^{b}	0.42 ± 0.05	77.8 ± 14.7 ^{a}	5.1 ± 0.8 ^{ab}	0.38 ± 0.04

All data are Mean ± SE calculated from 5 replicates of each 3 repeats. Different alphabets in the same column showed significantly different level at $p< 0.05$.

Table 2 Effect of NAA on growth and development of *Kalanchoe rhombopilosa* explants after cultured onMS medium for 8 weeks

Concentration (mg/l)		% shoot regeneration	No. of shoot	Shoot length (cm)	No. of leaves per shoot	% root regeneration	No. of roots	Root length (cm)
TDZ	NAA							
0	0	100.0 ± 0.0	2.9 ± 0.5	0.38 ± 0.04 ^{a}	3.1 ± 0.2 ^{a}	86.7 ± 6.7 ^{c}	6.5 ± 0.7 ^{b}	0.78 ± 0.07 ^{c}
	0.05	100.0 ± 0.0	2.8 ± 0.7	0.37 ± 0.04 ^{a}	3.0 ± 0.1 ^{a}	93.3 ± 6.7 ^{b}	11.6 ± 0.4^{a}	1.21 ± 0.04 ^{a}
	0.1	100.0 ± 0.0	3.3 ± 0.6	0.31 ± 0.02 ^{b}	2.8 ± 0.1 ^{b}	100.0 ± 0.0 ^{a}	12.1 ± 0.9 ^{a}	1.14 ± 0.06 ^{b}

All data are Mean ± SE calculated from 6 replicates of each 3 repeats. Different alphabets in the same column showed significantly different level at $p< 0.05$.

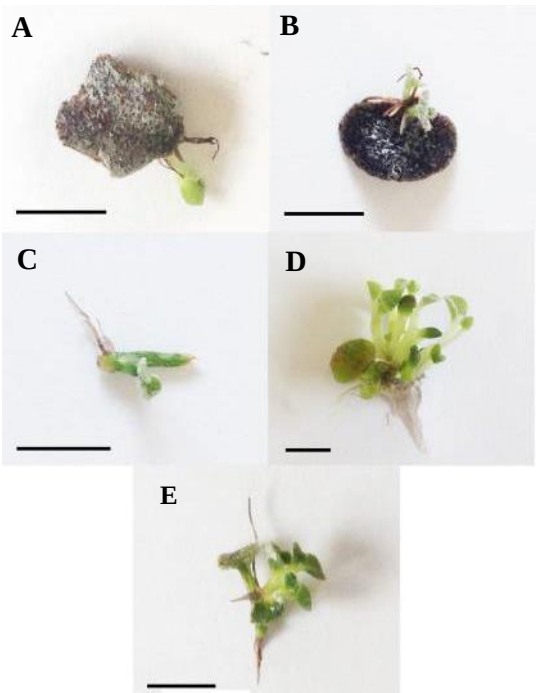

Fig. 1 *Kalanchoe rhombopilosa* explants, ventrally (A) and dorsally (B) attached leaf blade, vertically (C) and horizontally (D) placed stems, and shoot tips (E), after cultured on MS medium for 9 weeks (bar = 5 mm)

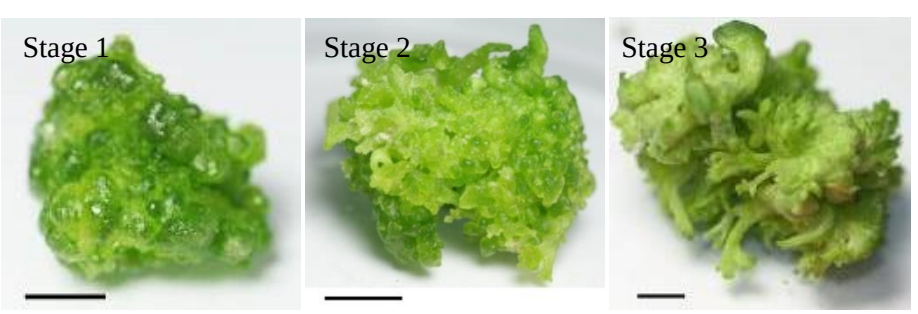

Fig. 2 Developmental stage of callus observed on *Kalanchoe rhombopilosa* explants after cultured for 8 weeks (bar = 5 mm)

Table 3 Effect of TDZ and NAA on callus development of *Kalanchoe rhombopilosa* explants after cultured on MS medium for 8 weeks

Concentration(mg/l)		Fresh weight (g)	% callus	Callus diameter (cm)	Callus developmental stage		
TDZ	NAA				Stage1	Stage2	Stage3
0.1	0	5.38 ± 0.33 ^{a}	100.0 ± 0.0	3.39 ± 0.08	37.7 ± 5.0 ^{bc}	10.3 ± 1.5 ^{cd}	51.3 ± 4.6^{a}
	0.05	4.66 ± 0.38 ^{ab}	100.0 ± 0.0	3.54 ± 0.04	46.3 ± 14.5 ^{abc}	26.2 ± 7.2 ^{ab}	27.5 ± 7.3 ^{b}
	0.1	5.78 ± 0.32 ^{a}	100.0 ± 0.0	3.49 ± 0.06	66.7 ± 4.1 ^{ab}	23.0 ± 3.6 ^{abc}	10.3 ± 4.5 ^{bc}
0.5	0	4.31 ± 0.11 ^{ab}	100.0 ± 0.0	3.10 ± 0.09	65.0 ± 5.6^{ab}	18.3 ± 2.0 ^{abc}	16.3 ± 3.8 ^{bc}
	0.05	4.97 ± 0.86 ^{ab}	100.0 ± 0.0	3.26 ± 0.24	63.0 ± 2.5 ^{ab}	25.3 ± 4.3 ^{ab}	11.7 ± 2.0 ^{bc}
	0.1	5.45 ± 0.09 ^{a}	100.0 ± 0.0	3.41 ± 0.04	71.3 ± 8.5 ^{ab}	20.3 ± 8.1 ^{abc}	8.3 ± 4.8 ^{bc}
1.0	0	5.19 ± 0.51 ^{ab}	100.0 ± 0.0	3.41 ± 0.18	61.7 ± 8.4 ^{ab}	15.8 ± 4.5 ^{bc}	17.5 ± 6.6 ^{bc}
	0.05	5.24 ± 0.14 ^{ab}	100.0 ± 0.0	3.23 ± 0.11	57.2 ± 12.6 ^{ab}	31.0 ± 8.9 ^{a}	11.8 ± 3.8 ^{bc}
	0.1	4.69 ± 0.30 ^{ab}	100.0 ± 0.0	3.16 ± 0.03	74.0 ± 4.0 ^{ab}	19.7 ± 3.4 ^{abc}	7.0 ± 0.6^{bc}
2.0	0	4.53 ± 0.39 ^{ab}	100.0 ± 0.0	3.13 ± 0.15	70.7 ± 1.3 ^{ab}	19.3 ± 1.8 ^{abc}	8.0 ± 3.1 ^{bc}
	0.05	5.01 ± 0.38 ^{ab}	100.0 ± 0.0	3.35 ± 0.07	78.0 ± 5.3^{a}	18.3 ± 5.5 ^{abc}	3.0 ± 0.6 ^{c}
	0.1	5.04 ± 0.58 ^{ab}	100.0 ± 0.0	3.21 ± 0.16	64.7 ± 2.4 ^{ab}	25.0 ± 2.9 ^{ab}	9.7 ± 1.5^{bc}
4.0	0	4.34 ± 0.45^{ab}	100.0 ± 0.0	3.05 ± 0.18	64.7 ± 2.7^{ab}	28.0 ± 2.3 ^{ab}	7.3 ± 1.3 ^{bc}
	0.05	3.74 ± 1.02 ^{b}	100.0 ± 0.0	2.87 ± 0.24	68.0 ± 3.1 ^{ab}	28.7 ± 2.4 ^{ab}	3.3 ± 0.7 ^{c}
	0.1	4.41 ± 0.80 ^{ab}	100.0 ± 0.0	3.03 ± 0.24	64.3 ± 5.5 ^{ab}	30.3 ± 4.2 ^{ab}	5.3 ± 2.4 ^{bc}

All data are Mean ± SE calculated from 6 replicates of each 3 repeats. Different alphabets in the same column showed significantly different level at $p< 0.05$.

Fig. 3 Effect of TDZ and NAA on growth and development of *Kalanchoe rhombopilosa* explants after cultured for 8 weeks (bar = 5 mm)

Table 4 Growth and development of *Kalanchoe rhombopilosa* explants after cultured for 8 weeks on MS medium supplement with coconut water (CW), potato extract (PE), or banana homogenate (BH) in different concentration

Treatment	% shoot regeneration	No. of shoots	Shoot length (cm)	No. of leaves per shoot	% root regeneration	No. of root	Root length (cm)	% callus
Control	100.0 ± 0.0	2.9 ± 0.5 $^{a-c}$	0.38 ± 0.04 ^{ab}	3.1 ± 0.2 ^{ab}	86.7 ± 6.7	6.5 ± 0.7 ^{a}	0.78 ± 0.07 ^{a}	26.7 ± 6.7 $^{c-e}$
CW 5%	100.0 ± 0.0	3.7 ± 1.1 ^{a}	0.31 ± 0.05 $^{a-d}$	2.7 ± 0.3 $^{a-c}$	100.0 ± 0.0	3.4 ± 0.8 $^{c-f}$	0.48 ± 0.05 ^{bc}	20.0 ± 11.5 $^{c-e}$
CW 10%	93.3 ± 6.7	3.2 ± 0.3 ^{ab}	0.33 ± 0.06 $^{a-c}$	2.8 ± 0.2 $^{a-c}$	66.7 ± 17.6	4.4 ± 0.8 $^{b-e}$	0.46 ± 0.11 ^{bc}	73.3 ± 6.7 ^{a}
CW15%	80.0 ± 11.5	2.6 ± 0.2 $^{a-d}$	0.41 ± 0.06^{a}	3.6 ± 0.6 ^{a}	73.3 ± 17.6	6.0 ± 0.1 ^{ab}	0.59 ± 0.02 ^{b}	40.0 ± 20.0 ^{bc}
CW20%	86.7 ± 13.3	2.2 ± 0.2 $^{b-e}$	0.33 ± 0.02 $^{a-c}$	3.2 ± 0.4 ^{ab}	93.3 ± 6.7	4.7 ± 0.2 $^{b-d}$	0.45 ± 0.04 ^{bc}	66.7 ± 6.7 ^{ab}
PE 5%	93.3 ± 6.7	2.6 ± 0.3 $^{a-d}$	0.31 ± 0.04 $^{a-d}$	2.7 ± 0.1 $^{a-c}$	86.7 ± 6.7	5.5 ± 0.5 ^{ab}	0.46 ± 0.02 ^{bc}	33.3 ± 13.3 ^{cd}
PE10%	80.0 ± 11.5	2.2 ± 0.3 $^{b-e}$	0.23 ± 0.03 $^{c-g}$	2.4 ± 0.2 $^{b-d}$	80.0 ± 11.5	4.5 ± 0.5 $^{b-e}$	0.44 ± 0.05 ^{bc}	26.7 ± 6.7 $^{c-e}$
PE 15%	100.0 ± 0.0	1.7 ± 0.1 $^{c-e}$	0.25 ± 0.02 $^{c-f}$	2.7 ± 0.2 $^{a-c}$	93.3 ± 6.7	3.7 ± 0.4 $^{c-f}$	0.35 ± 0.04 ^{cd}	13.3 ± 13.3 $^{c-e}$
PE 20%	93.3 ± 6.7	2.4 ± 0.5 $^{a-e}$	0.28 ± 0.01 $^{b-e}$	2.8 ± 0.1 $^{a-c}$	93.3 ± 6.7	5.0 ± 0.1 $^{a-c}$	0.56 ± 0.01 ^{b}	6.7 ± 6.7 ^{de}
BH5%	80.0 ± 11.5	1.1 ± 0.1 ^{e}	0.20 ± 0.04 $^{d-g}$	2.1 ± 0.5 ^{cd}	73.3 ± 13.3	3.8 ± 0.7 $^{c-f}$	0.27 ± 0.05 ^{de}	33.3 ± 6.7 ^{cd}
BH 10%	100.0 ± 0.0	1.3 ± 0.2 ^{de}	0.18 ± 0.03 $^{e-g}$	1.9 ± 0.1 ^{cd}	93.3 ± 6.7	3.2 ± 0.4 $^{d-f}$	0.18 ± 0.03 ^{e}	6.7 ± 6.7 ^{de}
BH 15%	73.3 ± 6.7	1.4 ± 0.1 ^{de}	0.14 ± 0.02 ^{fg}	1.5 ± 0.3 ^{d}	93.3 ± 6.7	2.7 ± 0.5 ^{f}	0.27 ± 0.02 ^{de}	0.0 ± 0.0 ^{e}
BH 20%	86.7 ± 6.7	1.3 ± 0.1 ^{de}	0.12 ± 0.01 ^{g}	1.8 ± 0.2 ^{cd}	93.3 ± 6.7	2.8 ± 0.2 ^{ef}	0.25 ± 0.03 ^{de}	6.7 ± 6.7 ^{de}

All data are Mean ± SE calculated from 6 replicates of each 3 repeats. Different alphabets in the same column showed significantly different level at $p < 0.05$.

CONCLUSION

The present study provided beneficial methods for *Kalanchoe rhombopilosa* propagation. For *in vitro* shoot and root multiplication, the recommended explants were stem segments which were subsequently cultured on MS medium supplemented with 0.1 mg/l NAA. The suitable planting materials for plantlet exposure were sand: coconut husk: volcanic rock (1:1:1).

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