Plant Gene and Trait 2020, Vol.11, No.6, 1-9
http://genbreedpublisher.com/index.php/pgt

Research Article Open Access

Morphological and Histological Analysis of Somatic Embryogenesis in *Haworthia cooperivar*

Zhu Jieqiong [1,2], Zhao Xiaomei [1], Lin Guangzhe [1], Yan Li [1], Tong Debin [3], Lian Yuji [1]

1 College of Life Sciences Linyi University, Linyi, 276000, China
2 College of Life Sciences Shan dong Normal University, Jinan 250014, China
3 Linyi Xianzhu Biotechnology Co. Ltd, Linyi, 276000, China

Corresponding author email: lianyuji@lyu.edu.cn

Plant Gene and Trait, 2020, Vol.11, No.6 doi: 10.5376/pgt.2020.11.0006
Received: 05 Jun., 2020
Accepted: 22 Aug., 2020
Published: 12 Sep., 2020

Preferred citation for this article:
Zhu J.Q., Zhao X.M., Lin G.Z., Yan L., Tong D.B., and Lian Y.J., 2020, Morphological and histological analysis of somatic embryogenesis in *Haworthia cooperivar*, Plant Gene and Trait, 11(6): 1-9 (doi: 10.5376/pgt.2020.11.0006)

Abstract The fleshy leaves were used as materials for induction and proliferation somatic embryonic callus and plant regeneration. The morphological and histological characteristics of granular embryonic callus were studied by light microscopy using Spuur resin section. The results showed that the yellowish and granular embryonic callus was induced on modified MS medium were supplemented with 1.5 mg/L 6-BA and 0.5 mg/L IAA, the highest induction ratio was 66.7%. The highest proliferation net weight (14.73 g) was presented at modified MS medium were added 1.5 mg/L 6-BA an d 0.4 mg/L IAA. Milky white globular or heat shape somatic embryo was differentiated from calls that originate d from outer or inner-epidermis, the embryonic callus was composed of cells with big nuclear, vigorous division and containing starch granules. The inner embryonic cell clusters showed spiral or oval shapes. These structures were further developed to somatic embryos, which were transformed to complete clumps of small plants from the surface of callus. Healthy developed seedlings rooted on MMS+ 0.1mg/L IAA. This research provides technical support for the rapid propagation and molecular breeding of *Haworthia cooperivar.*

Keywords *Haworthia cooperivar*; Somatic embryo; Histological analysis; Plant regeneration

Haworthia cooperivar is a perennial succulent herb belongs to Liliacea, *Haworthia*, which is an excellent variety selected after hybridization of Yulu (Xie and Xu, 1994, China Agricultural Press, pp.1-87). Its leaves are crystal clear and look like ice lamp. It is the best of the Yulu family and has high ornamental value (Zhou et al., 2017). Under natural conditions, *Haworthia cooperivar* mainly propagates by seeds, but the seed setting rate is relatively low and the period of emergence is relatively long. At present, *Haworthia cooperivar* mainly uses ramets, leaf cuttings and other propagation methods under artificial cultivation conditions, but it has some problems, such as poor ability to grow lateral buds, low propagation coefficient and slow growth rate, which cannot meet the market demand (Song, 2014). Plant tissue culture technology is a rapid and effective method to obtain cloned plants, including somatic embryogenesis and organogenesis (Rosa et al., 2015), which has the advantages of large-scale regeneration, germplasm conservation and genetic transformation (Ogawa et al., 2014; Teixeira et al., 2014; Nguyen et al., 2015). Plant somatic embryo regeneration has the advantages of single primordial embryogenic cell, consistent genetic background among individuals, complete embryo structure, rapid seedling formation, large reproduction, and can be used for artificial seed production (Zhai et al., 2011). At present, the tissue culture research of *Haworthia cooperivar* mostly use the inflorescence (Guo et al., 2016), flower branches (Zhu et al., 2012) and flower stems (Zhang et al., 2017; Zhou et al., 2017) as explants to induce adventitious buds or adventitious roots by using different plant growth regulators (Zhang et al., 2016). Research on the induction of somatic embryos is limited to the induction methods of friable callus (Yan et al., 2017), and there is no report on the tissue structure of somatic embryos in *Haworthia cooperivar*. In order to explore the somatic embryogenesis of *Haworthia cooperivar*, establish an efficient plant regeneration system *in vitro*, and provide a theoretical basis for *in vitro* rapid propagation and molecular breeding of *Haworthia cooperivar*, the leaves of *Haworthia cooperivar* were used as explants for induction embryogenic callus and somatic embryo with plant growth regulator. And the morphological, cytological and histological characteristics of somatic embryogenesis in *Haworthia cooperivar* were studied by section, staining and observation.

1 Results and Analysis

1.1 Effects of different cytokinin treatments on somatic embryonic callus induction of *Haworthia cooperivar*

The explants were inoculated on MMS medium with different combinations of plant growth regulators and observed after 56~70 d. The results showed that no callus differentiation was observed on the medium without plant growth regulators. Different colors and shapes of calli were produced on the medium supplemented with different kinds of cytokinins. When the explants were cultured on the medium supplemented with 1.5 mg/L 6-BA and 0.5 mg/L IAA for 15 d, the base of leaf explants began to expand, and yellow calli appeared at the swelling part. After about 42 d, they gradually changed into light yellow granular calli with moist surface, soft and fragile texture, and differentiated small buds could be observed on some calli. After 56 d, smooth milky white granular structure appeared on the light yellow granular callus. Under the microscope, globular shape somatic embryo (Figure 1A) and heat shape somatic embryo (Figure 1B) were observed, and the callus induction rate was 66.67% (Table 1).

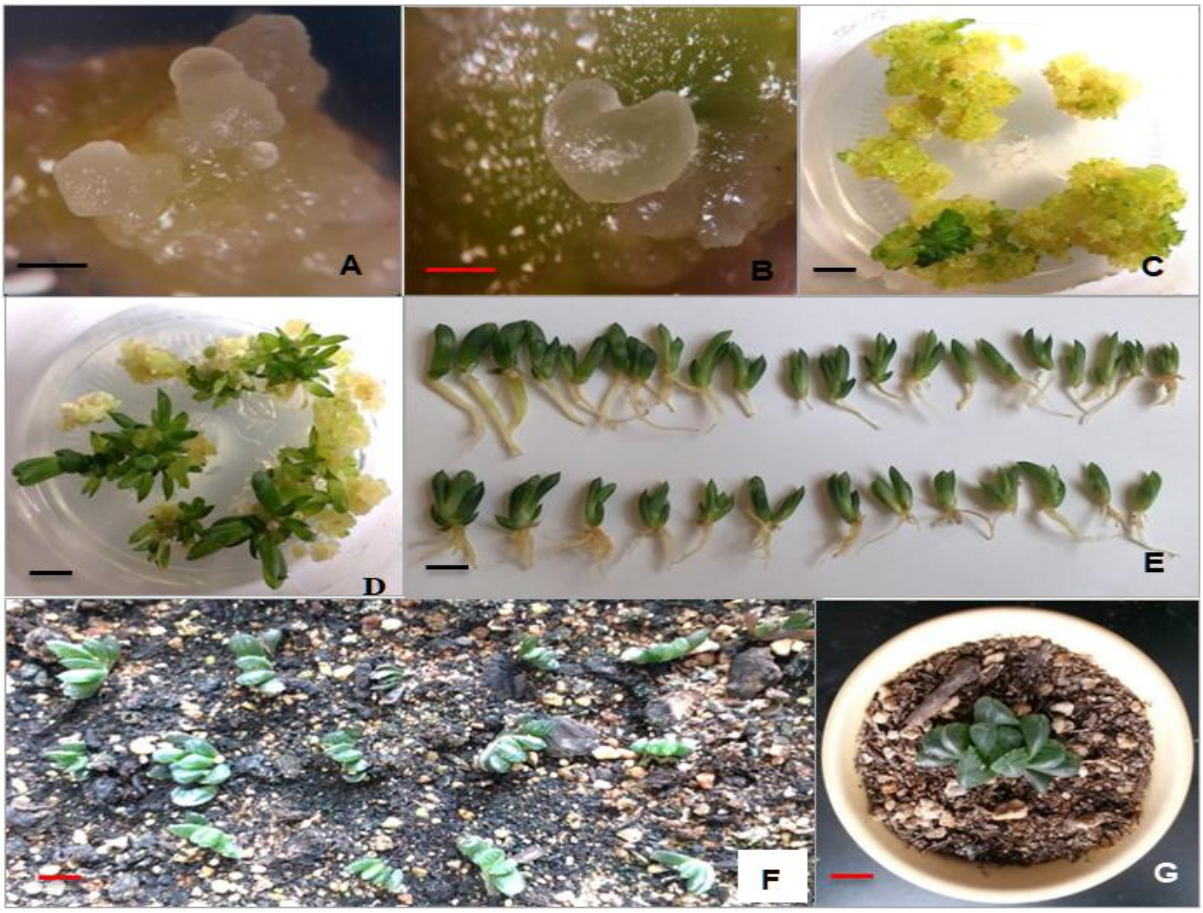

Figure 1 Somatic embryogenesis and plant regeneration of *Haworthia cooperivar*

Note: A: Globular shape somatic embryo; B: Heat shape somatic embryo; C, D: Maturation and germination of somatic embryo; E: Regenerated plantlets; F, G: SE derived plantlets were transplanted after acclimation. A, B: Bars=1 mm; C-D: Bars=0.25 cm

Table 1 Effects of different cytokinin treatments on somatic embryonic callus induction of *Haworthia cooperivar*

Cytokinin	IAA (mg/L)	No. of explants	Induction ratio (%)	Growth state
0	0	30	0	Non callus develope
6-BA 1.5	0.5	30	66.67±0.6 a	Yellowish-white friable callus with granular structures
ZT 1.5	0.5	30	16.67±7.64 b	Compact green callus
KT 1.5	0.5	30	22.86±12.64 b	Compact green callus

Note: Different lowercase letters indicate significant differences at $p<0.05$

When the explants were cultured on MMS medium supplemented with KT or ZT for about 15 d, the incision of explants was slightly expanded, but no callus occurred. After 60 d of culture, a small number of compact green callus were differentiated from some explants. Embryoids were not observed in the observation room under the microscope. The callus induction rate was 16.67% and 22.86%, respectively.

1.2 Embryonic callus proliferation

1.2.1 Effects of different concentrations of 6-BA on the embryonic callus proliferation

The effects of different concentrations of 6-BA on the color, morphology, texture, and growth rate of embryonic callus were quite different. The results of callus proliferation were shown in Table 2. When the concentration of 6-BA was 0.5~1.5 mg/L, the proliferation was not obvious at 7~14 d, it was vigorous after 21 d, and it was faster at 56 d, with the net proliferation weight of 9.36 g, 11.14 g and 14.73 g, respectively. The difference between treatments was significant. The callus were yellowish-white friable with granular structures. And small buds could be observed on the surface of callus (Figure 1C). The green buds and callus were not tightly combined, and the buds would fall off from the callus with a slight touch of tweezers.

Table 2 Effect of different concentration of 6-BA combine d with 0.1 mg/L IAA on proliferation of somatic embryonic callus of *Haworthia cooperivar*

Pant growth regulator (mg/L)		Proliferation weight of somatic embryonic callus (g)				
6-BA	IAA	14 d	28 d	42 d	56 d	70 d
0.5	0.1	1.33±0.12cd	4.39±0.38cd	9.33±0.32b	9.36±0.46c	7.43±0.26c
1.0	0.1	1.37±0.15c	5.70±0.18a	8.91±0.41bc	11.14±0.54b	9.45±0.48b
1.5	0.1	1.98±0.11b	5.36±0.15b	10.63±0.48a	14.73±0.45a	13.52±0.24a
2.0	0.1	2.25±0.37ab	4.25±0.26de	6.55±0.35de	6.31±0.48 de	4.36±0.32 de
2.5	0.1	2.50±0.16a	4.49 ±0.24c	6.72±0.32d	6.57±0.38 d	4.41±0.17 d

Note: Different lowercase letters indicate significant differences at $p<0.05$

When the concentration of 6-BA was 2.0~2.5 mg/L, the proliferation speed was obvious at 14 d, and it was the most at 42 d. The net weight of proliferation was 6.55 g, and 6.72 g, respectively. After that, the callus grew slowly and stopped growing gradually. And the difference between treatments was not significant. The callus gradually showed a hollow bubble structure with yellowish-white soft shape, and there was no green bud. After 42 d, the texture of callus was thick and hollow gradually. After 56~60 d, the callus almost stopped growing and began to brown. The results showed that the low concentration of 6-BA could promote the proliferation of embryonic callus and maintain the characteristics of embryonic callus. However, embryonic callus is easy to lose embryogenesis under the stimulation of higher concentration of 6-BA, which was not conducive to the proliferation of callus.

1.2.2 Effects of different concentrations of IAA on the embryonic callus proliferation

Different concentrations of IAA had little effect on the color, morphology, and texture of embryonic callus, but had some different effects on callus proliferation. When the concentration of IAA was 0.1~0.2 mg/L, the proliferation of callus was more vigorous, and the proliferation was the most after 56 d, which was 13.37 g and 14.22 g, respectively, and then decreased slowly. The callus was uniform in structure and color, and still maintained the characteristics of yellowish, loose texture, and relatively hard granular embryonic callus. Green bud spots could be observed on the surface of callus, most of which were callus proliferation. When IAA concentration was 0.3~ 0.5mg /L, the proliferation of callus was relatively slow, and the proliferation was 11.97 g, 10.96 g and 11.02 g at 56 d, respectively, with no significant difference between treatments. Although the embryonic callus still maintained the characteristics of yellowish, loose texture and friable callus with granular structures, they tended to mature and become smaller plants. The results showed that IAA concentration had a certain effect on the proliferation of embryonic callus. Low concentration of IAA could promote the proliferation of callus. Although the higher concentration of IAA could increase the proliferation of early callus, the proliferation of callus tended to differentiate into adventitious buds. Therefore, IAA concentration of 0.1 ~ 0.2 mg/L was suitable for the embryonic callus proliferation of *Haworthia cooperivar* (Table 3).

Table 3 Effect different concentration of IAA combined with 1.5 mg/L 6-BA on proliferation of somatic embryonic callus of *Haworthia cooperivar*

Pant growth regulator (mg/L)		Proliferation weight of somatic embryonic callus (g)				
6-BA	IAA	14 d	28 d	42 d	56 d	70 d
1.5	0.1	2.00±0.11a	6.02±0.17a	12.63±0.27a	13.37±0.33ab	12.03±0.24a
1.5	0.2	1.90±0.02b	5.31±0.12bc	10.57±0.47b	14.22±0.15a	13.40±0.31b
1.5	0.3	1.32±0.11cd	4.34±0.23d	8.75±0.11d	11.97±0.83b	9.76±0.06c
1.5	0.4	1.39 ±0.23c	5.22±0.29c	9.00±0.31c	10.96±0.39bc	8.93±0.44d
1.5	0.5	1.92±0.23ab	5.37±0.29b	8.71±0.31 de	11.02±0.39bc	8.23±0.44 de

Note: Different lowercase letters indicate significant differences at $p<0.05$

1.3 Plant regeneration of somatic embryo

When the callus were transferred to MMS culture medium supplemented with 0.1~0.2 mg/L IAA, the plant regeneration of somatic embryo was more obvious. When the callus was cultured for 21~28 d, complete clumps of small plants were accumulated on the surface of callus (Figure 1D; Figure 1E). From 42 to 56 d after culture, the roots of small plants were strong and abundant, and their growth was relatively vigorous. On average, 21~31 small plants could be isolated from each callus, and most of the embryonic callus were differentiated into complete small plants (Table 4). When embryonic callus were cultured on the medium supplemented with 0.3~0.4 mg/L IAA for 42~56 d, the number of small plant on the callus was less, and the young roots were thin and short. Some seedlings were rootless seedlings with incomplete root development, and more calluses had not differentiated into small plants. On average, 2~6 plantlets could be isolated from each callus. When the callus was cultured on MMS medium supplemented with 0.5 mg/L IAA for 10 d, the first complete small plant was observed, and it was not appeared. After 35 d, green buds were differentiated from the callus, and cultured for 42~56 d, it was found that most of the small plants differentiated from the callus were rootless. The seedlings were thick and few in number, and the combination with the callus were loose.

Table 4 Maturation of somatic embryo

Concentrations of IAA (mg/L)	No. Callus clumps	No. Mature plantlets	Growth stage
0.1	18	31.30±1.21a	Numerous plantlets roots with strong and vigorously
0.2	18	21.67±0.87b	grow
0.3	18	6.67±1.62c	Small amount of plantlets with thin roots
0.4	18	2.33±1.89d	
0.5	18	6.67±2.12c	Big and small amount of plantlets without roots

Note: Different lowercase letters indicate significant differences at $p<0.05$

1.4 Transplantation of *in vitro*-cultured plant

The plantlets were taken out from the test tube, washed out the medium with tap water, and dried in the dark for 1~2 d. After that, it was transplanted to the substrate and sprayed with water to keep the substrate moist. In the early stage, it is necessary to avoid direct sunlight, gradually strengthen the light intensity, and maintain ventilation. After 2 weeks, the leaves became strong and green, and the survival rate was as high as 100% (Figure 1E; Figure 1F).

1.5 Results and analysis of tissue section

The genesis of somatic embryos in *Haworthia cooperivar* is an indirect way through which the explants' leaves dedifferentiate to form embryonic callus on induction medium and the embryonic callus dedifferentiate to form somatic embryos. The results of longitudinal section of granular structure showed that the granular callus was composed of cells with obvious nucleus, large nucleus and nucleolus, dense cytoplasm and starch granules. The outer layer of this granular structure is composed of closely arranged and regular epidermoid structure (Figure 2A). Developed from outer epidermis are clusters of embryonic cells consisting of 7~8 cells (Figure 2B). A somatic embryonic center derived from a cell can be observed below it (Figure 2C). These irregular small cell clusters with vigorous division were formed by single cells dividing in the flat and vertical circles, with no obvious

connection area with other cells, showing irregular arrangement and relatively loose distribution, and no obvious vascular bundle structure was found. The inside of the granular structure is composed of a number of cells surrounded by the anticlinal division like epidermis. Such cells have clear boundaries, cell size is basically the same, nucleoli are obvious, and cell division is vigorous. As can be seen from the growth outline of the cell mass, its growth direction is the rotation growth from inside to outside, and there are few connections between the cells mass. The vertical cell division forms epidermis, while the horizontal cell division increases the number of cells, which is a vigorous meristematic cell. And metaphase chromosomes can be observed (Figure 2C; Figure 2D; Figure 2E). Vascular bundle and duct system were not observed in the sections, and the vertically dividing pre-procambial cells, which were significantly smaller than the surrounding cells, could be observed in the areas with relatively vigorous division (Figure 2F).

The longitudinal and transverse sections of globular structure (Figure 2G; Figure 2H) have obvious and closely arranged epidermal cells. The size of the cells under the epidermis is basically the same. Most of the cells have obvious nuclei, with obvious nucleoplasm, mostly located in the center. And most of the cells undergo longitudinal or transverse divisions are meristematic cells. Obvious starch granules can be found, and the number is more than other types of cells.

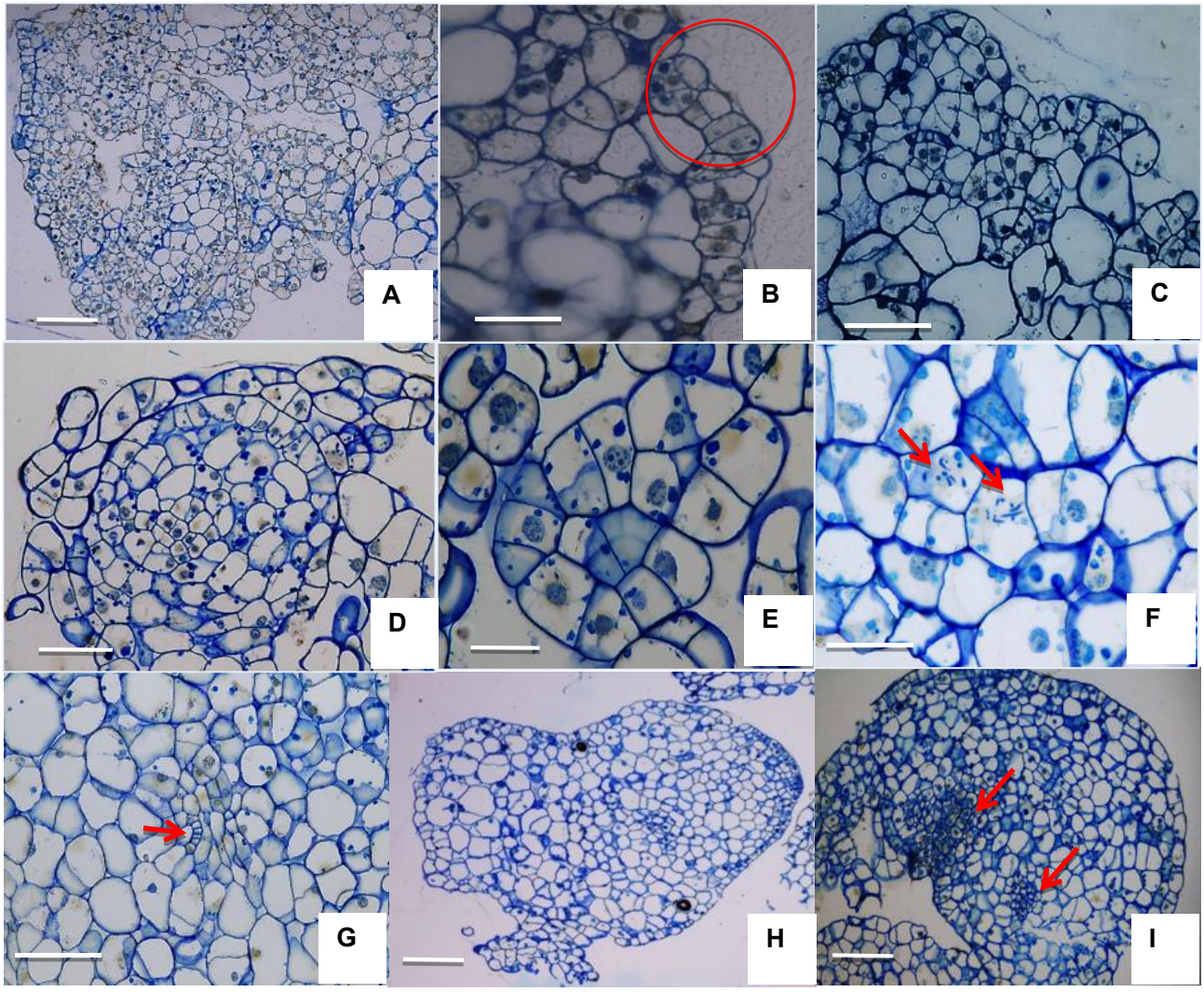

Figure 2 Section of somatic embryogenesis in *Haworthia cooperivar*

Note: A: Section of globular structure; B, C: Meristematic cells that developed from outer epidermis; D, E: Meristematic cells that developed from outer epidermis; F: Metaphase chromosomes in vigorous divided cell mass; G: Pre-procambial cells; H, I: Structure of non-embryonic callus; Scale bar: A, H-I: 200 μm; (D): 150 μm; B-C, G: 100 μm

The longitudinal section of some granular structures showed that the single granular structure was separated from the surrounding tissues, and the outer surface of the granular structure was epidermis with similar cell size, regular shape, and tight arrangement. The cells in the medial epidermis were arranged irregularly with loose distribution and large intercellular space. They were vacuolated parenchyma cells, but no obvious vascular bundle structure was observed (Figure 2G). Pre-procambial cells with obvious nucleus and dense cytoplasm were observed to divide along the vertical or horizontal direction in the middle (Figure 2H; Figure 2I).

2 Discussion

Somatic embryogenesis is one of the effective ways for plant regeneration (Zhai et al., 2011). However, many factors affect the occurrence of somatic embryogenesis. Plant growth regulators are involved in cell cycle regulation and cell division, and are essential factors for somatic embryo induction (Francis and Sorrell, 2001; Fehér et al., 2003; Gaj, 2004). It has been reported that 2,4-D alone or in combination with other plant growth regulators is most widely used in somatic embryo induction (Gumerova et al., 2003; Umehara and Kamada, 2005), and NAA were also frequently used, while IAA was rarely used (Hou et al., 2015). Yan et al. (2017) reported that the embryonic callus induction and embryonic changes in *Haworthia cooperivar* depend on the ratio of BA/2,4-D. In this experiment, the leaves in *Haworthia cooperivar* were used as explants to induce embryonic callus successfully by using the improved MS medium supplemented with 6-BA combination IAA, and the embryonic callus could be maintained for more than 3 years. The results showed that the concentration of 6-BA had a great influence on the induction, proliferation and embryonic maintenance of embryonic callus. When the concentration of 6-BA was 1~1.5 mg/L, the effects of the proliferation and embryonic maintenance of embryonic callus were relatively good. Although the amount of proliferation of callus was relatively large when the concentration of 6-BA was increased, the embryonic gradually lost during the culture process. The effect of IAA on the proliferation of embryonic callus showed that the concentration of 0.1~0.2 mg/L was suitable for the proliferation and maintenance of embryonic callus. When the IAA concentration was increased, somatic embryo maturation and small plant conversion were more vigorous.

Somatic embryogenesis mainly includes four processes: somatic embryo induction, embryonic maintenance and culture, embryo differentiation and somatic embryo regeneration (Ibaraki and Kurata, 2001). The embryonic callus has the morphological characteristics of dense, whitish granular, slow growth or loose arrangement and fragile (Sun et al., 2003; Yang et al., 2003). In the experiment, the leaves in *Haworthia cooperivar* showed similar changes after two months of culture on the medium combined with 6-BA and IAA, forming granular callus with slow growth, yellowish and loose arrangement. Granular callus were further developed into typical globular shape and heat shape structures, which were then transformed into green mature embryos. Somatic embryogenesis was not observed in the greenish dense, hard, or ivory soft water-impregnated callus. This is consistent with the results reported by Xiao et al. (2016) and Cao et al. (2016).

The results of tissue sections of embryonic callus in *Haworthia cooperivar* showed that after the formation of embryonic callus, not only the embryonic cell clusters with deep staining, large nucleus, dense cytoplasm and close arrangement occurred on the surface of the callus, but also the embryonic cell clusters composed of small cells with vigorous division and close arrangement occurred in the interior. The cell clusters grew in a round or spiral shape, which then developed into a somatic embryo. In addition, pre-procambial cells consisting of vertically dividing small cells were observed inside the vigorously dividing callus, which can act as pluripotent or totipotent stem cell-like cells to cause organ differentiation or somatic embryogenesis. Such pre-procambial cells readily respond to the auxin/cytokinin ratio, leading to organ differentiation or somatic embryonic development. This differentiation is usually determined by the direction and location of pre-procambial cells division (Risopatron et al., 2010). The cells from inner epidermis of embryonic callus in *Haworthia cooperivar* were irregularly arranged, loosely distributed, and had large intercellular spaces. They were elongated or large, highly vacuolated large parenchymal cell clusters without obvious nuclear structure, which was similar to the results reported by Ren et al. (2019).

In addition, the accumulation of starch granules during somatic embryogenesis is related to the differentiation of somatic embryos. And the dynamic changes of starch granule accumulation during somatic embryogenesis have been reported in studies on somatic embryo induction in coconut (*Cocos nucifera* Lz) trees (Verdeil et al., 2001) and palm (*Elaeis guineensis*) trees (Schwendiman et al., 1988; Kanchanapoom and Domyoas, 1999). And it has been pointed out that the accumulation of starch granules is related to cell division, mainly concentrated near the center of vigorous cell division to provide energy for cell growth, and its accumulation is a sign of somatic embryo differentiation and development (Ji et al., 2019). In the experiment, a wide distribution of starch granules could be observed in the cell mass of embryonic callus with vigorous division, but not in the non-embryonic callus. The results indicated that the accumulation of starch granules in embryonic callus might be an important factor to ensure the success of somatic embryogenesis.

In this experiment, we successfully induced somatic embryos with the explant leaves in *Haworthia cooperivar*, constructed the plant regeneration system, and studied the morphological and histological characteristics of somatic embryogenesis. The experimental results provided a theoretical basis for the rapid propagation, germplasm preservation, breeding, and mass production of *Haworthia cooperivar*.

3 Materials and Methods

3.1 *Haworthia cooperivar* materials

In 2017, Linyi Xianzhu Biotechnology Co. Ltd provided potted *Haworthia cooperivar.* Selected healthy and complete leaves and gently rub surface stains with washing powder water. Rinsed under running water for 15 min after washing and set aside. On the ultra-clean bench, sterilized the surface by adding 1 drop (about 50 μL) Tween-20 0.15% corrosive sublimate for 5 min, then rinsed repeatedly with sterile water for 5~6 times, placed in the sterilized stainless steel plate, dried the surface water with sterile filter paper, and set aside.

The medium used for the experiment was modified MS (Murashige and Skoog, 1962). The modified MS medium (Mo dified MS, MMS) was to adjust the concentrations of thiamine hydrochloride, pyridoxine hydrochloride, nicotinic acid and calcium pantothenate under the condition of MS basic culture, and the adjusted concentrations were 10.0 mg/L, 1.0 mg/L, 1.0 mg/L and 1.0 mg/L, respectively. 30 g/L sucrose and 8.0 g/L agar powder were added into the culture medium, pH 5.6~5.8, at 104 kPa, 121℃ for 20 min autoclavous steam sterilization. Callus induction, somatic embryo induction and maturation were carried out at (25±2)℃ temperature, 30~50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ of light intensity, 16 h of light and 8 h of darkness per day.

3.2 Induction of embryonic callus

The sterilized leaves were cut lengthwise with an anatomic knife and inoculated with MMS+1.5 mg/L 6-BA +0.5 mg/L IAA, MMS+1.5 mg/L ZT+0.5 mg/L IAA, MMS+1.5 mg/L KT+0.5 mg/L IAA medium. MMS was used as the control group to screen the optimal combination of plant growth regulators for embryonic callus induction. 10 explants were inoculated for each treatment with 3 replicates. Observed the growth of callus, and counted the induction rate and analyzed after 60 d.

3.3 Proliferation of embryonic callus

About 1.5 g of yellowish, granular, and loose embryonic callus were divided into 8~10 pieces and transferred to the culture medium for culture. Embryonic callus proliferation medium was MMS+ (0.5, 1.0, 1.5 2.0, 2.5) mg/L 6-BA+0.1 mg/L IAA, MMS+1.5 mg/L 6-BA+(0.1, 0.2, 0.3, 0.4, 0.5) mg/L IAA. Callus were separated from the medium on ultra-clean bench, and then transferred to a sterile petri dish with the weight removed for weighing, and the data were recorded before the callus was re-inoculated onto the original medium. Every 14 d, fresh weight was weighed, and the growth state of callus was observed. Until the growth of callus entered the decline period (about more than two months), each treatment was repeated for 3 times.

3.4 Plant regeneration

Embryonic callus with good growth status were selected and transferred to MMS+(0.1, 0.2, 0.3, 0.4, 0.5) mg/L IAA+30 g/L sucrose +8.0 g/L agar. After 56 d of culture, the maturity rate and the average number of seedlings were counted to screen the best medium for seedlings. All treatments were repeated for 3 times.

3.5 Domestication and transplantation

The glass culture bottle was moved to a cool outdoor place for about 1 week. Then opened the cap of the culture bottle and place it for 3~7 days until the tissue culture seedlings become strong. Then washed the basal medium with water and dried the seedlings in the shade for 3~7 days. Then the seedlings were transplanted to the substrate, which was a mixture of peat soil, river sand, and perlite, with a volume ratio of 5:3:2.

3.6 Observation of organization structure

Fresh globular shape callus particles were selected and fixed, embedded, trimmed and sliced according to the method of Lian et al. (2013). The embedded tissue was cut into semi-ultrathin slices of 1 μm by a nanometer slicer (Leica, Germany). After staining, the sections were observed under Olympus optical microscope (Olympus BX51, Japan), and photographed, recorded.

3.7 Statistical analysis of data

The following formula is used for data statistics.

Callus induction ratio= (Number of explants with callus/Total number of inoculated explants)×100%

Net proliferation of embryonic callus (Fresh weight) = Quality of callus after proliferation- Quality of inoculated callus

Duncan's multiple analysis method was used for significance difference analysis, and the minimum significance difference was shown at the level of 5%.

Authors' contributions

ZJQ designed and carried out this experiment. ZJQ, ZXM, and YL completed the somatic embryo induction and tissue section treatment of *Haworthia cooperivar*. TDB and LGZ completed domestication and transplantation and management. LYJ conceived and directed this study, guided experiment design, data analysis, and helped to draft and revise the manuscript. All authors read and approved the final manuscript.

Acknowledgments

This study was supported by Project entrusted by Linyi University (No.HX160402).

Reference

Cao Y., Chen Z.H., Ou D.W., Zhang S.C., and Yang C.W., 2016, The establishment of teosinte tissue culture and callus section observation, Huanan Shifan Daxue Xuebao (Journal of South China Normal University (Natural Science Edition)), 48(6): 19-24

Fehér A., Pasternak T.P., and Dudits D., 2003, Transition of somatic plant cells to an embryogenic state, Plant Cell Tiss. Org. Cult., 74: 201-228

Francis D., and Sorrell D.A., 2001, The interface between the cell cycle and plant growth regulators: a mini review, Plant Growth Regul., 33: 1-12
https://doi.org/10.1023/A:1010762111585

Gaj M.D., 2004, Factors influencing somatic embryogenesis in duction and plant regeneration with particular reference to *Arabidopsis thaliana* (L.) Heynh, Plant Growth Regul., 43: 27-47
https://doi.org/10.1023/B:GROW.0000038275.29262.fb

Gumerova E.A., Galeeva E.I., Chuyenkova S.A., and Rumyantseva N.I., 2003, Somatic embryogenesis and bud formation on cultured *Fagopyrum esculentum* hypocotyls, Russian J. Plant Physiol., 50: 640-645
https://doi.org/10.1023/A:1025640107932

Guo S.H., Zhu Y.X., and Guan Y.J., 2016, Key techniques for rapid propagation of *haworthia cooperivar* pilfera of *Liliaceae*, Zhongguo Nongxue Tongbao (Chinese Agricultural Science Bulletin), 32(34): 85-89

Hou J.Y., Wu Y., Shen Y.C., Mao Y.J., Liu W.B., Zhao W.W., Mu Y., Li, M.H., Yang M.L., and Wu L.F., 2015, Plant regeneration through somatic embryogenesis and shoot organogenesis from immature zygotic embryos of *Sapium sebiferum* Roxb, Sci. Hortic., 197: 218-225
https://doi.org/10.1016/j.scienta.2015.09.040

Ji Y.L., Chen F.J., Deng W., and Zhang D.C., 2019, Histocytological observation of somatic embryogenesis in *Sorghum bicolor* (L.), Shengwu Ziyuan (Biotic Resources), 41(4): 371-375

Kanchanapoom K., and Domyoas P., 1999, The origin and development of embryoids in oil palm (*Elaeis guineensis Jacq*) embryo culture, Sci. Asia, 25: 195-202
https://doi.org/10.2306/scienceasia1513-1874.1999.25.195

Ibaraki Y., and Kurata K., 2001, Automation of somatic embryo production, Plant Cell Tiss Org. Cult., 65(3): 179-199
https://doi.org/10.1023/A:1010636525315

Lian Y.J., Lin G.Z., and Zhao X.M., 2013, Histology and development analysis of meristematic nodules from cultured *Pulsatilla koreana*, Zhiwu Xuebao (Chinese Bulletin of Botany), 48(5): 540-549
https://doi.org/10.3724/SP.J.1259.2013.00540

Murashige T., and Skoog F., 1962, A revise d medium for rapid growth and bioassays with tobacco tissue cultures, Physiol. Plant, 15(3): 473-497
https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Nguyen Q.T., Bandupriya H.D.D., López-Villalobos A., Sisunandar S., Foale M., and Adkins S.W., 2015, Tissue culture and associated biotechnological interventions for the improvement of coconut (*Cocos nucifera* L.), A Review, Planta, 242(5): 1059-1076
https://doi.org/10.1007/s00425-015-2362-9
PMid:26189000

Ogawa Y., Shirakawa M., Koumoto Y., Honda M., Asami Y., Kondo Y., and Hara-Nishimura I., 2014, A simple an d reliable multigene transformation method for switch grass, Plant Cell Rep., 33(7): 1161-1172
https://doi.org/10.1007/s00299-014-1605-8
PMid:24700247

Ren H.Y., Gong G.H., Wang Y.K., Zhao A.L., Xue X.F., 2019, Somatic embryogenesis and development in *Ziziphus jujuba* Mill.: histological observation, Nongxue Xuebao (Journal of Agriculture), 9(3): 32-35

Risopatron J.P.M., Sun Y., and Jones B.J., 2010, The vascular cambium, molecular control of cellular structure, Protoplasma, 247(3-4): 145-161
https://doi.org/10.1007/s00709-010-0211-z
PMid:20978810

Rosa Y.B.C.J., Bello C.C.M., and Dornelas M.C., 2015, Species- dependent divergent responses to in vitro somatic embryo in duction in *Passiflora* spp, Plant Cell Tiss. Org. Cult., 20: 69-77
https://doi.org/10.1007/s11240-014-0580-7

Schwendiman J., Pannetier C., and Michaux-Ferriere N., 1988, Histology of somatic embryogenesis from leaf explants of the oil palm *Elaeis guineensis*, Ann. Bot., 62(1): 43-52
https://doi.org/10.1093/oxfordjournals.aob.a087634

Song Y., 2014, Study on tissue culture and rapid propagation technology of *Haworthia cooperivar*, Xiandai Nongye Keji (Modern Agricultural Science and Technology), (18): 164-164

Sun Y., Zhang X., Jin S., Liang S., and Nie Y., 2003, Somatic embryogenesis and plant regeneration in wild cotton (*Gossypium klotzschianum*), Plant Cell Tiss. Org. Cult., 75(3): 247-253

Teixeira da Silva J.A., Zeng S., Galdiano R.F., Dobránszki J., Cardoso J.C., and Vendrame W.A.. 2014, In vitro conservation of *Dendrobium* germplasm, Plant Cell Rep., 33(9): 1413-1423
https://doi.org/10.1007/s00299-014-1631-6
PMid:24845051

Umehara M., and Kamada H., 2005, Development of the embryo proper and the suspensor during plant embryogenesis, Plant Biotechnol., 22: 253-260
https://doi.org/10.5511/plantbiotechnology.22.253

Verdeil J.L., Hocher V., Huet C., Gros demange F., Escoute J., and Ferriere N.M., 2001, Ultrastructural changes in coconut calli associated with the acquisition of embryogenic competence, Ann. Bot., 88: 9-18
https://doi.org/10.1006/anbo.2001.1408

Xiao W., Tu H.Y., and Zhang A.L., 2016, Embryogenic callus induction and plant regeneration from *Hedychium coronarium* via somatic embryogenesis, Yuanyi Xuebao (Acta Horticulturae Sinica), 43(8): 1605-1612

Yan X.F., Liu Y.J., Huang J.X., Yang J.H., Li J.K., and Wu C.X., 2017, Induction method of loose embryogenic callus of *Haworthia cooperivar*, Tianjin Nongye Kexue (Tianjin Agricultural Sciences), 23(7): 21-24, 36

Yang Y.G., Guo Y.M., Guo Y., Guo Z.C., and Lin J.X., 2003, Regeneration and large-scale propagation of *Phragmites communis* through somatic embryogenesis, Plant Cell Tiss. Org. Cult., 75(3): 287-290
https://doi.org/10.1023/A:1025871015405

Zhai Y., Zhang Z.Q., Jia M., Wang Y., Song X.D., and Zhou L., 2011, Plant regeneration and somatic embryogenesis of *Lilium* spp, Xibei Zhiwu Xuebao (Acta Botanica Boreali-Occidentalia Sinica), 31(4): 834-841

Zhang H.L., Liu Y.J., Huang J.X., Yang J.H., Li J.K., and Yan X.F., 2017, Study on the influence factors of morphogenesis of *Haworthia cooperivar* tissue culture seedling, Tianjin Nongye Kexue (Tianjin Agricultural Sciences), 23(7): 17-20

Zhang J.X., Liu Y.J., Yang J.H., Qin Y.J., Zhang C., and Li J.L., 2016, Effects of hormones on differentiation of adventitious bud and adventitious root of *Haworthia cooperivar*, Tianjin Nonglin Kexue (Tianjin Agriculture and Forestry Science and Technology), 252(4): 4-6

Zhou H.C., Liu Y.J., Huang J.X., Yang J.H., Li J.K., and Wu C.X., 2017, Study on in vitro mutagenesis of *Haworthia cooperivar*, Tianjin Nongye Kexue (Tianjin Agricultural Sciences), 23(7): 25-28

Zhu T.H., Lu J.M., Sun Q., Wang S.Y., Yao D.B., Gu J.P., and Tang G.J., 2012, Study on the culture of *Haworthia fasciata* in vitro, Shanghai Jiaotong Daxue Xuebao (Journal of Shanghai Jiaotong University (Agricultural Science)), 30(3): 45-48