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Full Length Research Paper

Aerenchyma formation and increased accumulation of free proline in roots of xerophytic *Aloe vera* L. cultured in nutrient solutions

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Aloe vera* L. is a xerophyte with a crassulacean acid metabolism pathway. Due to its importance in traditional medicine, healthcare, cosmetics and food products, aloe production is currently at an accelerated pace worldwide. Field production, however, has been limited by its sensitivity to chilling temperature and also by low yield and low plant quality. To overcome these limitations, this study tested the possibility of aloe cultivation in solution culture in a protected environment. Chinese *Aloe* or Buyecheng *Aloe*, both are *A. vera*, and were grown in four solution media or one of the media at different temperatures and pH levels. Time of primary root appearance and/or root growth was quantified. To identify possible mechanisms underlying aloe adaptation to aquatic stress, Chinese aloe were grown in a developing solution culture system and also in soil for 60 days and microstructure, ultra structure, genomic deoxyribonucleic acid (DNA) profile, free proline and protein contents of Chinese aloe roots adapted to aquatic stress were compared to those grown in soil. Results showed that aloes could be aquatically cultivated even though it is a xerophyte. Nutrient solutions, solution pH and cultivation temperature affected root initiation and root growth. Microstructure and ultra structure comparisons of roots produced in solution to roots grown in soil suggested that aloes adapted to aquatic stress by the formation of aerenchyma. Genomic DNA fragmentation of roots formed in solution showed apparent DNA ladders in the adaptive roots, suggesting that the aerenchyma formation was through programmed cell death. Meanwhile, free proline and protein contents in aquatic roots were significantly higher than those of roots grown in soil. As far as known, this is the first documentation of aerenchyma formation in a succulent crassulacean acid metabolism (CAM) plant for adaptation to aquatic stress. The formation of aerenchyma is structure along the increased of proline accumulation metabolically and could be the mechanisms underlying aloe adaptation to aquatic stress.

Key words: Aerenchyma, *Aloe*, aquatic root, free proline, hydroponic culture, xerophytes.

INTRODUCTION

Aloe vera L., a member of the family Liliaceae, has tropical approximately 325 species originating largely in and southern Africa (Huxley, 1994). Among the species, *A. vera* L. is most widely cultivated for its thick fleshly leaves from which gelatinous substances are extracted. *A. vera* gel has been widely used as a traditional medicine to induce wound healing and as an anti-cancer and anti-virus agent (Hamman, 2008). Some of its medicinal properties have been attributed to aloin, a C-glycoside derivative of anthraquinone (Reynolds, 1985; Choi et al., 1996). *A. vera* gel has also been used in

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cosmetic and food products (Reynolds and Dweck, 1999). Because of its economic importance, *A. vera* production is currently at an accelerated pace worldwide (Eshun and He, 2004). Field production of aloe, however, has encountered several difficulties including low yield, low percentage of gel extraction and leaf contamination by pathogens and/or pests (Simmonds, 2004). Additionally, due to its tropical origin, *Aloe* cannot tolerate temperatures close to freezing (Wang, 2007), which has significantly restricted its production acreage. We propose the production of aloes through solution culture under protected environmental conditions. Under such conditions, high quality and high yield aloes could be produced on a year-round basis. However, there is little information available thus far regarding aquatic culture of aloes.

This is probably attributable to the fact that all aloes are xerophytes with CAM pathway (Newton, 2004). A distinct feature of many xerophytes is their development of water storage tissue or aqueous tissue consisting of large thin-walled cells in which water is held in mucilage (Newton, 2004). Accumulation of water in the aqueous tissue results in succulent leaves, as in aloes, or stems, as in cacti (Willert et al., 1992), thus conserving water and surviving in areas of low or erratic precipitation (Reynolds, 1985). It was unknown if aloes could be cultured in solution, more specifically if adaptive mechanisms would develop in aloes for tolerating aquatic stress. Research on aquatic, semi-aquatic and even some non-aquatic plants has shown that metabolic and structural adaptations are developed when plants are challenged by waterlogged stress (Drew, 1997; Sairam et al., 2008). Metabolic adaptations include the induction of fermentation pathway enzymes (ethanol, lactic acid and alanine fermentation) (Parent et al., 2008) and also increased accumulation of free proline (Huang and Cavalieri, 1979; Aloni and Rosenshtein, 1982; Verbruggen and Hermans, 2008). Structural modifications include aerenchyma and adventitious root formation (Arunika et al., 2001; Evans, 2003). Aerenchyma, a porous cortical tissue with enhanced gas exchange with the atmosphere (Thomas et al., 2005; Steffens et al., 2011), is particularly common in plant adaptation to waterlogged stress (Evans, 2003). Aerenchymatous phellem enables hypocotyl-to-root O2 transport in *M. siculus*. Phellem increases radically under stagnant conditions and will contribute to water logging tolerance by enhancing root aeration (Teakle et al., 2011).

Ethylene is believed to play a role in aerenchyma formation, probably by triggering programmed cell death in specific cortical cells (Drew et al., 2000; Gunawardena et al., 2001; Enstone et al., 2003; Sairam et al., 2008). To explore the potential of aquatic production of aloe, this study tested the effects of nutrient solutions, solution pH and temperature on root growth of two aloe varieties and analyzed anatomical and physiological changes during aloe adaptation to solution culture.

MATERIALS AND METHODS

Plant materials and culture solutions

One year old healthy Chinese aloe and Buyecheng aloe (both are *A. vera*) were used in this study. Aloes were washed with tap water until they were soil free. All roots were removed using sterile scissors; subsequently aloes were planted in four liquid media: M_1, basal medium with addition of 5.0 µM indole-3-butyric acid (IBA); M_2, tap water only; M_3, basal medium only; and M_4, tap water with 5.0 µM IBA. The basal medium was composed of 4.5 mM $Ca(NO_3)_2$, 3.0 mM KNO_3, 1.0 mM KH_2PO_4, 1.5 mM K_2SO_4, 2.0 mM $MgSO_4$, 30 µM Fe-Na- ethylene diamine tetraacetic acid (EDTA), 10 µM $MnSO_4$, 40 µM H_3BO_3, 2.0 µM $ZnSO_4$, 0.3 µM $CuSO_4$ and 0.5 µM Na_2MoO_4.

Factors influencing root growth

Three experiments were conducted to investigate factors influencing aloe growth in solution culture: (1) To determine media effect, Chinese aloe plants were transplanted in the four liquid media with pH adjusted to 6.5 and grown in a temperature of 20°C, light intensity of 300 µmol m^{-2} s^{-1} and relative humidity of 80%. Days required for the appearance of primary roots were recorded. (2) To test solution temperature effects, Chinese aloe and Buyecheng aloe were planted in M_4 solution with temperatures set at 15, 20 and 28°C, respectively, pH at 6.5 and grown under the same light intensity and relative humidity as the first experiment. Again, days for the appearance of primary roots were recorded. (3) To assay pH effect, Chinese aloe plants were grown in M_4 with pH set at 5.5 and 7.5, respectively. Temperature was set at 28°C and light intensity and relative humidity were the same as the previous two experiments. Days of primary root appearance, number of roots, root length and root fresh weights were recorded at 10 and 15 days. No air was pumped into the culture solutions during the experiments. Each experiment was arranged in a completely randomized design with three replications.

Anatomical analysis of roots

Plants of Chinese aloe were planted in M_4 solution with a pH of 6.5 and grown under a light intensity of 300 µmol m^{-2} s^{-1}, temperature of 28°C and relative humidity of 80% until plants had at least five roots. The plants were then moved to M_3 solution with a pH of 6.5 and cultured under the same growth environment for up to 60 days. The control was Chinese aloe grown in soil. Root samples were taken 6 and 60 days after primary root appearance, respectively for anatomical analysis of roots and also for genomic DNA, free proline and protein extractions as described below. The experiment was arranged in a completely randomized design with three replications. Roots formed in solution and soil cultures at the ages of 6 and 60 days were dissected and vacuum infiltrated with 3% (w/v) paraformaldehyde (Sigma, St Louis, MO, USA) and 0.25% glutaraldehyde (Sigma, St Louis, MO, USA) in phosphate buffer saline (PBS)f or 30 min (pH 7.2). The fixed tissues were renewed with fresh solution and post fixed in 1% OsO4 (Sigma, St Louis, MO, USA) in PBS (pH 7.2). The tissues were washed in PBS, dehydrated in a graded ethanol series and embedded in EPON812 (Emicron, USA). Half-thin sections (100 nm) were examined at every stage, the observations and photographic recordings were performed with an Olympus BX51 microscope. Ultra-thin sections (50 to 70 nm) were double stained with 2% (w/v) uranyl acetate (Sigma, St Louis, MO, USA) and 2.6% (w/v) lead citrate (Sigma, St Louis, MO, USA) aqueous solution and examined with a transmission electron microscope (Hitachi, H-8100, Tokyo, Japan) at 100 kV.

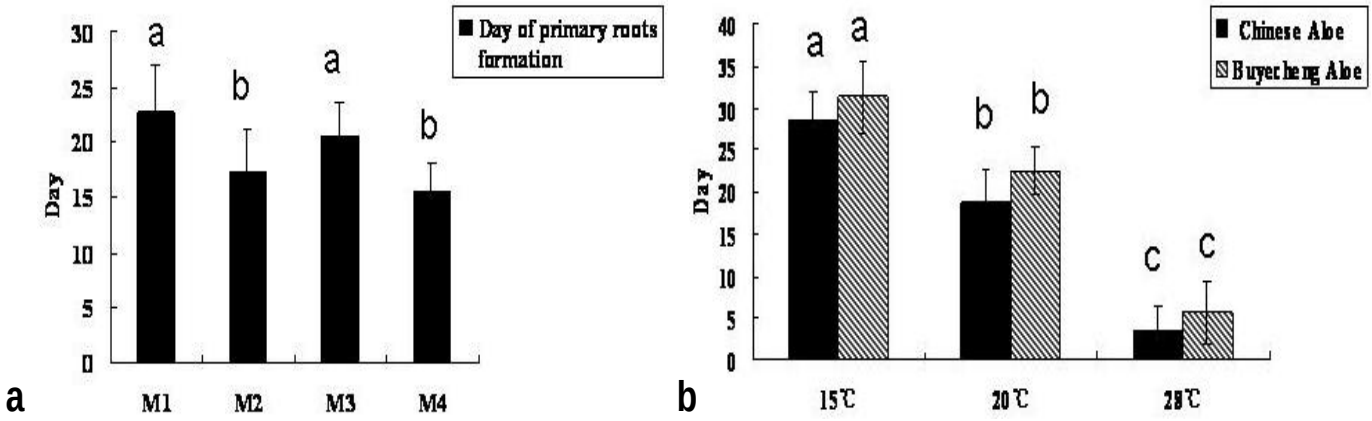

Figure 1. The effect of cultural solutions (media M_1, M_2, M_3 and M_4) on the time of primary root appearance from Chinese aloe cultured at 20°C; **a** and variety (Chinese aloe and Buyechng aloe) and temperature effects on the time of primary root formation when they were cultured in medium 3 or M_3; b. Different letters on the top of bars indicate significant difference at $P = 0.05$.

Free proline assay

Roots were collected 60 days after Chinese aloe were grown in solution and soil cultures. Free proline was extracted from the roots and quantified using the acid ninhydrin method described by Tang et al. (2008).

Soluble protein analysis

The soluble protein analysis was conducted according to the method described by Riccardi et al. (1998), where 200 mg of fresh roots from Chinese aloe grown 60 days in solution and soil culture were collected, homogenized with 5 ml distilled water and centrifuged at 8000 rpm for 10 min at 4°C. The resultant supernatants, 1.0 ml each, were mixed with 5.0 ml Coomassie brilliant blue G-250 for 2 min and analyzed at 595 nm. The amount of soluble protein was calculated according to a standard curve.

DNA extraction and gel electrophoresis

Fresh roots of Chinese aloe were grown 60 days in solution and soil cultures were cut and milled into powder in liquid nitrogen. DNA was extracted from the roots based on the method described by Kidwell and Osborn (1992). Briefly, after centrifugation, equal volumes of phenol, chloroform and isoamylalcohol were added and centrifuged at 4°C. The pellets were mixed with 2 times of the volume of ethanol to precipitate under -20°C. Pellets were washed with 70% ethanol, cleaned and dried, dissolved in 30 TE buffer and stored in refrigerator at 4°C. DNA was separated on a 0.7% agarose gel (Biowest, Spanish), then it was stained with ethidium bromide and photographed under ultraviolet (UV) transmission light.

Statistical analysis

All data were analyzed by the V8 statistic analysis system for Windows (SAS Institute Inc, USA) to determine the significant differences (defined as $P < 0.05$). All data were subjected to analysis of variance (ANOVA) using the Sigma Plot 8.0 Dome statistical package and the statistical analysis system (SAS) software. Means were compared using Duncan's multiple range tests at the 5% level of probability.

RESULTS

Factors influencing root initiation and growth in solution culture

Nutrient solutions significantly affected root initiation. The days required for the first appearance of primary roots in Chinese aloe cultured in M_4 and M_2 solutions were 15 and 17 days, respectively compared to 21 and 23 days cultured in M_3 and M_1 solution, respectively (Figure 1a). Cultivation temperatures also affected root formation regardless of aloe variety (Figure 1b). The primary root appearance required 28.6 and 31.5 d for Chinese aloe and Buyecheng aloe, respectively after culture in M_3 solution at 15°C. Leaves of both varieties at this temperature became amaranthine and withered. When the two varieties were grown at 20°C, days for primary root formation was reduced to 18.7 and 22.5 d for Chinese aloe and Buyecheng, respectively, and there were no stressful symptoms on aloe leaves. The primary root appeared even quicker, only 3.4 and 5.0 d for Chinese aloe and Buyecheng aloe, respectively after culture at 28°C. As already mentioned, the two varieties differed in root initiation. Chinese aloe formed its primary roots quicker than Buyecheng aloe at 15 and 20°C. When both were cultured at 28°C, the time of the primary root appearance was not significantly different. Solution pH had significant effects on root formation as well. The first appearance of primary roots from Chinese aloe cultured in M_4 solution with a pH 5.5 was faster than those cultured at pH 7.5 (Figure 2a). Root numbers 15 days after culture, however, were similar regardless of pH level (Figure 2b). Root lengths recorded after 10 and 15 days of culture were significantly greater when plants were cultured at pH 5.5 compared to those cultured at pH 7.5 (Figure 2c). Fresh weight increase at pH 5.5 was significantly greater than that at pH 7.5 10 days after

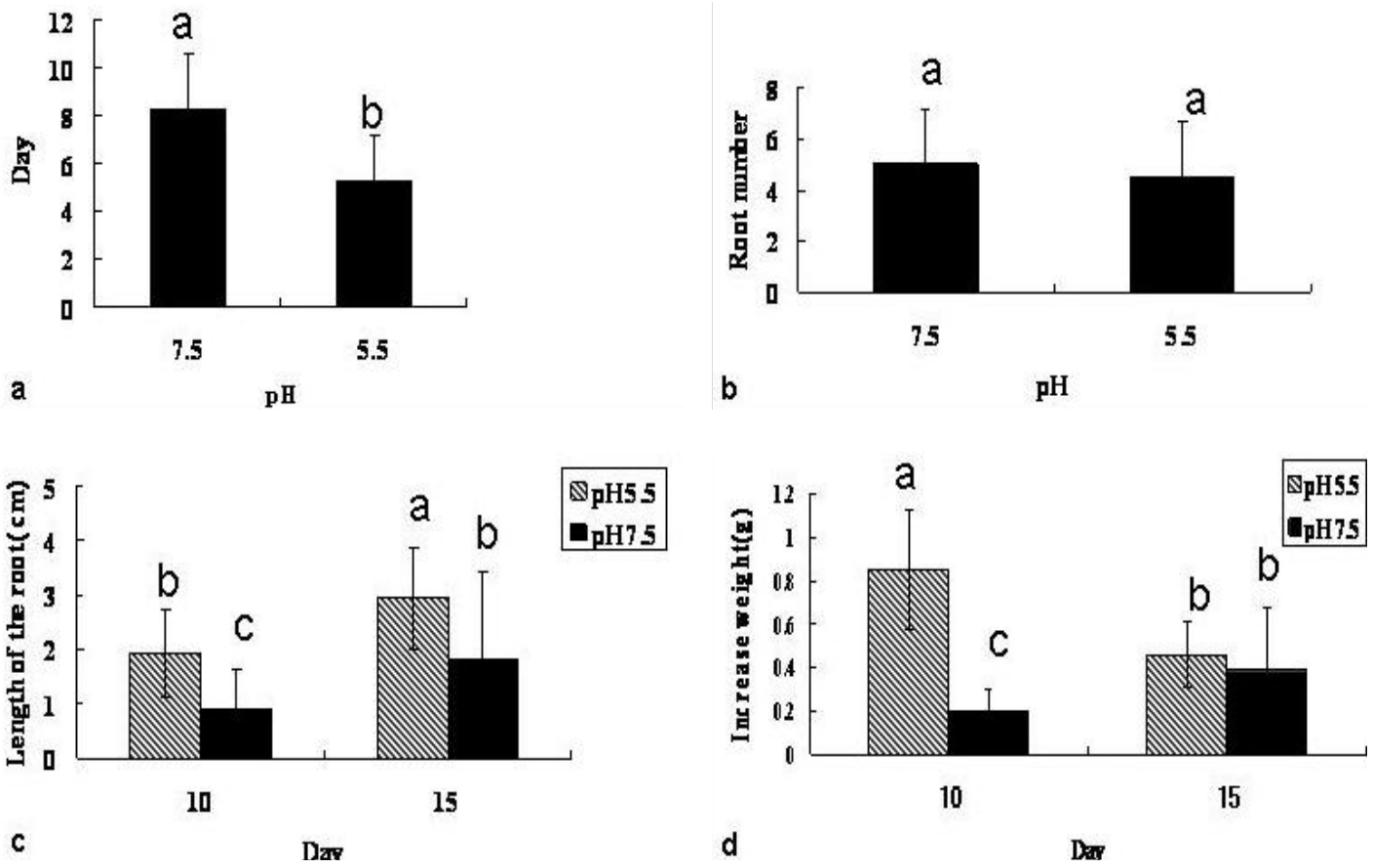

Figure 2. The effect of medium pH on root initiation and growth of Chinese aloe cultured in medium 2 (M_2) with pH at 5.5 and 7.5, respectively and a temperature of 28°C. The time of primary root appearance; a. the number of roots produced when cultured at pH 5.5 and 7.5; b. total root length (cm) produced at pH 5.5 and 7.5 sampled days 10 and 15 after culture; c. and fresh weight (g) of Chinese aloe at pH 5.5 and 7.5 sampled days 10 and 15 after culture; d. Different letters on the top of bars indicate significant difference at $P = 0.05$.

culture. However, on day 15, the fresh weight produced at pH 7.5 was similar to that produced at pH 5.5 (Figure 2d).

Anatomical comparison of roots produced in solution to those produced in soil cultures

The primary roots formed 6 days after Chinese aloe plants were cultured in M_4 solution with a pH of 6.5 were white without lateral roots (Figure 3 a1). Roots of the plants grown in the solution for 60 days became brown and many lateral roots were produced along the taproot (Figure 3 b1). The surface of roots grown in soil was coarse and crooked with some lateral roots (Figure 3 c1). Histological analysis showed that roots produced 6 days after growth in solution had a thin epidermis and a well developed cortex and vascular system (Figure 3 a2). Additionally, central marrow cells were tightly packed (Figure 3 a3). The roots grown in solution for 60 days had a thicker epidermis and fewer xylem cells and also produced aerenchyma in the cortex cell zone (Figure 3 b2). The aerenchyma produced by the pericycle radically displaced the endodermis and the cortex, causing the epidermis to break away from the tissue (Figure 3 b3). For plants grown in soil for 60 days, their cortex cells were closely knitted together without gaps (Figure 3 c2). There was also abundant xylem cell polarization, but no aerenchyma occurred in the pericycle (Figure 3 c3). Ultra-structural analysis of Chinese aloe roots grown in soil showed that cortex cells with cytoplasm accumulation around cell walls were closely connected (Figure 4 a1). Nuclei and some organelles, such as ribosomes and mitochondria were observed in the cytoplasm of the cortex cells (Figure 4 b1). Nuclei with intact membranes, smooth surfaces and also dispersed chromatins were observed in pericycle cells.

The cortex cells contained large amounts of cytoplasm and a large vacuole that restricted cytoplasm to the cell periphery (Figure 4 c1). As to the primary roots of Chinese aloe grown in M_4 solution for 6 days, concentric circles of membranes were occasionally observed in the

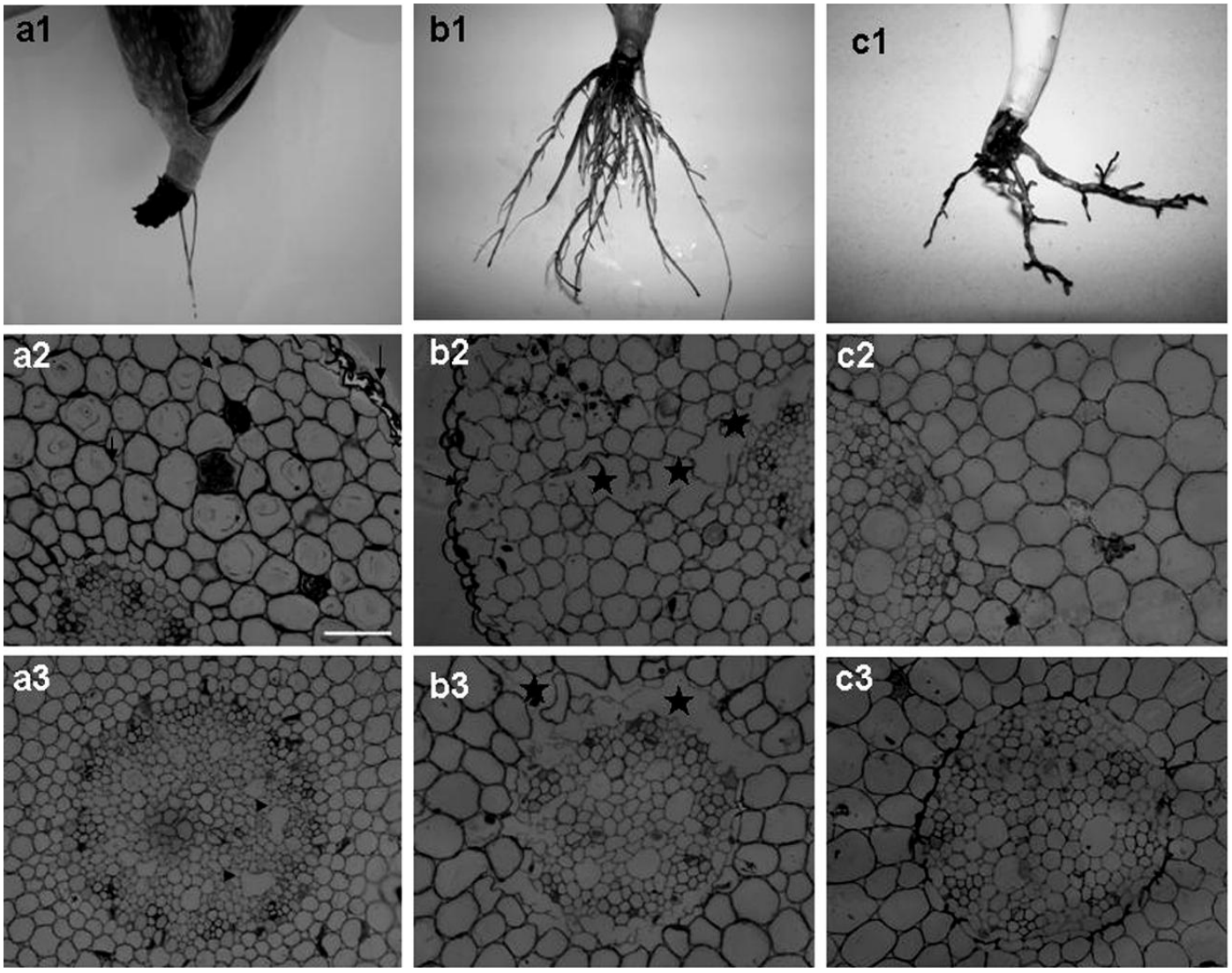

Figure 3. Microstructure analysis of roots of Chinese aloe when cultured in solution and soil, of which; a1 to a3 are morphology and cross sections of roots 6 days after culture in solution where a1 is primary roots; a2 is cross section, the epidermis was thin (indicated by the arrow) but the cortex and vascular system differentiated normally, and; a3 is magnified vascular system developed with close juncture. In the second column; b1 to b3 are morphology and cross sections of roots 60 d after culture in water where; b1 is roots 60 d after culture in water, the surface became brown with many lateral roots; b2 is the epidermis cells that became thicker, some cortex cells formed aerenchyma (star), and; b3 is a vascular system surrounded by cortex cells with increased cell volume and intercellular space, and aerenchyma (indicated by stars) produced around the pericycle. In the right column; c1 to c3 are morphology and cross section of root 60d after culture in soil where; c1 is coarse and crooked roots with lateral roots produced when cultured in soil, their cortex cells in c2 were closely knitted together without gaps, and c3 is the cortex cells and the pericycle cell developed without aerenchyma. Bar = 100 μm.

cells, which were empty, membrane-bound structures. Some cells contained granular material instead of recognizable organelles (Figure 4 a2). There was some nuclear fragmentation around the membrane; but tonoplast membranes appeared to have disintegrated and the organelles were swollen and distorted (Figure 4 b2). The nuclei of the pericycle cells had an inflexed-membrane and it appeared that more organelles were present than in those cells of soil-grown roots, especially mitochondria and chloroplasts. However, no obvious nuclear fragmentation was observed at this stage (Figure 4 c2). Cell walls were intact in these cells, but were detached from the neighboring radial files of cells in the cortex. For roots of Chinese aloe grown in solution for 60 days, cells with collapsed walls attached to one another within the same radial file of cells. Many of the cortex cells collapsed, creating the gas spaces characteristic of aerenchymatic tissue (Figure 4 a3). The earliest distinction between normal pericycle cells and lysing cells was the appearance of unusual-looking nuclei and the

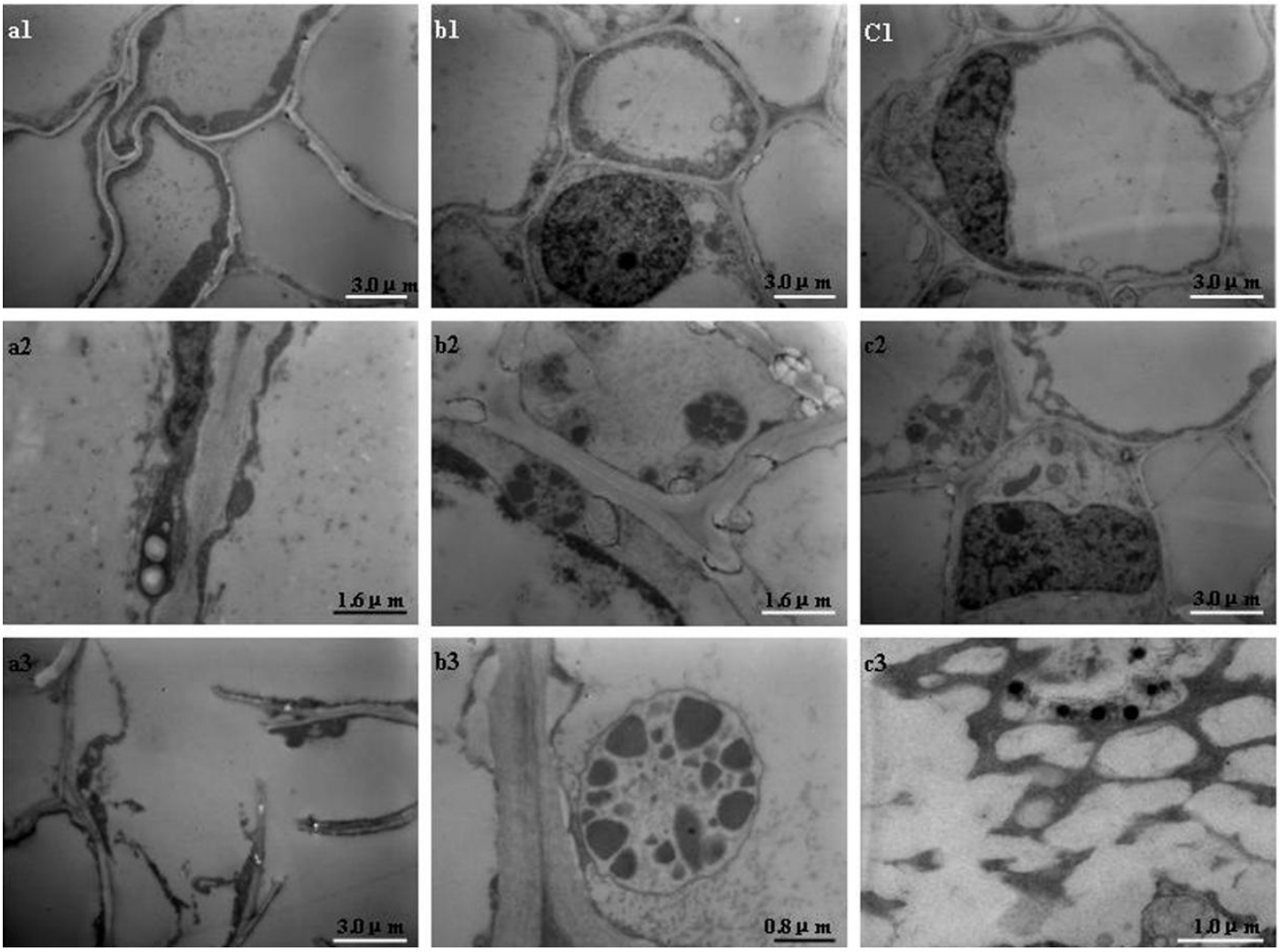

Figure 4. The ultra-structure of roots of Chinese aloe when cultured in solution and soil, of which; a1 to c1 are cell structures of roots produced in soil, where a1 indicates the cortex cells closely connected; b1 shows the rounded nucleus in cortex cells; and c1 presents the nucleus of the pericycle cell. In the second panel, a2 to c2 are cell structures of primary roots 6 days after culture in solution where; a2 is the cortex cell of primary roots, in which concentric circles of membranes were observed; b2 exhibits the nucleus of cortex cells, degraded organelles and early signs of chromatin condensation, and c2 shows the normal nucleus of pericycle cells. In the bottom panel, a3 to c3 are cell structures of roots 60 days after culture in M_3; in a3 many of the cortex cells had collapsed, creating the gas spaces, and b3 is the deterioration of nuclear membranes and general nuclear fragmentation root cells, and c3 shows lysing pericycle cells where apparent mixture of nuclei and vacuoles were also observed as lysis progressed.

deterioration of the nuclear membrane resulted in nuclear fragmentation around the membrane (Figure 4 b3). Organelles, especially nuclei, were rarely seen in these lysing pericycle cells and the apparent mixing of nuclei and vacuoles was also observed as lysis progressed (Figure 4 c3).

Fragmentation of DNA extracted from roots grown in water and soil

To verify the nature of the lysed cells, DNA was extracted from roots of Chinese aloe grown in both solution and soil. The laddering of DNA or the degradation of genomic DNA into internucleosomal fragments was detected in the elongation zone of roots grown in solution for 60 days (Figure 5a, lanes 1 to 4). But there was no DNA laddering in root tip of roots grown in solution for 60 days (Figure 5b, lane 5). DNA smearing was detected in the elongation region of roots grown in solution for 6 days (Figure 5b, lane 6); but no DNA smearing was found in the root tip grown in solution for 6 days (Figure 5b, lane 7). There was no DNA degradation in cortex cells of roots (1.50 cm from root apex) grown in soil (Figure 5b, lane 8) as well as the elongation zone of roots grown in soil (Figure 5b, lanes 8 to 9).

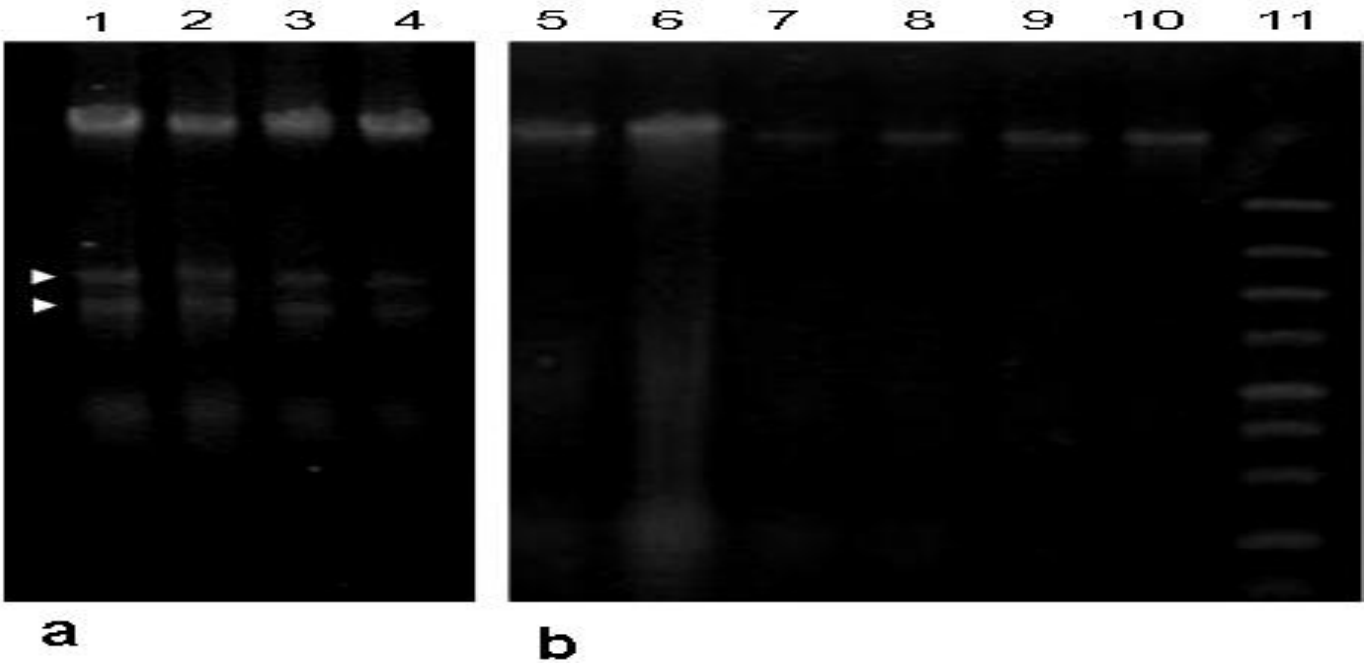

Figure 5. Fragmentation of genomic DNA in a 2% agarose gel where lanes 1 to 4 are DNA of roots (the elongation region) 60 days after cultured in M_3 in which clear DNA ladders (indicated by the arrow head) were observed; lane 5 is DNA of root tip formed in water after 60 days, DNA smearing was not detected; lane 6 is DNA from elongation region of primary roots 60 days after culture in M_3, DNA smearing was detected; lane 7 is DNA of primary root tip 6 days after culture in solution; lane 8 is DNA of root tip formed in soil, DNA smearing was not detected; lanes 9 to 10 are DNA from elongation region of roots grown in soil, DNA smearing was also not detected; and lane 11 is DNA marker.

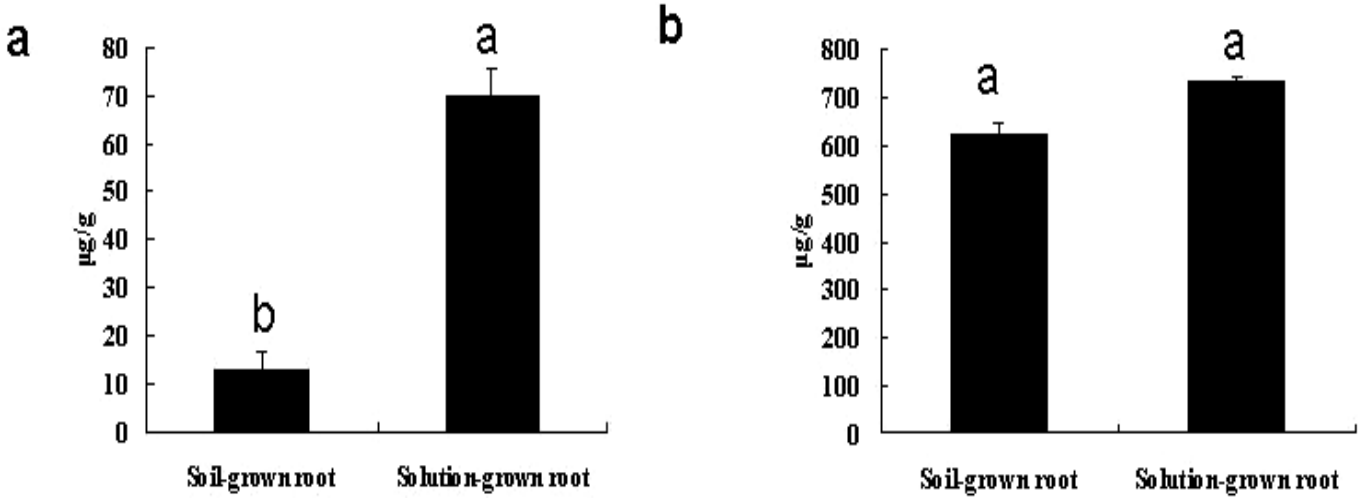

Figure 6. The content of free proline; a and protein; b in roots of Chinese aloe 60 days after culture in water and soil at 28°C. Different letters on the top of bars indicate significant difference at $P = 0.05$.

Free proline and protein contents of roots grown in water and in soil

Free proline content of Chinese aloe roots grown in both solution and soil was analyzed (Figure 6a). The proline content increased dramatically in roots grown in water and was five times higher than that of roots grown in soil. At the same time, the protein content of roots grown in water also increased compared to that of roots grown in soil (Figure 6b).

DISCUSSION

Aloes can be cultured in solution

This study showed that *A. vera* can be produced in solution culture even though it is a xerophyte and CAM plant species. Our results indicated that nutrient solution, plant variety, pH and temperature significantly affect the time of primary root appearance, root length and root numbers or root fresh weight. The fast rooting in tap water suggests that nutrient depletion promotes root initiation, which concurred to the observation reported by Forde and Lorenzo (2001). Supplementation of IBA did not promote root formation since there were no significant differences in the time of primary root appearance between IBA-treated and no IBA-treated solutions. Aloe root growth required temperatures at 20°C or higher due to its tropical origin. Additionally, acidic solution promote root initiation but subsequent growth required pH at 7.5 as root fresh weight produced in solution with pH 7.5 became similar to those at pH 5.5. Based on the results obtained from the three experiments conducted in this study, a solution culture system for aloes was proposed. Briefly, (1) Young aloes should be washed free of soil; and all roots should be removed, providing mechanical wounding for plants to adapt to aquatic solutions. (2) The aloes should first be cultured in M_4 at 28°C and pH of 5.5 for root initiation. (3) After root initiation, rooted aloes should be cultured in M_4 with pH of 7.5 until about five roots appear with a total root length of approximately 15 cm. (4) Plants then should be cultured in M_3 solution. (5) The nutrient solution (M_3) should be replaced monthly to promote healthy growth. High quality and high yield Chinese aloes were produced in our preliminary evaluation of this culture system (data not shown).

Adaptations of aloe to solution culture

Two mechanisms were identified when Chinese aloe plants were cultured in the aforementioned solution culture system: structurally, the formation of aerenchyma and physiologically the accumulation of free proline in roots. Aerenchyma is considered an efficient mechanism occurring in aquatic and semi-aquatic plants to ameliorate low oxygen stress as it facilitates gas exchange between aerial and submerged plant parts by reducing the diffusion resistance to gas exchange imposed by cells (Arunika et al., 2001; Steffens et al., 2011). Aerenchyma also occurs in nonaquatic plants, such as *Zea mays* (Drew, 1997; Drew et al., 2000 and Duan et al., 2010), *Spartina patern* (Burdick, 1989), *Luffa cylindrica* (Shimamura et al., 2007), and *Rumex palustris* (Laan et al., 1991; Pierik et al., 2009), but not all plants such as *Brassica napus* (Voesenek et al., 1999). Our study showed that *A. vera*, as a xerophyte with a CAM metabolic pathway, also produced aerenchyma for adaptation to oxygen stress in solution culture. Considering the fact that aloes have a strong capability to adapt to drought conditions, it is perplexing why aloes also develop aerenchyma for waterlogged stress. Zhu et al. (2010) reported that a corn inbred line with high root cortical aerenchyma produced higher shoot biomass and more seed when grown under drought conditions and proposed that the improved drought tolerance is associated with high aeremchyma formation.

It is unknown at present if drought and waterlogged stress produce some common strategies in plants in response to stressful conditions. Aerenchyma formation has been identified through at least two different processes: either schizogeny or lysigeny, or through a combination of the two processes (Drew, 1997; Drew et al., 2000; Gunawardena et al., 2001; Evans, 2003; Gladish et al., 2006). Schizogenous production of aerenchyma involves cell wall reorganization and cell separation, whereas lysigenous aerenchyma is formed as a consequence of programmed cell death (PCD) and cell wall autolysis (Drew, 1997; Gunawardena et al., 2001; Evans, 2003). In the present study, aerenchyma formation in aloe roots appeared to occur through the process of lysigeny as PCD was identified during aerenchyma formation. With PCD, a variety of other changes in cell structure followed the initial changes in nuclear structure. The degenerating cells had thinner primary cell walls and less electron-dense middle lamellae. Cells in the root tip cortex (first 1.50 cm of root) were without intercellular spaces, aerenchyma started developing 2 cm back from the root apex. Through PCD, initially formed primary roots gradually changed into adaptive roots characterized by the appearance of aerenchyma. DNA fragmentation also supported the observation that aerenchyma formation in aloe was through lysigeny process (Figure 5). Lysigeny is also a process for aerenchyma formation in *Arabidopsis*, rice, maize and soybean when challenged by low oxygen stress (Mühlenbock et al., 2007; Steffens et al., 2011; Yamauchi et al., 2011).

Another mechanism employed by Chinese aloe in adaptation to aquatic stress is the accumulation of free proline in roots. The free proline content in roots grown in solution was five times greater than that of roots grown in soil and also the protein content in roots grown in solution increased substantially compared to that found in roots grown in soil. Proline accumulation has been documented in plants exposed to high salinity, drought, heavy metals, cold, pathogen infection, UV irradiation as well as hypoxia (Verbruggen and Hermans, 2008). As proposed by Claussen (2005), there is a general positive correlation between stress pressure and proline accumulation. It is possible that waterlogged conditions invoke oxidative stress in aloes, resulting in the production of reactive oxygen species such as O_2^-, H_2O_2, and ^-OH. Proline, as a scavenger of reactive oxygen species, at an increased biosynthesis may reduce the

damage caused by reactive oxygen species. Taken together, this study showed that aloes can be produced in solution culture under protected environmental conditions. The formation of aerenchyma along with the elevated free proline accumulation could be mechanisms identified thus far in aloes for adaptation to aquatic stress. Nutrient solutions, solution pH and temperature can significantly affect root initiation and root growth. Further research on these factors influencing aloe yield is warranted before its commercial production in solution culture.

Abbreviations: DNA, Deoxyribonucleic acid; **CAM**, crassulacean acid metabolism; **IBA**, indole-3-butyric acid; **EDTA**, ethylene diamine tetraacetic acid; **PBS**, phosphate buffer saline; **UV**, ultraviolet; **PCD**, programmed cell death

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