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ORIGINAL PAPER

# In vitro sucrose concentration affects growth and acclimatization of *Alocasia amazonica* plantlets

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**Abstract** Plantlets of *Alocasia amazonica* were regenerated on the MS medium supplemented with different concentrations (0–9%) of sucrose. An absence of sucrose in the growth medium induced generation of leaves, however, it decreased multiplication. On contrary, sucrose supply of 6% or 9% enhanced multiplication but hampered photoautotrophic growth (generation of leaves). Increasing sucrose supply also increased sugars and starch content and number of stomata and decreased water potential and size of stomata during in vitro growth period. During ex vitro acclimatization, shoot length, root length, leaf number and root number of *Alocasia* plantlets grown with 3% sucrose, were found to be better among the other studied sucrose concentrations. Under ex vitro acclimatization, number of stomata, contents of various carbohydrates in the leaves were increased but size of stomata decreased with increasing sucrose supply during in vitro growth period. Moreover, water potential of leaves of plantlets, which have been grown with a sucrose concentration other than 3%, was decreased. During in vitro growth, net $CO_2$ assimilation rate ($P_N$), transpiration (E), stomatal conductance ($g_s$) and variable fluorescence to maximum fluorescence ratio (Fv/Fm) were unaffected, however, during acclimatization these were changed and maximum $P_N$, E, and $g_s$ were observed in the plantlets micropropagated with 3% sucrose. Fv/Fm was decreased severely in the plantlets micropropagated with 6% sucrose during acclimatization. Thus a sucrose concentration of 3% in the medium is appeared to be better among studied concentrations for both in vitro growth and ex vitro acclimatization of *A. amazonica* plantlets.

**Keywords** In vitro culture · Carbohydrates concentration · Stomatal development · Osmotic stress · Photosynthesis · Transpiration

**Abbreviations**

| | |
|---|---|
| BA | Benzyl adenine |
| DAA | Days after acclimatization |
| E | Transpiration |
| Fv/Fm | Variable fluorescence to maximum fluorescence ratio |
| $g_s$ | Stomatal conductance |
| PFD | Photon flux density |
| $P_N$ | Net $CO_2$ assimilation rate |
| RH | Relative humidity |

## Introduction

*Alocasia amazonica* (Araceae) commonly called 'Elephant's ear', is an evergreen ornamental perennial plant with a long rhizome and a short stem (Jo et al. 2008a). The cross hatch pattern of white lines over its shiny dark green foliage gives it an ornamental value. Alocasias are mainly propagated through corms and seeds, but the inefficiency of this conventional propagation method is major constraint in its commercial multiplication (Jo et al. 2008a). In vitro methods of propagation provide an alternative to meet the demand of the market. In vitro multiplication of Alocasias

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has been reported by few workers from axillary bud explants (Thao et al. 2003a, b; Jo et al. 2008a). Thao et al. (2003a) not only achieved high frequency shoot regeneration with 5 mg $l^{-1}$ benzyladenine (BA) on MS media using an initial axillary bud explant excised from corm of *A. amazonica* but also achieved rooting in the regenerated shoot in a hormone free MS medium besides 100% successful acclimatization potential. Moreover, Thao et al. (2003b) successfully induced tetraploidy in *A. michotitziana* through colchicine and oryzalin treatments. Adelberg and Toler (2004) reported better multiplication of *Alocasia* explant in thin film liquid media at low explant densities than on semi-solid agar media. They also observed that additional supply of sugar increased dry mass of *Alocasia* explants even at higher explant density. Greater growth in thin-film liquid culture of *Alocasia* is most probably due to easier accessibility of sugar in liquid compared to solid medium (Adelberg and Toler 2004). Jo et al. (2008a) indicated use of hydroponic bioreactor system for large scale rapid multiplication of *A. amazonica*. Moreover, Jo et al. (2008b) also reported the best growth and development of *A. amazonica* growing under 15 and 30 µmol $m^{-2}$ $s^{-1}$ photon flux densities and short (8/16 h) and equinoctial (12/12 h) photoperiods.

Plants growing under tissue culture conditions are semi-autotrophic (Hazarika 2003) and leaves formed during in vitro growth may never attain photosynthetic competence (Van Huylenbroeck and Debergh 1996). Moreover, plantlets growing under in vitro conditions have limited accessibility to $CO_2$ inside the vessel (Hazarika 2003), Therefore, sugar is supplemented as a carbon source to maintain an adequate supply of carbon source for in vitro multiplication and growth of plant cell, tissue and organs or whole plantlets. Supplementation of sucrose in growth medium meets the energy demands for growth and physiological function (Hazarika 2003). For tissue culture, workers generally use 3% sucrose in the medium as per recommendation of Murashige and Skoog (1962). Besides serving as energy source, it also provides the carbon precursors for structural and functional components (Marino et al. 1993). Supplementation of sugar to the culture media also helps in the maintenance of osmotic potential of cells and conservation of water (Hazarika 2003). The conservation of water is essentially important for ex vitro settlement of plants, because in vitro grown plants lack a well developed cuticle and epicuticular wax (water housekeeping system) (Van Huylenbroeck et al. 2000). Exogenous supply of sugar increases starch and sucrose reserves in micropropagated plants and could favor ex vitro acclimatization and speed up physiological adaptations (Pospíšilová et al. 1999). Nonetheless, addition of sugar to the culture media has been shown to be negatively correlated with growth (Kwa et al. 1995), photosynthesis (Hdider and Desjardins 1994; Serret et al. 1997; Hazarika 2003) and expression of enzymes of the carbon assimilatory pathway (Kilb et al. 1996). Likewise environmental variables (light quality (Heo et al. 2006a, b) and its intensity (Lee et al. 2007; Jo et al. 2008b)) and exogenous phytohormones (Puthur and Thomas 2004; Yeo et al. 2004) also play very crucial role in the growth, development and acclimatization of plantlets. Since information regarding the responses of *A. amazonica* plantlets to in vitro sucrose supply and its effect on ex vitro acclimatization are largely unexplored the purpose of the present sets of investigations attempts to determine how in vitro sucrose concentrations in the growth media affects in vitro and ex vitro growth and physiological responses during acclimatization of *A. amazonica* plantlets.

## Materials and methods

### Plant material and maintenance of culture

Apical buds from corms of *A. amazonica* hybrid (*A. lowii* × *A. sanderana*) were excised and washed under running tap water. The explants were surface disinfected with 70% (v/v) ethanol for 10 s followed by surface sterilization with 3% (v/v) sodium hypochlorite solution for about 30 min and rinsed thoroughly with sterilized distilled water. After repeated washing in sterile distilled water, the explants were inoculated in a culture tube containing 20 ml of MS (Murashige and Skoog 1962) semisolid medium (with macronutrients, micronutrients and vitamins according to basal MS medium composition) supplemented with 2.0 mg $l^{-1}$ benzyl adenine (BA), 3% (w/v) sucrose and 7.5 g $l^{-1}$ agar (Phyto Agar, Duchefa, Haarlem, The Netherlands) and adjusted to pH 5.8 with 1 M KOH, before autoclaving at 121°C and 103 kPa pressure for 15 min. After 5 weeks of culture, newly formed cormlets (0.5 cm) were separated and further sub-cultured in polypropylene growth vessels (107 mm × 107 mm × 97 mm, Osmotek, Israel) containing 50 ml MS basal medium supplemented with 30 g $l^{-1}$ sucrose and 2.0 mg $l^{-1}$ BA. This procedure was repeated for five times and the cormlets obtained in fifth subculture served as experimental material. Cultures were maintained under white fluorescent lamps (30 µmol $m^{-2}$ $s^{-1}$ photon flux density, PFD) at 25°C/18°C (day and night) and a 16 h photoperiod.

### Experimental treatments

Uniform cormlets (0.5 cm diameter) from those obtained in fifth subculture were selected, excised and inoculated into 900 ml transparent glass vessels containing 200 ml semi-solid MS basal medium (hormone free) having varying

concentration of sucrose viz. 0.0%, 3.0%, 6.0% and 9.0% (w/v). To provide air exchange gas-permeable micro-porous filters (Mill-Seal, Millipore, Tokyo; pore size 0.5 μm) were attached on the holes of lid of the culture vessels (Fig. 1a, b). Acclimatization experiment was performed in the green-house under mixed natural and artificial light. Plantlets at the end of 5 week culture were transplanted to a peat and perlite (3:1) substrate and placed in a green-house (maximum 50 μmol $m^{-2}$ $s^{-1}$ PFD, 25°C air temperature, 70 ± 5% RH, day–night regime 16 h light and 8 h darkness). For regulation of light condition during day the tiers of polyethylene shadings was used. In order to assess the acclimatization performance, different parameters were taken into accounts, viz. shoot length, corm size, fresh and dry weight, root length and number, leaf number, survival rate, number and diameter of stomata, sugars concentration, net photosynthesis rate, transpiration, and variable to maximum chlorophyll fluorescence, at day 0 and 30 days of acclimatization (DAA).

## Plantlets growth and leaf water potential

For various measurements and analysis, plantlets after being taken out from the culture vessels were removed off the culture media adhering to them. Shoot length including corm and root length excluding corm were measured with scale, corm diameter with vernier caliper and for fresh and dry matter weight plantlets were weighed before and after oven-drying at 70°C for 72 h. Proliferation (%) was calculated as per formula

$$\frac{\text{New plantlets or cormlets formed}}{\text{Total cormlets inoculated}} \times 100$$

Survival (%) was determined by counting alive and dead plantlets 30 DAA. Leaf water potential was measured only in the plantlets produced leaves using WP4 Dewpoint Potential Meter (Decagon Devices, Inc. Pullman WA, USA). Leaf discs (1.0 cm) were cut from leaves using a perforator with an inner diameter 1.0 cm. Leaf discs were placed in a disposable sample cup (Aqua-lab, Pullman, WA, USA) and water potential recorded after an hour of incubation.

## Stomatal observations

Stomatal observation was made only in the plantlets produced leaves. For this, leaves were cut into 3 × 3 mm sections and fixed in a formalin–acetic acid–ethyl alcohol

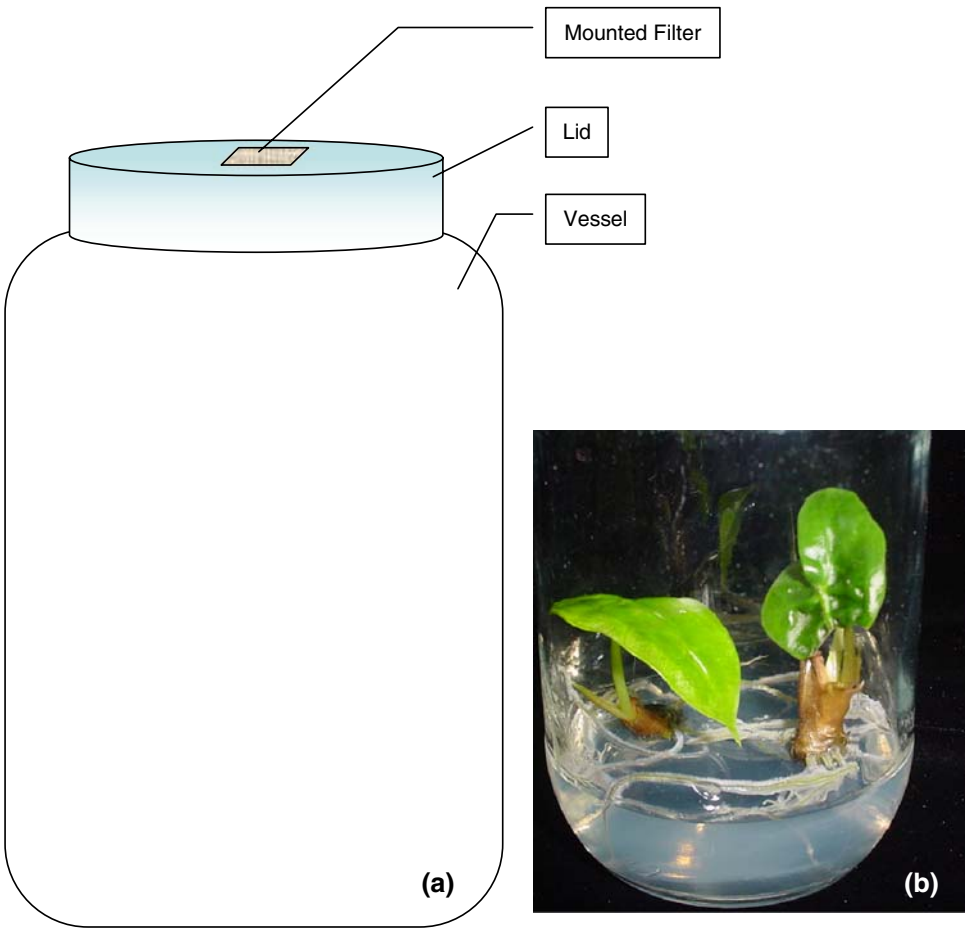


**Fig. 1** Diagrammatic representation of modified vessel (**a**) used for experimental purpose and *Alocasia amazonica* plantlets growing in the vessel (**b**) on the semisolid MS medium

(5:5:90) solution for 24 h. The leaf segments were washed three times with distilled water, stained for 15 min in a 0.01% (w/v) acridine orange and stained for another 15 min in a 0.01% (w/v) rhodamine. Finally they were thoroughly washed with distilled water before observation. Stomatal size and frequency were determined by microscopic examination of five leaf segments in a laser scanning confocal microscopic system (Bio-Rad MRC 1024 ES, UK) equipped with a Kr/Ar mixed gas laser (Bio-Rad MRC, UK). Samples were observed with a 20× dry objective lens (0.6–1.0 nuclear aperture, NA) by the method of Gray et al. (1999). The length and width of 50 stomata were measured and mean comparisons were made using the standard error of the mean.

### Estimations of sugars

Sugars were estimated by high performance liquid chromatography (HPLC) (Waters 600 s controller, Waters 626 pump, Waters C., Mildford, USA) equipped with refractive index detector (Refractometer Differential, Waters Co., Mildford, USA). Sugar and starch concentration were estimated only in the plantlets produced leaves. Leaves tissue (0.5 g) was homogenized in 3.0 ml of distilled water and homogenate was centrifuged at 4,000×*g* for 15 min. The supernatant was filtered through membrane filter (0.45 µm), and filtrate (20 µl) was injected in the HPLC carbohydrate column (300 × 7.8 mm Waters C., Mildford, USA). A mobile phase consisting of 75% (v/v) acetonitrile was used at a flow rate of 1.0 ml $min^{-1}$. The concentration of glucose, fructose and sucrose was quantified using Millennium 32 Chromatography, by determining peak area ratio based on corresponding sugar calibration values (Sigma Car-11 kit). Sucrose was not detected during acclimatization period. Results were expressed on the basis of percent fresh weight.

### Estimation of chlorophyll fluorescence, photosynthesis and transpiration

Chlorophyll fluorescence, photosynthesis and transpiration were measured only in the plantlets produced leaves. Chlorophyll fluorescence (Fv/Fm: variable and maximum fluorescence) was measured according to Kooten and Snel (1990) using a PAM chlorophyll fluorometer (PAM 2000, Heinz Walz, Effeltrich, Germany). Leaf samples were placed in the dark for 30 min followed by measurement of minimum fluorescence (Fo) at a 250 µmol $m^{-2}$ $s^{-1}$ PFD and maximal fluorescence (Fm) at a 2,400 µmol $m^{-2}$ $s^{-1}$ PFD under saturated pulse. Net photosynthesis ($P_N$), stomatal conductance ($g_s$) and transpiration (E) were measured using a portable photosynthesis system (LI-6400, LI-COR, Lincoln, NE). Photosynthetic measurements were made in the following conditions: $CO_2$ concentration of the in-flow air was at 400 µmol $mol^{-1}$, PFD was 100 µmol $m^{-2}$ $s^{-1}$ and RH 60%. Leaf temperature was 25°C.

### Statistical analysis

Experiments were set in a completely randomized design and there were 10 replications (n = 10) for each analysis (except those stated otherwise) and data were analyzed by analysis of variance (ANOVA) using the Statistical Analysis System (SAS, 1989) and tested for significance by Duncan's multiple range test (DMRT) at the 5% level.

## Results

### Effect of sucrose under in vitro conditions

An absence of sucrose in the growth medium induced formation of leaves, however, it hampered multiplication and increase in size of corm, fresh weight and dry weight of plantlets (Table 1). Supply of sucrose (above 3%) increased proliferation, corm size, fresh and dry weight and root numbers but decreased number of leaves formed (Table 1; Fig. 2a, b). Apart from these, sucrose supply also affected size and number of stomata of *A. amazonica* (Table 1). While number of stomata was increased, stomatal diameter and length were decreased with increasing sucrose supply (Table 1). Moreover, an enhanced supply of sucrose decreased leaf water potential (Fig. 3), and increased glucose, fructose, sucrose and starch concentrations in dose dependent manner in the leaves of *A. amazonica* (Fig. 4a). During in vitro conditions, supply of sucrose had no effect on Fv/Fm, net $CO_2$ assimilation rate ($P_N$), transpiration (E) and stomatal conductance ($g_s$) (Fig. 5a–d).

### Effect of in vitro sucrose supply on ex vitro acclimatization

Length of shoot and root, along with number of leaf and root of plantlets micropropagated with 3% sucrose were better compared to the plantlets raised from those propagated in sucrose free medium (0%) or even in the medium containing 6% or 9% (w/v) sucrose (Table 2). Corm size, biomass and number of stomata increased with increasing sucrose supply (Table 2; Fig. 2d). The size of stomata was found to be a maximum at 3% sucrose supply (Table 2). While leaf water potential was decreased with increasing sucrose supply under in vitro conditions (Fig. 3), under ex vitro, leaf water potential of plantlets grown in the medium containing sucrose concentration other than 3%

**Table 1** Effect of sucrose concentration on growth and stomatal attributes in the leaves of *Alocasia amazonica* plantlets at 60 days of in vitro culturing and 0 days after acclimatization

| Parameter (units) | Sucrose concentration (%) in in vitro growth medium | | | |
|---|---|---|---|---|
| | 0 | 3 | 6 | 9 |
| Shoot length (cm) | 4.1 a | 4.9 a | 3.0 b | 2.9 b |
| Root length (cm) | 2.1 c | 5.4 b | 5.6 ab | 6.0 a |
| Leaf numbers plant$^{-1}$ | 1.3 a | 1.0 ab | 0.3 bc | – |
| Roots numbers plant$^{-1}$ | 6.3 c | 9.0 b | 10.3 b | 13.0 a |
| Corm size (cm) | 0.51 c | 0.64 b | 0.78 a | 0.83 a |
| Fresh weight (g plant$^{-1}$) | 0.38 d | 0.49 bc | 0.56 b | 0.80 a |
| Dry weight (mg plant$^{-1}$) | 25.5 d | 62.9 c | 133.7 b | 226.3 a |
| Proliferation (%) | 0 a | 7.5 b | 35 c | 47.5 d |
| Number stomata (mm$^{-2}$) | 42.50 c | 84.00 b | 97.75 a | – |
| Diameter of stomata (μm) | 24.93 a | 22.14 b | 17.50 c | – |
| Length of stomata (μm) | 34.34 a | 30.88 b | 26.14 c | – |

The dash (–) in last column represents parameters not-detected

Data values are mean of replicates (n = 10). Values in the same row carrying different letters are significantly different at $P \leq 0.05$ by DMRT

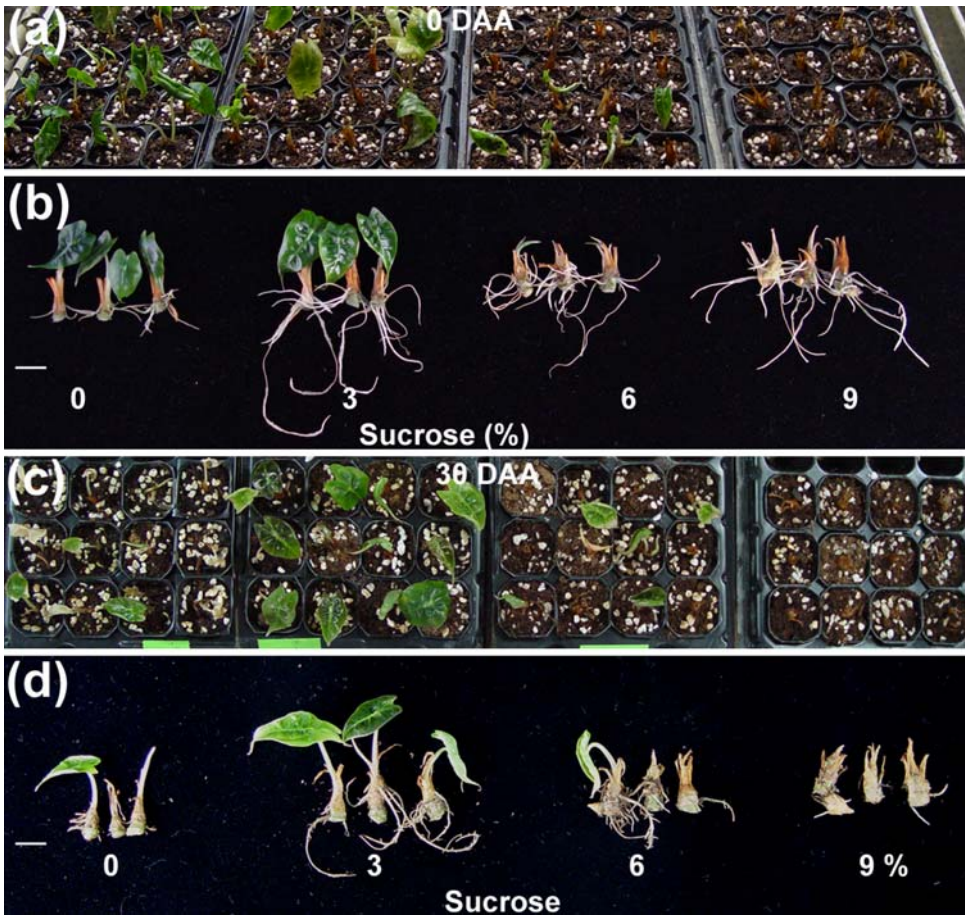


**Fig. 2** Effect of sucrose concentration (0%, 3%, 6% and 9%) on the growth of *Alocasia amazonica* plantlets at 0 days of acclimatization (**a**, **b**) and after 30 days of acclimatization (**c**, **d**). Bar = 2 cm

decreased (Fig. 3). Compared to in vitro, under ex vitro conditions, carbohydrates, except for starch, decreased in the leaves of *A. amazonica* plantlets (Fig. 4a, b) and a significant level of carbohydrates was maintained in the leaves of *A. amazonica* even after 30 DAA (Fig. 4b). During in vitro growth period, Fv/Fm (Fig. 5a) $P_N$ (Fig. 5b), E (Fig. 5c), and $g_s$ (Fig. 5d) remained unaffected, however, during ex vitro acclimatization maximum

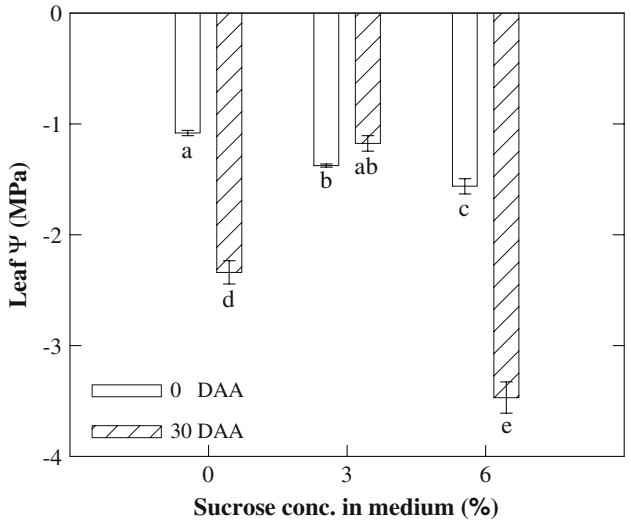


**Fig. 3** Effect of sucrose concentration on the water potential of leaves of *Alocasia amazonica* plantlets during in vitro (open bar, 0 days of acclimatization,) and ex vitro (hatched bar, 30 days of acclimatization) growth period in presence of 0%, 3%, 6% of sucrose in the growth medium. Data are mean ± SE of six replicates (n = 6). Bars with different letters are significantly different at $P \leq 0.05$ (DMRT) at an individual DAA

$P_N$ (Fig. 5b), E (Fig. 5c), and $g_s$ (Fig. 5d) were observed in the plantlets cultured with 3% sucrose. Fv/Fm decreased severely at 6% of sucrose supply during acclimatization (Fig. 5).

## Discussion

In this study, we observed the effect of sucrose concentration on the multiplication and growth of *A. amazonica* plantlets under in vitro and their subsequent acclimatization under ex vitro condition. An absence of sucrose induced generation of leaves, although, it hampered multiplication and decreased corm size, fresh weight and dry weight of plantlets. Sucrose supply of 6% or 9% increased multiplication, corm size, fresh weight, dry weight and root number; however, it decreased photoautotrophic growth (leaves). A similar reduction in the growth of plantlets was observed by Serret et al. (1997), when they incorporated sugar into the medium. It has previously been shown that decrease in sucrose concentration in the medium enhanced the photosynthetic ability of plantlets (Desjardins et al. 1995). Plantlets growing on the sucrose supplemented media exhibit reduced photosynthesis, probably, due to presence of sufficient energy source (sugars) for other metabolic activities (Rolland et al. 2002; Amiard et al. 2005). Another possible reason for slow growth of these plantlets might be impeded metabolic activities because of osmotic stress as indicated by decreased water potential.

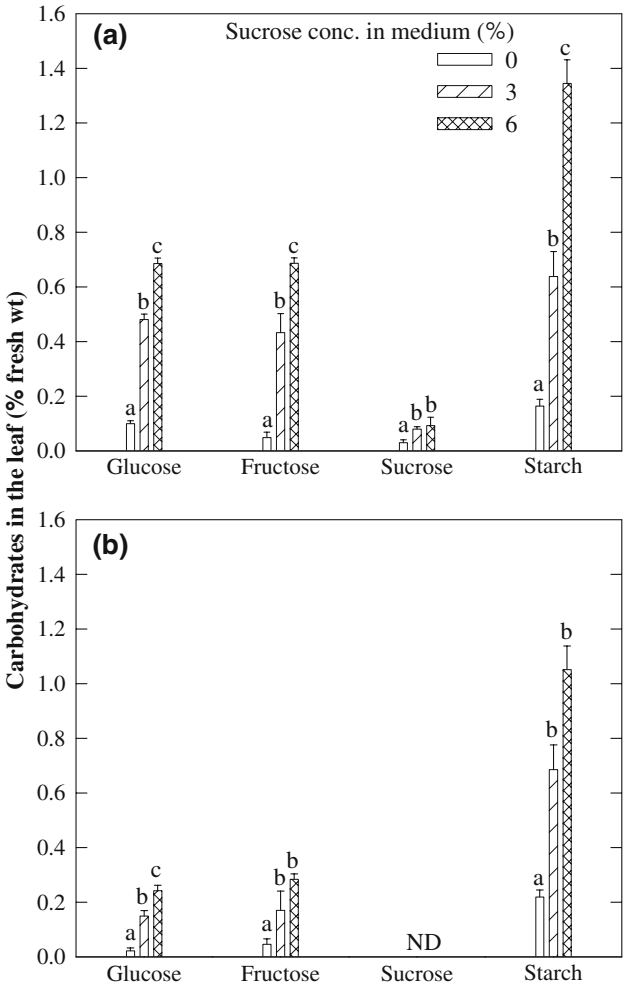


**Fig. 4** Effect of sucrose concentration on the various carbohydrates concentrations (% of fresh weight) in leaves of *Alocasia amazonica* plantlets during in vitro (**a**, 0 days of acclimatization, DAA) and ex vitro (**b**, 30 days of acclimatization, DAA) growth period in presence of 0% (*open bar*) 3% (*bar hatched to left*), 6% (*cross-hatched bar*) of sucrose in the growth medium. Data are mean ± SE of six replicates (n = 6). Bars with different letters are significantly different at $P \leq 0.05$ (DMRT) for an individual sugars

A decrease in the size and an increase in the number of stomata appear to be related with leaf expansion as supply of sugar delayed leaf expansion. Apart from these effects, an increase in sucrose supply also decreased leaf water potential and increased glucose, fructose, sucrose and starch content in a dose dependent manner in the leaf tissue of *A. amazonica*. Since, cytosolic status of sugars plays a very crucial role in the maintenance of osmotic potential of cytosol, the observed excessive accumulation of sugars might be responsible for lower water potential in these leaves.

When in vitro grown plantlets were transferred to ex vitro condition, best growth was observed in those plantlets which have been micropropagated with 3% sucrose.

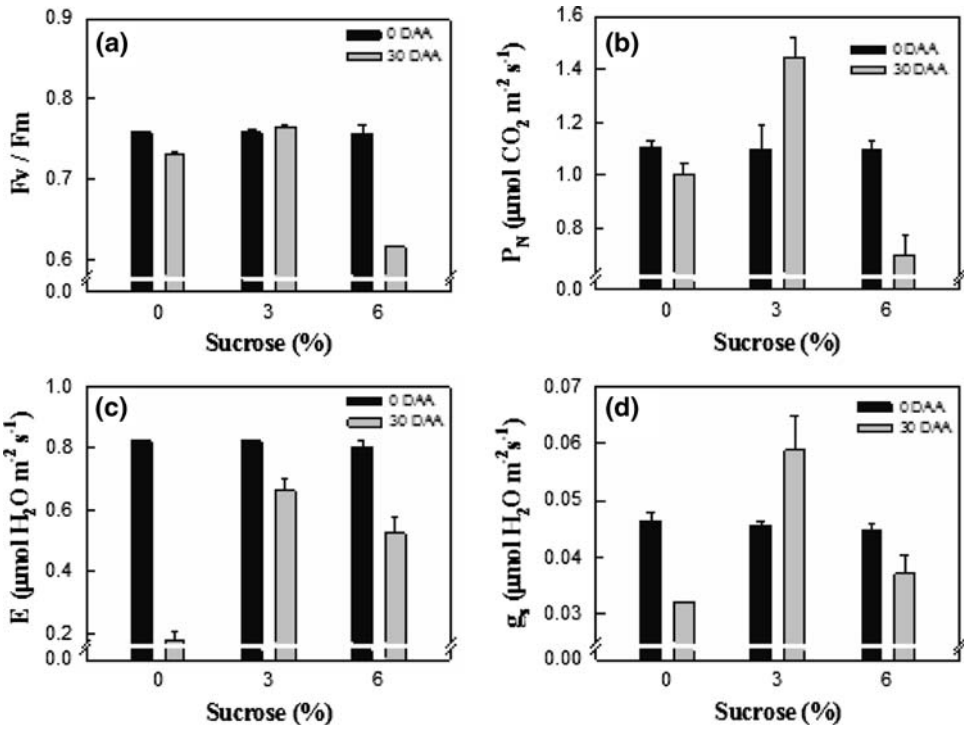


**Fig. 5** Effect of sucrose concentration on the chlorophyll fluorescence (Fv/Fm: variable and maximum fluorescence) (**a**), net photosynthesis (**b**), transpiration (**c**) and stomatal conductance (**d**) in the leaves of *Alocasia amazonica* plantlets during in vitro (0 days of acclimatization, black bar) and ex vitro (30 days of acclimatization, *gray bar*) growth period in presence of 0%, 3%, and 6% of sucrose in the growth medium. Data are mean ± SE of six replicates (n = 6)

**Table 2** Effect of sucrose concentration on ex vitro growth and stomatal attributes in the leaves of *Alocasia amazonica* plantlets at 30 days of acclimatization

| Parameter (units) | Sucrose concentration (%) in in vitro growth medium | | | |
|---|---|---|---|---|
| | 0 | 3 | 6 | 9 |
| Shoot length (cm) | 4.1 b | 5.9 a | 3.6 b | 3.1 b |
| Root length (cm) | 1.0 d | 5.1 a | 4.0 b | 1.7 c |
| Leaf numbers | 0.3 b | 1.0 a | 0.3 b | – |
| Roots numbers | 4.0 c | 8.0 a | 6.3 b | 5.7 b |
| Corm size (cm) | 0.59 c | 0.69 b | 0.72 b | 0.83 a |
| Fresh weight (g $plant^{-1}$) | 0.19 d | 0.52 b | 0.50 c | 0.56 a |
| Dry weight (mg $plant^{-1}$) | 16.9 c | 55.2 b | 58.4 b | 86.4 a |
| Survival rate (%) | 50 c | 85 a | 79 b | 79 b |
| Number stomata ($mm^{-2}$) | 69.75 b | 67.25 b | 92.50 a | – |
| Diameter of stomata (μm) | 21.01 b | 23.00 a | 19.36 c | – |
| Length of stomata (μm) | 24.32 c | 32.14 a | 28.38 b | – |

The dash (–) in last column represents parameters not-measured

Data values are mean of replicates (n = 10). Values in the same row carrying different letters are significantly different at $P \leq 0.05$ by DMRT

An increase in corm size was observed with an increase in the sucrose supply and thus corm appears to be major sink for carbohydrates in *A. amazonica*. Capellades et al. (1991) also observed increase in size and number of starch granules with increasing supply of sucrose in the culture medium. Biomass accumulation in the corms served as reserve energy source for sustenance. Moreover, an over-accumulation of sugars at higher sucrose supply might have induced osmotic stress and thus the corm showed little or no growth and remained alive as a dormant structure. A decrease in the number of roots in *A. amazonica* plantlets raised with 6% sucrose concentration, under ex vitro condition, suggests that these plantlets faced tremendous osmotic stress, which has been reflected as a decrease in water potential. A decrease in water potential of leaf of plantlets (those have been micropropagated with other than 3% sucrose) under

ex vitro conditions, suggests that plantlets grown in sucrose free or with 6% sucrose have undergone water stress. Increased number of stomata with increasing supply of sucrose suggests differentiation of stomata was also influenced by sucrose status of leaf tissue. Rooting is an energy requiring process thus an adequate level of carbon source is desired (Hazarika 2003; Zapata et al. 2003). A similar observation of root initiation was reported in apple (Lane 1978) and shoots without sucrose did not survive after transfer in the green house (ex vitro condition) (Zimmerman 1983). Decreased establishment of *Potentilla fruiticosa* and *Ficus lyrata* plantlets not conditioned with 2% or 4% sucrose was reported by Wainwright and Scrace (1989). Moreover, Langford and Wainwright (1987) found that sucrose supplied at a concentration of 3% in the medium increased the photosynthetic ability, thereby improving survival of plantlets**.** Increased photosynthetic ability and acclimatization of *Alocasia* plantlets micropropagated with 3% sucrose supply have further been supported by a relatively higher $P_N$, E, $g_s$ and variable to maximum fluorescence ratio (Fv/Fm). Decreased Fv/Fm at higher sucrose (6%) supply suggests poor acclimatization, photodamage of PS II apparatus and strong photoinhibition, which was also reflected by decreased net $CO_2$ assimilation rate ($P_N$). Down-regulation in above photosynthesis related parameters appear to be resulted from a lower sink demand of plants because of presence of enough sucrose in the plant system. It has been reported previously that photosynthesis is down-regulated in response to external sugar feeding of leaves or plants, inhibition of sucrose export resulting from overexpression of an apoplastic invertase, elevated $CO_2$ levels, and inhibition of export by cold-girdling petioles (Rolland et al. 2002; Amiard et al. 2005). Furthermore, Mehta et al. (2000) reported that sucrose supply of 6% induced browning of media, which appear to be detrimental for growth of the shoots. The present observation with *Alocasia* is similar to Van Huylenbroeck and Debergh (1996). They reported that plants micropropagated with 3% sucrose attained full photosynthetic capacity after 1 week of growth. Hazarika et al. (2000) have demonstrated that in vitro preconditioning of *Citrus* microshoots with 3% sucrose concentration is advantageous for ex vitro survival and acclimatization. Although, many authors reported that sugar supply is beneficial for acclimatization of plantlets, they did not provide exclusive data related with osmotic disturbance (water potential), concentration of various sugars, chlorophyll fluorescence (Fv/Fm), $P_N$, E, and $g_s$ in plantlets micropropagated on sucrose-less or high sucrose containing growth medium. On the basis of these experimental evidences we are suggesting that an adequate concentration of sugars appears to be important for the regulation of osmotic and energy balance and acclimatization of *A. amazonica* plantlets.

## Conclusions

A sucrose concentration of 3% in the medium is better among the studied concentration for both in vitro growth and ex vitro survival of *A. amazonica* plantlets. Absence or more than 3% of sucrose decreased survival of plantlets and also induced osmotic stress in *A. amazonica*.

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