

Full Length Research Paper

# Potato seed tuber production from *in vitro* and apical stem cutting under aeroponic system

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Low productivity of potato in Malawi is mainly due to lack of quality seed tuber coupled with the absence of a potato seed certification programme which leads to farmers achieving less than 7 t ha<sup>-1</sup> against potential yield of 40 t ha<sup>-1</sup>. With regards to this, an assessment of potato (*Solanum tuberosum* L) seed tuber production under aeroponics in Malawi was conducted in order to assess aeroponics as a system of producing minitubers in Malawi. *In vitro* plantlets and apical stem cuttings of three clones (CIP381381.13, CIP381381.20 and CIP395016.6) were used as source material for the aeroponic study in the greenhouse. A two factor factorial experiment arranged in a completely randomised design (CRD) with four replicates was laid out. Data collected included the following: Percentage plants survival to harvest, root length, plant height, number of minitubers per plant, date from transplanting to first tuberisation, number of harvests and tuber weights. Days to first tuberisation from both material sources was observed 28 days after transplanting. The results show that the *in vitro* plant material source yielded significantly better seed potato tuber numbers per plant (24.3) than apical stem cuttings (3.4) (p<0.05). Among the *in vitro* clones, CIP 381381.13 gave significantly higher tuber numbers (30.0) per plant as compared to the other clones. This indicates that *in vitro* plantlets have potential to give a viable material for seed potato tuber production under aeroponics.

**Key words:** Seed potato, aeroponics, apical stem cuttings, *in vitro* plantlets, *Solanum tuberosum*, Malawi.

## INTRODUCTION

As a major food crop, potato ranks fourth in terms of production as compared to cassava, sweet potato and maize in Malawi (Ministry of Agriculture and Food Security, Malawi, 2007). It is an important food and cash crop in Malawi with an average annual national production of 527,830 tons, giving an average yield of 7 tons/ha which is far much below the potential yield of 40 t ha<sup>-1</sup> (Soko, 2004). Potato seed production in Malawi is basically informal, with most cases recycling from previous crop harvest (Demo et al., 2006; Kagona, 2008; Saini, 2008). Selection of seed is based only on size, with small ones been preferred as they cannot sell at the markets. This contributes to disease build up as little or no phytosanitary

measures are taken into account leading to significant yield losses in the subsequent potato production.

Currently, the common method for propagation of commercially important potato cultivars is through tubers. However, this propagation method has encouraged accumulation of tissue borne viruses, fungi and bacteria in subsequent seasons. This has led to significant losses in yield and tuber quality over seasons (Nyende et al., 2005). Rooted apical stem cuttings are the easiest and cheapest means of propagating potato (Struik and Wiersema, 1999). They have faster regeneration potential and are true to type. Their ability to regenerate rapidly gives them great potential for conservation of potato clones and production of potato seed. If rooted apical stem cuttings originate from true to type and pathogen-free tubers, they serve as an efficient means of producing basic seed under strict management practices, such as

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**Table 1.** Nutrient solution formulation used in aeroponics.

Nutrient	Concentration (g/L)
Potassium nitrate	0.54
Ammonium nitrate	0.35
Calcium triple phosphate	0.61
Magnesium sulphate	0.44
Fetrilon Combi	0.012

Fetrilon Combi: micronutrient powder that has the following formulation: 9% magnesium, 3% sulphur, 4% iron, 4% manganese, 1.5% copper, 1.5% zinc, 0.5% boron and 0.1% molybdenum.

aeroponics (Novak et al., 1980; Naik and Karihaloo, 2007). Leaf and stem cuttings are highly appealing for fresh potato production due to their ability to escape soil-borne pathogens and the tendency of plantlets to produce few but large sized tubers (Struik and Wiersema, 1999). However, to supply healthy mother plants on a continuous basis using leaf and stem cuttings remain a challenge.

Tissue culture is used to mass propagate clonal materials in disease-free environments without the limitations of field propagation techniques of clonal planting material. *In vitro* plantlets are deemed to yield more under hydroponics and aeroponics but a wider use of tissue culture materials may be difficult if farmers are not familiar with growing techniques or if environmental conditions are not favourable for plant growth (Barak et al., 1996; Marissa et al., 2004).

Production of potato seed through aeroponics will promote seed availability of health potato seed. In addition, aeroponics allows easy identification and roguing of diseased plants. Furthermore, potato seed produced through this method could enjoy accelerated growth due to improved aeration of the roots and optimal nutrient uptake obtained from an atomized nutrient solution (Barak et al., 1996; Nichols, 2005). The objective of this study was to evaluate performance of selected potato clones derived from tissue culture and apical stem cuttings grown under aeroponics in order to establish their potential as a source of seed potato.

## MATERIALS AND METHODS

Tissue culture and apical stem cuttings of three potato clones from CIP Nairobi, Kenya regional centre; CIP 381381.20, CIP 381381.13 and CIP 395015.6 were used.

### Experimental site and design

#### Site

The aeroponics seed potato tuber production was conducted at Njuli Estate in Malawi (132 55' S 317 09' E; 1115 m above sea level) between the months of July and November, 2008. The annual rainfall in Njuli estate ranges from 1200 to 1300mm. Temperatures range between 13 and 35°C but may approach and surpass 38°C during October and November (Mkanda et al., 1995).

### Design

A two factor factorial experiment arranged in a completely randomized design (CRD) having four replications and twenty-four plots was used to evaluate the performance of clones obtained from tissue culture and apical stem cuttings under aeroponic system in the greenhouse. The plants were spaced at 25 cm between plants and 25 cm between rows. Each replicate contained twenty plants from each clone and the net plot contained five plants per clone.

### Plantlet and cuttings management

#### *In vitro* plantlets

Eighty *in vitro* plantlets from each clone were transplanted in wooden trays, 50 x 50 x 5 cm, containing well drained rooting substrate sand of less than 1 mm grain size for a period of two to three weeks to induce roots as well as acclimatization to the external environment and were spaced 5 cm between plants and 5 cm between grooves. During this period, the plants were watered with a nutrient solution (Table 1).

Before being transplanted to the aeroponics boxes, roots of the plantlets were washed with clean water to get rid of sand. Plantlets were then wrapped with a thin sponge around the plant crown and fitted into the holes of the Styrofoam. Each hole of the Styrofoam was covered with a black plastic to avoid light penetrating into the box, and to promote formation of shoots from stolons instead of tubers (Nugaliyadde et al., 2005).

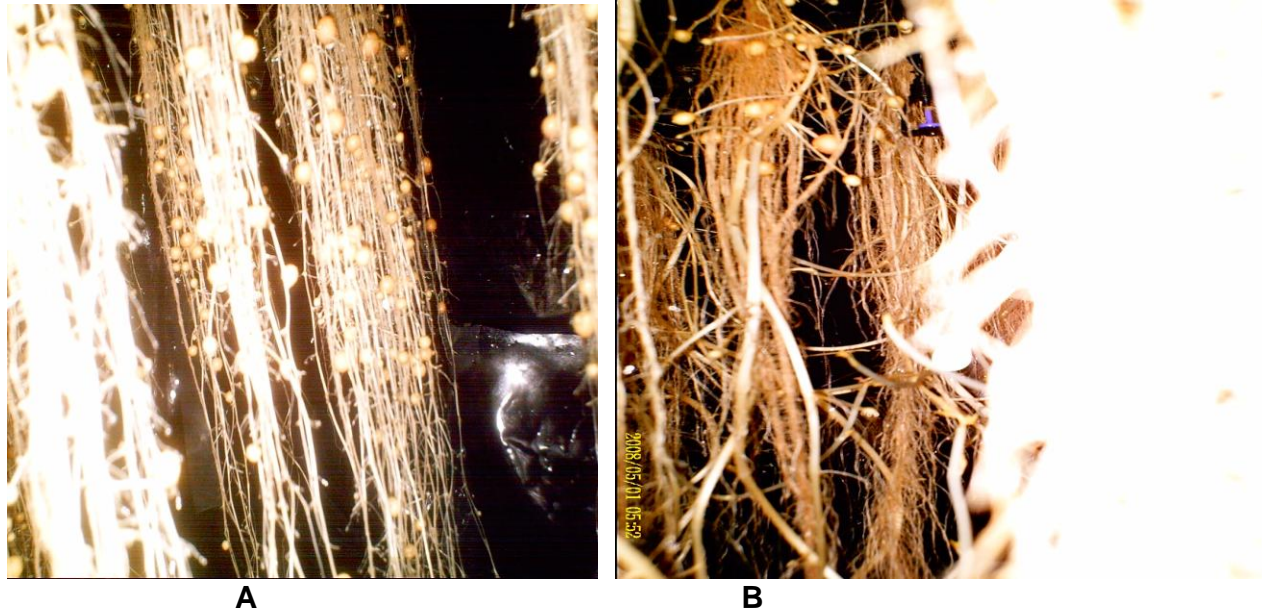
#### Apical stem cuttings

Tubers from clones CIP 381381.13, CIP 381381.20 and CIP 395015.6, were grown in pots to produce plantlets in a screen house for a period of 6 weeks. NPK (12:14:12) fertilizer was applied to the plantlets. At five to six leaf stage, apical stems were cut from the plantlets with a sterile surgical blade. The apical stem cutting consisted of at least one axial bud and two leaves. The apical stem cuttings were dipped in 1% indolebutyric acid (Agan Chemicals, Israel) and then rooted in wooden trays, 50 x 50 x 5 cm, containing well drained rooting substrate sand of less than 1 mm grain size for a period of three weeks, and were spaced at a square spacing of 5 x 5 cm. The apical stem cuttings were supplied with the nutrient solution (Table 1) at the time they were being rooted.

The apical stem cuttings were uprooted and washed with clean water to remove the sand that stuck on their roots. The uprooted cuttings were wrapped with a thin sponge around the plant crown and transplanted into the holes of the Styrofoam in the greenhouse. Black plastics were covered in each of the planting holes of the Styrofoam so as to avoid light penetrating into the spraying unit for efficient tuberisation of potato (Nugaliyadde et al., 2005).

#### The aeroponic system

The aeroponic system used for both the *in vitro* plantlets and apical stem cuttings was 7 m long, 1 m wide and 1 m high, giving a growth chamber of 7 m<sup>3</sup>. The system was equipped with pressure pump (KLB Engineering, South Africa), 400 g/L nutrient solution tank (Aero Plastics, Malawi), a spraying unit and a control panel with an automatic timer (Green Air Products, Oregon- USA). The growth chamber had a removable top cover having 2 cm diameter holes at a spacing of 25 x 25 cm to insert the plant. The spraying unit was placed at the bottom of the growth chamber (Figure 1). The inlet of this unit was connected to the water pump through a solenoid valve and the outlet to the nutrient solution tank to collect drop-out of the solution. The nozzles (Mazal Casting Company (Pty) Limited, South



**Figures 1.** Potato seed tuber production from *in vitro* (A) and apical stem cutting (B) under aeroponic system, respectively.

Africa) of the spray unit were fixed 60 cm equidistant apart to direct the nutrient solution towards the root zone at required time intervals. The timer which was connected to the pressure switch (Sunlight Supply, USA) and solenoid valve (American Hydroponics, Canada) was programmed to spray nutrient solution for 15 min and break for 15 min during the day time (8:00 am to 18:00 pm) and also spray for 15 min and break for 1 h during the night time (18:00 pm to 8:00 am) during plant growth. Electrical conductivity (EC) and the pH was monitored using EC meter and pH meter, respectively. The EC expressed in milliSiemens per centimeter (mS/cm) was never more than 1 mS/cm. The pH was maintained within the range of 6.8 and 7.1. The nutrient solution was replaced every month. During harvesting stage of mini-tubers, nutrient solution was changed every 20 days. A filter was fixed at the end of the draining pipe into the tank for retaining root pieces or other solids that could come off the system (glue, plastic, etc). The minimum and maximum temperatures during the experiment were recorded using minimum and maximum thermometer (Agason Ltd, Malawi), and was collected twice in a day, at 9:00 am and 2:00 pm.

#### Data collection and analysis

Data recorded included: Percentage plant survival at harvest, root length, plant height, days to first tuberisation, number of harvests and number of minitubers per plant. Root length was measured from the stem, suspended inside aeroponics box to the tip. Plant height was measured as the length from above the Styrofoam to the top tip. Days to first tuberisation was dated when at least 60% of sub plot had at least a tuber. Harvesting of minitubers started two months from the day of transplanting and then fortnightly, for both transplanted tissue culture and apical stem cuttings. Each time harvesting was done, harvesting sequence was recorded. Number of tubers per plant was counted after harvesting and the mean number of minitubers per plant was determined. Data was subjected to analysis of variance (ANOVA) to test the significance of the differences between treatments using Genstat Discovery Edition 3. Least significant difference (LCD) was used to separate the actual means that were significantly different (Genstat, 2009)

## RESULTS

### Plant survival

There was significantly ( $p < 0.05$ ) better survival rate from plants derived from apical stem cuttings than those derived from tissue culture under aeroponics conditions (Table 2). More than 90% of the plants derived from apical stem cuttings survived when transplanted into the aeroponics system as compared to 80% of the *in vitro* derived plants. No significant difference was observed between clones and similarly, and there was no significant difference ( $p < 0.05$ ) within clones obtained from apical stem cuttings in terms of plant survival rate under aeroponics (Table 3).

### Root length

Plants derived from apical stem cuttings gave significantly longer roots ( $p < 0.05$ ) as compared to those derived from tissue culture under aeroponics conditions (Table 2). However, there was no significant difference ( $p < 0.05$ ) within the clones from the same sources for both apical stem cuttings and tissue culture under aeroponics conditions (Table 2).

### Plant height

Apical stem cuttings grew significantly taller ( $p < 0.05$ ) than *in vitro* plantlets under aeroponics system of potato seed production (Table 2). However, there were no significant differences ( $p < 0.05$ ) between clones from *in vitro*

**Table 2.** Plant survival (%), root length (cm), plant height (cm), number of stolons, days to 1st tuberisation, number of harvests, number of tubers per plant and tuber weights (g) derived from *in vitro* plantlets and rooted apical stem cuttings.

Material source/clone	Plant survival (%)	Root length (cm)	Plant height (cm)	Number of stolons	Days to first tuberisation	Harvesting sequence	Mean tuber weight (g)	Mean number of tubers/ plant
<i>In vitro</i>								
CIP 381381.20	82.0	72.8	64.1	3.8	28.0	4.0	13.6	20.5
CIP 381381.13	81.3	71.5	64.1	8.5	31.3	4.0	14.3	30.0
CIP 395015.6	84.0	72.1	64.2	3.8	29.5	3.5	14.6	22.5
CIP 381381.20	91.5	116.0	142.6	2.0	28.8	1.3	11.9	3.8
CIP 381381.13	95.0	117.7	142.2	2.5	28.8	1.3	14.3	3.6
CIP 395015.6	98.3	119.0	143.6	2.3	28.8	1.0	13.6	2.9
Mean	88.7	94.8	103.5	3.8	29.2	2.5	13.5	13.9
LSD <sub>0.05</sub>	6.9	5.5	6.4	1.6	3.7	0.6	4.6	2.3

<sup>a</sup>Comparison of means by least significant difference at  $p>0.05$  within a column.

**Table 3.** Plant survival (%), root length (cm), plant height (cm), number of stolons, days to 1st tuberisation, harvesting sequence, tuber weights (g) and number of tubers/plant derived from *in vitro* plantlets under aeroponics conditions

Material source/clone	Plant Survival (%)	Root length (cm)	Plant height (cm)	Number of stolons	Days to first tuberisation	Harvesting Sequence	Mean tuber weight (g)	Mean number of tubers/plant
CIP 381381.20	82.0	72.8	64.1	3.8	28.0	4.0	13.6	20.5
CIP 381381.13	81.3	71.5	64.1	8.5	31.2	3.5	14.3	30.0
CIP 395015.6	84.0	72.1	64.2	3.7	29.5	4.0	14.6	22.5
Mean	82.4	72.1	64.1	5.3	29.6	3.8	14.3	24.3
LSD <sub>0.05</sub>	3.7	3	2.8	2.2	3.6	3.1	5.9	3.2

<sup>a</sup>Comparison of means by least significant difference at  $p<0.05$  within the column.

plantlets under aeroponics (Table 2). Similarly, there were no significant differences ( $p<0.05$ ) between clones obtained from apical stem cuttings in terms of height under aeroponics conditions.

### Number of stolons

Significant differences were observed between clones obtained from *in vitro* plantlets and apical stem cuttings in terms of development of stolons

under aeroponics conditions ( $p<0.05$ ). *In vitro* derived plants gave as high as 8 stolons against less than 3 stolons obtained from materials from rooted apical stem cuttings. *In vitro* plantlets gave significantly more stolon numbers ( $p<0.05$ ) than apical stem cuttings (Table 2). Amongst *in vitro* clones, CIP 381381.13 gave significantly higher stolon numbers (8.5) as compared to the other clones which gave less than 4 stolons. However, there were no significant differences ( $p<0.05$ ) between CIP 381381.20 and CIP 395015.6 (Table

3) in terms of stolon number under aeroponics conditions. On the other hand, there were no significant differences ( $p<0.05$ ) between clones obtained from apical stem cuttings in terms of stolon development under aeroponics (Table 2).

### Days to first tuberisation

There was no significant difference ( $p<0.05$ ) between the rooted apical stem cuttings and tissue

culture derived plants in terms of days to first tuberisation (Table 2). Similarly, there were no significant differences between clones within the same material sources ( $p < 0.05$ ) with respect to days to 1st tuberisation under aeroponics condition (Table 2).

### Harvesting sequence

There were highly significant differences between apical stem cuttings ( $p < 0.05$ ) and *in vitro* derived plants with regards to harvesting sequences (Table 2). On average, 4 harvests were realised from *in vitro* derived plants against a mean harvest of 1 realised from apical stem cuttings derived plants under aeroponics conditions. The *in vitro* derived plants gave significantly higher number of harvests than apical stem cuttings ( $p < 0.05$ ). Among the *in vitro* derived plants, there were no significant differences between the clones with respect to harvesting sequences (Table 3).

### Number of tubers per plant

There were significant differences between number of tubers ( $p < 0.05$ ) from *in vitro* derived plants and those from apical stem cuttings under aeroponics conditions (Table 2; Figure 1A and B). *In vitro* derived plant materials gave more than 24 tubers per plant as compared to less than 4 tubers per plant obtained from apical stem cutting derived plants under aeroponics conditions. Within the *in vitro* derived clones, there were significant differences between mean number of minitubers per plant from the clones ( $p < 0.05$ ). CIP 381381.13 gave significantly higher mean number of minitubers per plant (30.0) than CIP 381381.20 and CIP 395015.6 (Table 3). There were no significant ( $p < 0.05$ ) differences in mean number of minitubers per plant between CIP 381381.20 and CIP 395015.6 which gave 20.5 and 22.5 tubers per plant, respectively. Comparing the apical cuttings derived plants, there were no significant mean minituber numbers per plant between the clones (Table 2). The tuber number per plant for apical stem cutting materials ranged from 2.9 to 3.8.

### Tuber weight

There were no significant tuber weight (g) differences between minitubers produced from *in vitro* and rooted apical stem cuttings derived plants under aeroponics (Table 2). Similarly, there were no significant differences in mean tuber weights (g) within the clones of the same material source, that is, *in vitro* and rooted apical stem cuttings across the clones under aeroponics (Table 2).

## DISCUSSION

Plants derived from apical stem cuttings survived better

than those derived from tissue culture (Table 2). The results concur with those reported by Otazu (2007) and Otrshy (2006). This could be because apical stem cuttings derived plants were already acclimatised to an external environment in the screen house

Furthermore, apical stem cuttings derived plants gave higher mean height and root length as compared to those plants derived from tissue culture (Table 2). The increased biomass in plants derived from apical stem cuttings may have led to competition between tubers and leaves, in terms of sucrose unloading, with a bias towards the leaves, resulting in reduced tuber yield. These findings are also in line with those reported by Otazu (2007), who showed that *in vitro* plantlets yield more tubers than convention material sources when produced under aeroponics and hydroponics. Furthermore, the residual phytohormones from tissue culture contributed to significant differences in mean tuber number (Rolot and Seutin, 1999). The significant difference between the stolon numbers (Table 2) under *in vitro* and apical stem cuttings had a remarkable influence on the mean number of tubers obtained in the end. Stolons provide a structure where tuberisation occurs (Stallknecht and Farnsworth, 1982; Demo et al., 2006).

The first step in the formation of tubers is stolon formation (Struik and Wiersema, 1999). A stolon goes through different phases to become a tuber bearing stolon. These stages include: Induction, initiation, rapid growth and branching, cessation of longitudinal growth and swelling. Stolons within one plant or stem are not synchronised as far as these phases are concerned (Hussey and Stacey, 1981). The fewer stolon numbers observed in apical stem cuttings derived plants, could explain the reduced tuber numbers under apical stem cuttings. In this study, the low number of stolons corresponded to an increase in above ground biomass. This relationship could be due to competition for sucrose unloading between the storage organs (minitubers) and the above ground biomass (leaves and stems). Such competition for assimilates favours leaves which largely use the sucrose for respiration (Rykczevska, 2004; Jackson, 1999). Similarly, the number of stolons per stem declines with increasing stem branching. This relation is caused by the amount of assimilates available below ground growth (Rolot and Seutin, 1999).

Aeroponics provides room for sequential harvests as well as extended harvest period per plant that were realised (Manrique, 2000; Ranalli, 2007). At least three harvests were realised over a period of two months under *in vitro* derived plants. Plants in aeroponics system show secondary growth of stolons (Struik and Wiersema, 1999). In aeroponics, removal of tubers by repeated harvesting contributed to increase in tuber formation as it resulted in breaking apical dominance and hence increased yield through promotion of lateral tuber formation inside the aeroponics chamber (Otazu, 2007). Nevertheless, the low mean tuber numbers obtained from the apical stem

cuttings derived plants under aeroponics is slightly higher if the same apical stem cuttings are left to grow under sandy soils as they will only yield one tuber per plant (Otazu, 2007).

In comparing clones derived *in vitro* (Table 2), there were significant differences between tuber yields per plant between clones ( $p < 0.05$ ). CIP 381381.13 yielded more tubers than CIP 381381.20 and CIP 395015.6. There were no significant differences in terms of tuber yield between CIP 381381.20 and CIP 395015.6 ( $p < 0.05$ ). The results followed the same trend when comparison between mean stolon numbers per plant were analysed. Stolons provided a structure where tuberisation occurs (Demo et al., 2006). CIP 381381.13 had significantly higher mean stolon numbers as compared to other CIP clones ( $p < 0.05$ ) and thus giving significantly better yield than the rest of the clones. The results obtained under aeroponics concur with those obtained by Demo et al. (2006), who reported that when CIP 381381.13 is subjected to field conditions, it gives comparatively more stolons than CIP 381381.20 and CIP 395015.16 and consequently more tuber yield, with stolon numbers per plant being cultivar specific and also affected by environmental growing conditions as well as system of seed tuber production such as *in vivo* and *in vitro* (Rappaport et al., 1957; Hammes and Nel, 1975; Manrique, 2000; Otazu, 2007).

However, the mean yield of 24.33 tubers per plant is below the potential tuber yield per plant under aeroponics as reported by Stoner and Clawson (1998) that over 70 tubers per plant can be achieved. Temperature is by far the most critical factor for potato in the tropics (Rannali, 1997; Manrique, 2000). The adverse effects of temperature are magnified as growth proceeds below or above the critical range for plant growth (15 to 24°C). High temperatures adversely affect plant growth and tuber yield at least in three ways: through an overall reduction in growth, apparently in similar fashion to that produced by water stress; through a reduction in the photosynthetic rate and stomatal resistance and an increase in dark respiration; through a reduction in rapid tuber enlargement and maturity. These ways sum up and similarly promote flower production by reducing bud abortion, delay tuber initiation and reduces enlargement, and also delays senescence (Malagamba, 1983). Consequently, high temperature (day/night: 32/20°C) stresses during tuberisation cause yield reduction of over 50% (Ryckaczewska, 2004). The mean day temperatures experienced at the time of tuberisation (between February and March) was above 34°C.

## Conclusion

Furthermore, *in vitro* plant derived plants yielded more tubers than apical stem cuttings derived plants under aeroponics conditions. CIP 381381.13 gave significantly

higher tuber yield (30.0) than CIP 381381.20 (20.0) and CIP 395015.6. (22.5). Similarly, CIP 381381.13 gave significantly more stolon numbers than the other two clones used in the experiment. There were no significant differences in seed tuber weight between the two material sources and even within the clones. These results indicate that aeroponics has promise in improving potato seed production in Malawi.

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