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Development of an *in vitro* Culture Procedure using Seeds from *Zantedeschia aethiopica* 'Green Goddess' as Explants

In vitro Vermehrung von *Zantedeschia aethiopica* 'Green Goddess' mit Samen als Primärexplantat

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Abstract

Experiments were carried out to optimise the medium for the *in vitro* culture of *Z. aethiopica* 'Green Goddess' using seeds as explant and to promote multiple shoot development on germinating seeds. The germination on *in vitro* medium took place without problem. Optimal temperature, medium composition and cultural conditions for germination and for the further culture were investigated. Germination took place earliest at 20 °C, but highest germination rates occurred at 14 °C. Delayed germination and lower germination rates were observed on medium containing high agar concentration than on liquid medium or sand. Use of Agar-Roth led to the browning and death of resulting seedlings. On the other hand, shoots on medium gelled with Gelrite were unaffected. High sucrose levels also detrimentally affected germination.

High concentrations of BAP were required for seedlings to be stimulated into branching and to multiple shoot formation. Shoot formation occurred more frequently after direct sowing on phytohormone containing medium than after sowing on phytohormone free medium followed by reculture on phytohormone containing medium. Seedling decapitation resulted in increased number of axillary shoots.

Zusammenfassung

Ziel der Untersuchungen war, ein Verfahren für die *in vitro* Kultur mit Samen als Explantat zu entwickeln und die multiple Sproßbildung an Sämlingen zu fördern. Die Keimung auf *in-vitro*-Medium erfolgte problemlos. Optimale Mediumzusammensetzung, Temperatur- und Kulturbedingungen zur Keimung und zur weiteren Kultur wurden untersucht. Die Keimung erfolgte am frühesten bei 20 °C, jedoch trat der höchste Prozentsatz Keimung bei 14 °C auf. Verzögerte Keimung und niedrigere Keimrate wurden auf dem Medium mit hoher Agar-Konzentration beobachtet, im Vergleich zu flüssigem Medium oder in Sand. Agar als Medium führte zur Verbräunung und zum Absterben der resultierenden Sämlinge, Sprosse auf Gelrite-verfestigtem Medium blieben dagegen gesund. Eine hohe Zuckerkonzentration wirkte sich negativ auf die Keimung aus.

Hohe Konzentrationen von BAP waren nötig, um die Sämlinge zur Verzweigung und zur multiplen Sproßbildung anzuregen. Sproßbildung trat nach Direktaussaat auf phytohormonhaltigem Medium häufiger auf als nach Aussaat auf phytohormonfreiem Medium und nachfolgender Subkultur auf phytohormonhaltigem Medium. Entspitzen der Sämlinge führte zu einer vermehrten Anzahl an Seitentrieben.

Introduction

The propagation of *Zantedeschia aethiopica* 'Green Goddess' from seeds results in plants showing great variability in plant height, flowering period and quality. The *in vitro* culture using seeds as explant provides an opportunity of selecting for a particular characteristic e.g. early flowering, salt or temperature tolerance etc. for the development of improved clones. This is based on the hypothesis that early germination at unfavourable conditions such as low temperature or high salt levels may be linked to tolerance to these conditions (ZIMMER and BAHNEMANN, 1982). The selection of seeds germinating early may thus attain tolerant individuals. Multiple shoot development would realize rapid multiplication of the selected genotypes. The population built up would then be used for the verification of tolerance of the selected genotypes. This may enable relatively rapid production of clones as compared to conventional methods due to difficulties of crossing and the long generation time required. Multiple shoot formation on seeds is a phenomenon in which seeds germinating on an *in vitro* nutrient medium under hormone influence give rise to several shoots in some cases. This has been observed on several ornamental species such as *Anthurium scherzerianum* (SCHAPER and ZIMMER, 1989; ZENS and ZIMMER, 1986; ZIMMER and BAHNEMANN, 1982), "atmospheric" Tillandsias (ZIMMER *et al.*, 1993), 'Victoria' Cyclamen (ZIMMER and BUCHWALD, 1989) and *Dodecatheon meadia* L. (BEßLER, 1993). ISZAKE and IZHAR (1987) attained reduction in the breeding cycle in *Fragaria* through this methodology.

For *in vitro* selection and subsequent cloning of germinating seeds, the germination medium used should give a high germination rate and high production as well as optimum growth of regenerated shoots (FISCHER-KLÜVER, 1994). The *in vitro* culture of *Zantedeschia* seeds for the induction of multiple shoot formation has not yet been reported and therefore optimization of medium for germination is an important

prerequisite. The objective was therefore to develop a method of *in vitro* culture of *Z. aethiopica* 'Green Goddess' using seeds as explant and to promote multiple shoot development on germinating seeds

Materials and methods

Temperature effect

In order to determine the suitable temperature for *in vitro* culture using seeds as explant, seeds of early and late flowering varieties were cultured at temperatures of 14, 17, 20 and 23 °C in either phytohormone-free or medium containing 0,1 mg/l KNA (Potassium-α-naphthylacetate) and 1,0 mg/l BAP (Benzylaminopurine). Unless otherwise stated the following procedures were used throughout. Seeds were first washed for 30s in deionised water containing a drop of 'Tween 20' and rinsed prior to washing in 70 % alcohol for 30 seconds before treatment with a 4 % NaOCl (Sodium hypochlorite, a.i. 12 % Chlorox) solution for 5 minutes. Thereafter, treated seeds were transferred to a 1/2 MS (MURASHIGE and SKOOG, 1962) medium with microelements according KOCH (1974) in sterile glass culture vessels supplemented with 25 g/l sucrose, 30 g/l Fetrilon and 12 g/l Agar (Roth). The pH was set at 5,6. 25 ml aliquots of nutrient media were dispensed using a Kipp pipette into 150 ml glass culture vessels with "Twist Off" screw tops and autoclaved using a Stericovapoklav steam sterilisor at 121 °C (1.1 bar) for 20 minutes. Each treatment consisted of 200 seeds in 2 replications of 100 seeds each. The vessels were thereafter transferred to germination chambers with 16 h photoperiods using fluorescent lamps (Phillips, 36 W/83) at the respective treatment temperature.

As criteria for germination the parameters mean germination time and germination rate (%) were recorded. The mean germination time was calculated according to PIEPER (1909).

Agar concentration

As the germination on *in vitro* medium was not yet satisfactory, tests involving the germination on medium containing various agar concentrations as well as sand medium were undertaken. *Zantedeschia* seeds were cultured on medium gelled with either 12 g/l or 6 g/l Roth-Agar, in liquid media (rotary shaker 120 rotations/ minute) or sand containing either 1/2 MS macro elements "with salts" or without "without salts". A factorial combination of the phytohormones KNA (0 or 0,1 mg/l) and BAP (0 or 1,0 mg/l) was also included. Each treatment consisted of 200 seeds in 2 replications of 100 seeds each. Treatments were placed in a germination chamber at 20 °C.

Effect of sucrose, fetrilon (Iron) concentration and macroelement composition

To further improve the germination on *in vitro* medium, an experiment was conducted to compare the influence of sucrose, Fetrilon (Iron) and macroelement composition on the germination. 200 seeds/ treatment in 2 replications of 100 seeds were sown on either 1/2 MS or Knudson C medium supplemented with combinations of either 0, 15 or 30 g/l sucrose and 0, 15 or 25 mg/l Fetrilon and solidified using 6 g/l Agar (Roth).

Effect of Gelling substance and MS macroelement concentration on seedling quality

Shoots obtained after *in vitro* germination were cultured on MS media at full, half or quarter strength gelled with different types of agar; Roth, (Carl Roth GmbH), Serva, (Serva Feinbiochemica), Bacto-agar (Difco Laboratories) or Gelrite (Gellam gum) and supplemented with 15 g/l sucrose and 15 mg/l Fetrilon. Each treatment consisted of 15 shoots and the material was cultured for 16 weeks and subcultured every 4 weeks on the same medium. The shoot appearance was classified by visual colour rating, with 1 for green shoots and 5 for completely brown shoots.

Induction of multiple shoot formation

Tests for induction of multiple shoots on seeds sown on phytohormone containing medium

Tests were conducted for the induction of multiple shoots from seeds cultured *in vitro* on phytohormone containing medium. 200 seeds in 2 replications of 100 seeds each were sown in a medium containing 1/2 MS macroelements supplemented with 6 g/l agar (Roth), 15 mg/l Fetrilon, 15 g/l sucrose and with the phytohormones KNA at 0, 0,2, 0,4, 0,8 and BAP at 1,0 mg/l and 0, 0,5, 1,0 and 2,0 mg/l in factorial combination. Culture vessels were placed at 20 °C and 16 h photoperiods.

Culture at increasing levels of BAP

As the levels of BAP used to this point did not lead to production of numerous shoots that could enable rapid multiplication *in vitro*, new experiments were conducted in which higher levels of BAP were utilised. Seeds were sown on 1/2 MS medium supplemented with 15 mg/l Fetrilon, 15 g/l sucrose and 2,0 g/l Gelrite and containing BAP at 0, 5, 10, 15, 20, 25 and 30 mg/l. Additionally seeds were germinated initially on a phytohormone-free medium prior to transfer to the BAP treatments above. 50 seeds were used for each treatment. After completion of germination, seedlings were recultured in the same medium and observed for side shoot production.

Decapitation

Tests were also conducted to determine the potential of decapitation of shoots in order to increase multiplication. Seven-week-old seedlings totalling 147 previously germinated on a phytohormone-free medium were decapitated and cultured on 1/2 MS medium containing 1,0 mg/l BAP supplemented with 15 mg/l Fetrilon, 15 g/l sucrose and 2,0 g/l Gelrite. Care was taken to ensure that the apical meristem was completely removed. After 5 weeks, the number of shoots produced was determined.

Data analysis was conducted using SPSS Windows version 8.00 (11.May 1998) SPSS Inc., 1989–1997. Mean separation was conducted using the Tukey's HSD Test. Percent values were transformed using the arcsine transformation, while very small values were transformed using the $\log_{(x+1)}$ transformation before analysis.

Results

Germination of seed

Temperature effect

Seeds of the early flowering variety germinated significantly earlier than the late variety (Table 1). The earliest germination took place at 20 °C. Lowering the temperature to 14 °C or raising to 23 °C delayed germination. On the other hand, the culture temperature significantly influenced the germination rate. At temperatures of 14 and 17 °C significantly higher germination percentage was obtained than at 20 or 23 °C for both early and late flowering varieties. At 23 °C the germination percentage was significantly lower than at 20 °C. The germination rate was significantly higher on medium without phytohormone. Inclusion of hormones in the medium delayed germination regardless of temperature or seed variety, but the difference was not significant.

Agar concentration

The agar concentration influenced the mean germination time (Table 2). Seeds germinated significantly later in 12 g/l agar as compared to either 6 g/l agar, liquid medium or control. Germination was also significantly delayed on inclusion of 6 g/l agar as compared to liquid and sand medium. Inclusion of nutrient salts in the medium also significantly delayed germination. Inclusion of phytohormones in the medium did not influence the mean germination time significantly. The percent germination at 60 days was similarly influenced by the treatments.

Seedlings germinating in agar containing medium were observed to develop whitish spots or ring-like patterns which led to browning and to necrosis of the leaves at later stages and shoot growth was detrimentally affected.

Effect of sucrose, fetrilon (Iron) concentration and macroelement source

Increasing sucrose concentrations led to significant reduction in the germination percentage obtained after 60 days (Table 3). Seeds on medium without sucrose attained the highest percent germination. The germination level on medium containing 30 g/l sucrose was significantly lower than at 0 g/l and 15 g/l sucrose. The Fetrilon content and macroelement composition did not significantly influence the germination level. In contrast, the mean germination time was significantly influenced by Fetrilon content, sucrose concentration and macroelement composition. Seeds sown on medium containing 0 g/l sucrose germinated significantly earlier than those on 15 and 30 g/l sucrose and those at 15 g/l sucrose significantly earlier than at 30 g/l sucrose. The mean germination time was significantly affected by Fetrilon (iron) levels. Germination occurred significantly earlier following addition of Fetrilon. Germination took place significantly earlier on medium containing 1/2 MS macroelements than on Knudson macroelements.

Table 1. Influence of seed variety and temperature on the *in vitro* germination of *Z. aethiopica* 'Green Goddess'
Der Einfluß der Sorte und Temperatur auf die in vitro Keimung von Z. aethiopica 'Green Goddess'
ELF = Early Flowering Variety, LFV = Late Flowering Variety

		Temperature					
Parameter	Seed type	14 °C	17 °C	20 °C	23 °C	Mean	HSD
Percent germination after 60 days[r]	EFV	66,0 d	61,0 cd	43,0 c	24,0 b	48,5 b	
	LFV	13,0 ab	15,0 ab	8,0 a	5,0 a	10,3 a	
	Mean	39,5 c	39,0 c	25,5 b	14,5 a		
Mean germination time (days)	EFV	36	31	22	27	29 a	
	LFV	36	35	38	36	36 b	
	Mean	36	33	30	32		20

Effects of phytohormones are summarized, *Phytohormonbehandlungen zusammengefasst.*

Table 2. Influence of agar and nutrient salts on the in vitro germination of *Z. aethiopica* 'Green Goddess'
Der Einfluß von Agar und Nährsalzen auf die in vitro Keimung von Z. aethiopica 'Green Goddess'

		Agar concentration					
Parameter	Salt content	12 g/l	6 g/l	0 g/l (liquid)	0 g/l (sand)	Mean	HSD[c]
Percent germination after 60 days[r]	without salts	11,8 a	97,0 c	99,0 c	98,8 c	76,6 b	
	with salts	28,3 b	39,5 b	98,4 c		55,4 a	
	Mean	20,0 a	68,3 b	98,7 c	98,8 c		
Mean germination time (days)	without salts	29	17	12	15	18 a	
	with salts	31	31	20		27 b	
	Mean	30 c	24 b	16 a	15 a		6,9

Phytohormone combinations are summarized, *Phytohormonbehandlungen zusammengefasst.*

Table 3. Influence of sucrose and macroelement composition on the in vitro germination of *Z. aethiopica* 'Green Goddess'
Der Einfluß von Saccharose und Makroelement-Zusammensetzung auf die in vitro Keimung von Z. aethiopica 'Green Goddess'

		Sucrose concentration				
	Medium	0 g/l	15 g/l	30 g/l	Mean	HSD^c
Percent germination (after 60 days)^r	1/2 MS	98,7 b	96,3 b	66,7 a	87,2	
	Knudsons C	97,3 b	93,3 b	63,7 a	84,8	
	Mean	98,0 b	94,8 b	65,2 a		
Mean germination time (days)	1/2 MS	14	20	25	20 a	
	Knudsons C	15	22	28	22 b	
	Mean	14 a	21 b	27 c		2,3

Effects of Fetrilon treatments summarized, *Fetrilon-Behandlungen zusammengefasst.*

Tables 1, 2 and 3; Mean separation according to Tukey's $HSD_{(0,05)}$. Mean of 2 replications of 100 seeds each. Regressionanalysis on arcsine transformed values. Means of main effects bearing a common letter in respective row or column are not significantly different at $HSD_{(0,05)}$.
Tabelle 1, 2 und 3; Mittelwertsvergleich nach Tukey's $HSD_{(0,05)}$. Mittelwerte von 2 Replicationen mit jeweils 100 Samen. Regressionsanalyse an winkel-transformierten Werten. Mittelwerte der Hauptwirkungen mit gleichem Buchstabe in jeweiliger Zeile bzw. Spalte sind nicht signifikant abweichend bei $HSD_{(0,05)}$.

Table 4. Influence of gelling agent and MS concentration on the in-vitro growth of *Z. aethiopica* 'Green Goddess' seedlings
Der Einfluß von Geliermittel und MS-Konzentration auf das in-vitro-Wachstum von Z. aethiopica 'Green Goddess' Sämlingen

		Gelling agent					
Parameter	MS level	Agar Roth	Agar Serva	Difco-Bacto	Gelrite	Mean	HSD^c
Leaf appearance	1 MS	4,8	2,2	2,3	2,4	2,9 b	
(visual colour rating)	1/2 MS	4,1	2,0	2,0	1,1	2,3 a	
1 = Green	1/4 MS	4,8	2,5	3,0	1,3	2,9 b	1,0
5 = Completely brown	Mean	4,6 d	2,2 b	2,4 b	1,6 a		
Shoot weight (mg)	1 MS	696	993	898	779	841 b	
	1/2 MS	557	812	732	826	732 a	
	1/4 MS	491	646	675	905	639 a	
	Mean	581 a	817 b	768 b	836 b		183
Root weight (mg)	1 MS	359	598	577	357	473 b	
	1/2 MS	189	283	253	289	253 a	
	1/4 MS	130	249	338	385	276 a	
	Mean	226 a	377 b	389 b	344 b		169

Mean separation according to Tukey's $HSD_{(0,05)}$. n = 15. Means of main effects bearing a common letter in respective row or column are not significantly different at $HSD_{(0,05)}$. HSD^c = HSD for combinations.
Mittelwertsvergleich nach Tukey's $HSD_{(0,05)}$. n = 15. Mittelwerte der Hauptwirkungen mit gleichem Buchstabe in jeweiliger Zeile bzw. Spalte sind nicht signifikant abweichend bei $HSD_{(0,05)}$. HSD^c = HSD für Kombinationen

Influence of Gelling substance and MS macroelement concentration on the quality of seedlings

The type of gelling agent and MS macroelement concentration significantly influenced the leaf appearance as measured by visual colour rating (Table 4). Leaf browning was lowest in Gelrite gelled medium, attaining significantly "better" scores than the other gelling agents. There was no significant difference in leaf appearance following the use of agar (Serva) and Bactoagar, while agar (Roth) gave significantly "worse" leaf appearance scores than the other gelling agents. The leaf appearance was best on 1/2 MS, giving significantly better scores than on full or 1/4 MS. On the other hand, the gelling agent and MS concentration also significantly influenced the shoot fresh weight. Use of agar (Roth) resulted in significantly lower shoot fresh weight of than the other gelling agents. A full MS concentration resulted in significantly higher shoot fresh weight than 1/2 and 1/4 MS macroelement concentration. Similar results were obtained on root weight.

Induction of multiple shoot formation

Tests for induction of multiple shoots on seeds sown on phytohormone containing medium

Application of BAP and KNA at different levels and combinations generally resulted in a slight delay of germination and reduction in the germination percentage (data not shown). However, the differences were not significant. Germinating seeds predominantly produced single shoots (Fig 1). The number of seeds producing more than one shoot after 60 days was negligible taking place in only 6% of the seedlings (data not shown). Moreover, increasing BAP levels resulted in the inhibition of root growth and led to swelling at the base of the seedlings (Fig 1).

With increasing BAP concentrations, in several instances, germinating seeds darkened and died shortly thereafter. The production of side shoots was observed to occur after a prolonged period of growth on *in vitro* medium. The number of side shoots per seedling after 22 weeks of culture was still low regardless of BAP lev-

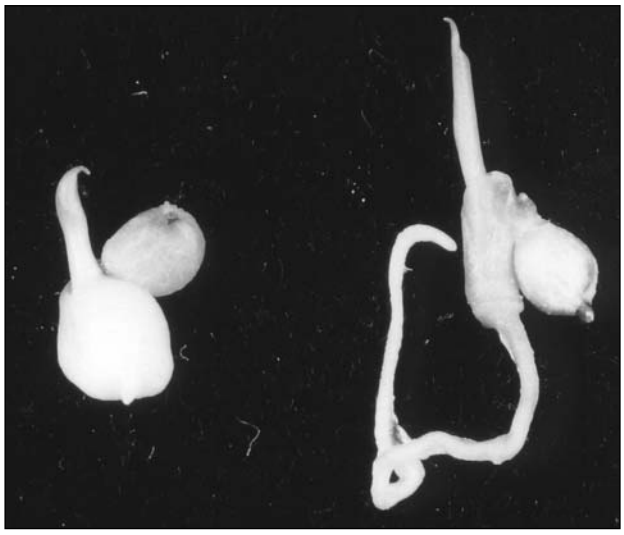


Fig 1. *Zantedeschia aethiopica* 'Green Goddess' germination in vitro. Left, 1,0 mg/l BAP; Right, no phytohormone
Zantedeschia aethiopica 'Green Goddess' Keimung in vitro. Links, 1,0 mg/l BAP; Rechts, Kein Phytohormon

el. However, over the next several weeks, the production of side shoots increased, such that after 36 weeks of culture, the number of side shoots per seedling were considerable (Table 5). In seedlings sown directly on phytohormone containing medium, the number of side shoots produced increased with increasing BAP levels until 20 mg/l after which it declined. Typical multiple shoot formation was observed on a number of shoots, resulting in the formation of up to 18 shoots/plant. Direct sowing on phytohormone containing medium resulted in significantly higher side shoot number than when seeds initially germinated in phytohormone-free medium were transferred onto phytohormone containing medium. The number of shoots bearing 10 or more side shoots was about 5%, showing this to be a rare characteristic.

Decapitation

More than 65% of the shoots produced 5-10 shoots per seedling on decapitation. The seedlings producing more than 5 shoots were separated and recultured into fresh medium. After further 4-6 weeks the side shoots were also decapitated to induce further side shoot production. The cycle could be repeated several times, producing similar numbers of shoots at each cycle. A decapitation and reculture cycle over a period of 13 months could yield about 1555 shoots arising from a single seedling. During the multiplication period, callus development was observed to arise in several clones. This took place usually at the cut edge of shoots after separation and subculture into new medium. The callus developed into a clump that could be separated and subcultured into new medium enabling a very rapid multiplication. At later stages, shoots arose from this clump, which developed into normal shoots. Through callus development, very high multiplication rates could be attained *in vitro*. In one case 211 shoots were obtained within 3 months. These shoots rooted without any problem and showed no malformation on transfer to greenhouse. However, only few seedlings formed callus. Of the 147 decapitated shoots, callus formation was only observed in 13 instances.

Discussion

Z. aethiopica 'Green Goddess' germinated *in vitro* over a wide range of temperatures, but the earliest germination was attained at 20 °C. This is within the range of 18 –22 °C recommended for greenhouse sowing (VON HENTIG and HEIMANN, 1973). At lower temperatures of 14 °C, germination was delayed greatly. This is because lower temperatures retard the rate of metabolic activities. Raising the temperature to 23 °C led to lower germination.

Higher germination rates obtained with lower agar content, liquid medium or sand revealed that, high agar content detrimentally affected seed germination. Agar may form a compact substrate, impeding the absorption of water and nutrients by the seeds. GEORGE (1993) reported that the decrease in water potential that results from the matrix potential of agar, limits ion uptake, affecting rate of water influx into tissues.

Increased sucrose concentration significantly reduced germination percentage and mean germination time. High levels of sucrose increased the medium osmotic potential, thereby restricting the absorption of water by the seeds and therefore delaying the germination. The osmotic potential of a nutrient medium is the sum of osmotic potentials of the agar and other

Table 5. Influence of BAP concentration on the mean side shoot number in *Z. aethiopica* 'Green Goddess'
Der Einfluß von BAP-Konzentration auf die Anzahl Seitentriebe in Z. aethiopica 'Green Goddess'

		Phytohormone							
Parameter	Shoots	0 mg/l	5 mg/l	10 mg/l	15 mg/l	20 mg/l	25 mg/l	30 mg/l	Mean
Side shoot no.	Direct	1,1	1,2	1,1	1,1	1,1	1,3	1,3	1,2
(22 weeks)[r]	Pre-germinated	1,0	1,2	1,2	1,1	1,1	1,1	1,2	1,1
	Mean	1,1 a	1,2 ab	1,2 ab	1,1 a	1,1 a	1,2 ab	1,3 b	
Side shoot no.	Direct	1,1 a	3,3 e	3,4 e	5,3 g	5,5 g	3,6 de	2,1 b	3,5 b
(36 weeks)[r]	Pre-germinated	1,1 a	4,0 f	2,7 cd	2,6 c	2,1 b	1,8 b	1,8 b	2,3 a
	Mean	1,1 a	3,7 d	3,1 c	4,0 d	3,8 cd	2,7 b	2,0 b	

Mean separation according to Tukey's $HSD_{(0,05)}$. n = 50. [r] Analysis on $\log_{(x+1)}$ transformed values. Means of main effects bearing a common letter in respective row or column are not significantly different at $HSD_{(0,05)}$.
Mittelwertsvergleich nach Tukey's $HSD_{(0,05)}$. n = 50. [r] Analyse an $\log_{(x+1)}$ transformierten Werten. Mittelwerte der Hauptwirkungen mit gleichem Buchstabe in jeweiliger Zeile bzw. Spalte sind nicht signifikant abweichend bei $HSD_{(0,05)}$.

constituents such as minerals and sugars, with sugars having a higher relative influence than macro-salts (PIERIK, 1997). PIERIK and STEEGMAN (1975) reported that at an osmotic potential of 3 bar, growth and organ formation ceased due to cessation of water uptake.

Use of Gelrite led to the best performance, giving the highest *in vitro* shoot fresh weight and the least browning of shoots than Difco-Bacto, Roth or Serva agar. PODWYSZYNSKA (1992, 1994) and PODWYSZYNSKA and OLSZEWSKI (1995) observed stronger growth of Araceae species e.g. *Homalomena* sp. on Gelrite medium compared to agar medium while shoots of many rose cultivars on agar solidified media showed symptoms of chlorosis, shoot tip necrosis and leaf yellowing. The influence of agar brand on cultures is probably due to different impurities, rigidity of gel and varying availability of water, ions and cytokinins (PODWYSZYNSKA and OLSZEWSKI, 1995). SCHERER (1988) reported higher water availability in Gelrite than in agar due to more lightly bound water resulting from the more elastic gels. On the other hand, better *in vitro* growth and regeneration and lower incidence of vitrification has been observed on agar than in Gelrite (HAUSER *et al*., 1995; PASQUALETTO *et al*., 1986).

Increasing concentration of MS macroelements improved shoot growth. HAUSER *et al.* (1995) also reported distinctly greater shoot formation on leaf explants of *Pelargonium domesticum* on full strength than on 1/2 MS medium. The concentration of MS macroelements did not affect *in vitro* seedling growth in Gelrite medium, indicating that in Gelrite nutrient availability and therefore favourable shoot growth could be attained at a lower MS concentration.

Inclusion of BAP into the medium was important for the outgrowth of lateral buds and the formation of multiple shoots. However, high levels of 15–20 BAP mg/l were required and only few seedlings produced true multiple shoots. At lower levels of BAP little or no side shoot development was observed possibly due to high levels of apical dominance present in the shoots. BAP at higher concentration induced multiple shoots in almond, chestnut and Soya beans, while lower concentrations were required for cucumber, pumpkin and melon seeds and none were formed in eggplants and tomatoes (HISAJIMA, 1981). HISAJIMA (1982) observed that at lower levels of BAP lateral buds developed, while typical multiple shoots were observed in high level of BAP.

Z. aethiopica 'Green Goddess' apparently requires very high levels of BAP to overcome the apical dominance. Lateral shoot production and multiple shoots appeared several weeks after germination. This may be due to the formation of lateral buds on the developing rhizome located at the base of the seedlings. HUSSEY (1976) obtained secondary axillary branching at higher BAP concentrations evidently formed as apical dominance was progressively reduced in both the main shoot and released laterals. Considerable time is also required for the induction of buds in some other plants. In another Aroid, *Spathiphyllum*, the initiation period was 3 months on buds and stem pieces and 6 months on inflorescence explants (FONNESBECH and FONNESBECH, 1979). Multiple shoot formation on germinating seeds has been reported in other Aroids. *A. scherzerianum* seeds grown on 0,1 mg KNA and 1,0 mg BAP resulted in multiple shoot formation, leading to 2000 plantlets in 15 months (ZIMMER and BAHNEMANN, 1982). ZENS and ZIMMER (1988a) obtained shoot regeneration on *A. scherzerianum* seeds on *in vitro* medium after 6 weeks. In another Aroid, *Philodendron selloum,* callus was observed at the base of 90,5 % of shoots, but no multiple shoot formation (FISCHER and ZIMMER, 1987).

The production of side shoots was greater when seeds were directly cultured in phytohormone containing medium than when first germinated in phytohormone-free medium. This may be due to the higher accumulation of phytohormones in the shoots of seeds directly sown on phytohormone containing medium. It is therefore recommendable to sow seeds directly in phytohormone containing medium.

High levels of BAP also led to root inhibition. HUSSEY (1976) also observed root inhibition following BAP application on various monocots; *Gladiolus*, *Iris*, *Sparaxis* and *Schizostylis*. ZENS and ZIMMER (1988b) also observed stagnation of root growth in *A. scherzerianum*. Root induction is normally promoted by auxin. High levels of BAP thus increase the cytokinin to auxin ratio, consequently hindering the root growth.

Decapitation of shoot tips resulted in increased lateral shoot production. This may be due to the apical dominance being overcome following the removal of the apex, thus enabling the outgrowth of the lateral meristems. Similarly, decapitation of seedlings in egg plants (*Solanum melongena* L.) and tomato (*Lycopersicon esculentum* L.) resulted in the development of 3–30 shoots within 6 weeks on MS medium (FÁRI *et al*., 1991). IZSAK and IZHAR (1983) obtained 5–10 shoots from strawberry seeds germinated on *in vitro* medium. Decapitation of shoot tips could thus be used for shoot multiplication in *Z. aethiopica* 'Green Goddess' with greater consistency.

On the other hand, division and separation of shoots resulted in callus formation in some shoots, this taking place in about 10 % of the decapitated shoots. This enabled a more rapid *in vitro* multiplication of 'Green Goddess'. However, callus formation may result in somaclonal variation (e.g. LÖRZ, 1984). But plants regenerated from the callus tissue obtained did not show any differing characteristics on cultivation in greenhouse conditions. ZENS and ZIMMER (1988b) similarly reported that clones of *A. scherzerianum* developed from callus material displayed uniformity in greenhouse conditions. They concluded that the inclination to somaclonal variation was low in *A. scherzerianum*. The same could be the case with *Z. aethiopica* 'Green Goddess'.

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