Selection for early flowering, temperature and salt tolerance of Zantedeschia aethiopica 'Green Goddess'

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Selection for Early Flowering, Temperature and Salt Tolerance of *Zantedeschia aethiopica* 'Green Goddess'

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Abstract

***Zantedeschia*, an important cut flower that shows high variability in plant height, flowering period and other characteristics when grown from seeds. Availability of variability, however, provides opportunity for the selection for such characteristics as early flowering, temperature or salt tolerance. In-vitro germination may be utilized in the selection for early germination under unfavourable conditions and selected seedlings rapidly multiplied by multiple shoot formation. This study described the selection procedures using early germinating seeds of *Zantedeschia* 'Green Goddess' on their flowering time, temperature and salt tolerance. The seeds were sown on in-vitro Murashige and Skoog (MS) medium at 20°C and 16h light; early germinating seeds transferred singly to new medium. For temperature tolerance, seeds were sown on media maintained at 10, 15 and 20°C. For salt tolerance, seeds were sown on medium containing 0, 40, 80, 120, 160 and 200 mmol/L NaCl. The selected seedlings were then multiplied in-vitro to develop clones, which were tested for flowering, temperature tolerance and salt tolerance against clones which germinated later. The results showed that clones, which were selected from early-germinated seedlings, grew faster, flowered earlier and produced more flowers than clones that germinated later. Early germinated seedlings at low temperatures achieved greater growth at lower night temperatures than those germinated at high temperatures. Clones selected after germination at higher levels of sodium chloride (NaCl) attained greater growth on media containing salt than those that germinated on salt free medium.**

INTRODUCTION

Zantedeschia aethiopica 'Green Goddess' grown from seeds are variable, and this variability provides an opportunity to select desirable characteristics such as early flowering and salt or temperature tolerance. The possibility of clonal production using in vitro germinated seeds on which multiple shoots formation has enabled rapid production of new and genetically improved clones (Zens and Zimmer, 1988). As soon as sufficient material is available, various tests on the suitability and horticultural value of the clones can be conducted. Zens and Zimmer (1988) used in vitro culture techniques to develop clone varieties of *Anthurium scherzerianum*. Schaper and Zimmer (1991) obtained clones of *A. scherzerianum*, which differed substantially from each other in their flowering behaviour.

High medium salinity has been observed to cause high incidence of plant death in *Zantedeschia* (Funnell, 1993). Cell and tissue culture techniques have been utilised in the development of salt-tolerant lines through somaclonal variation (Ashraf, 1994). Stepwise subjection of callus cultures to increased levels of NaCl has been used to isolate salt tolerant lines of *Arachis hypogaea* (Sarin et al., 1991), *Vitis vinifera* (Lebrun et al., 1985), *Brassica napus* and *Brassica campestris* (Chandler et al., 1988). Due to the genetic variability in a seedling population, seeds could be used for the selection for salt tolerance in vitro, being directly sown in salt containing media in vitro and early germinated seeds selected for cloning and evaluation.

The objective of the study was to develop new cultivars derived from clones *Z. aethiopica* 'Green Goddess' that are early flowering, temperature and salt tolerant through

selection of seeds germinating early on in vitro medium under unfavourable conditions of temperature and salt, and their rapid multiplication in vitro by multiple shoot formation.

MATERIALS AND METHODS

Development of Early Flowering Clones

One hundred seeds of *Z. aethiopica* 'Green Goddess' were washed in de-ionized water containing a drop of Tween 20, washed in 70% alcohol for 30 seconds, and then sterilized for 5 min in a 5% NaOCl solution (sodium hypochlorite a.i. 12% Cl, Carl Roth GmbH). Sterilized seeds were washed twice using autoclaved de-ionised water before initiating culture on a hormone-free ½ MS (Murashige and Skoog, 1962) medium at 20°C and 16h photoperiod as described (Ngamau, 2001b). Early germinating seeds were selected and transferred singly into new ½ MS medium containing 1.0 mg/L benzyl-aminopurine (BAP, Serva) for further development. Plants were subcultured every 5 weeks into new medium. Following decapitation and separation of shoots, clones were multiplied.

Germination was 82%. The first one third germinated seedling were categorized as early, the second as medium and the third as late germinating After securing a minimum of 80 uniform plantlets/clone, clones were selected for the in vivo tests. Four clones A15, B19, B33 and D3, selected from early germinating seedlings, were tested against E2, a clone from late germinated seedlings, and F2, plants grown from 6-month old rhizomes. The selected clones were rooted on ½ MS medium containing 0.4 mg/L of potassium-α-naphthylacetate (KNA, K & K Laboratories) for 3 weeks and transplanted in 10 cm diameter pots in commercial potting media (Einheitserde Typ T Topferde, A. Stangenberg GmbH) and acclimatized for 4 weeks in the greenhouse under polythene-covered tent. Each treatment consisted of 20 plants and they were completely randomized between selected clones. Flowering begun after about 6 months after transfer to the greenhouse and at flowering, parameters observed included; flower number/plant, plant height, flower emergence (visible spathe), flowering (flower opening), spathe length, scape length, number of visible side shoots, developed side shoots and side shoot height.

Development of Temperature Tolerant Clones

One hundred seeds of *Z.* 'Green Goddess' were sown at 10, 15 and 20°C (control) on phytohormone-free media described above. Early germinating seeds (30-40) were selected, coded according to germination temperature and germination time, re-cultured singly onto ½ MS medium containing 1.0 mg/L BAP and transferred to culture conditions described above for multiplication. Resulting shoots were separated and re-cultured every 4 or 5 weeks, with selected clones further multiplied to obtain a minimum of 120 plantlets per clone before rooting and acclimatization as above.

Clones A8, B17 that germinated early at 10°C and 15°C, respectively, and 24°C and 20°C were selected and transferred to the greenhouse in 14 cm diameter pots. The plants were placed in a daylight chamber of a greenhouse set at a minimum day temperature of 20°C between 8h to 16h. Light levels averaged 55 $\mu mol.m^{-2}.s^{-1}$ in the daylight chamber. They were moved in the evenings to dark chambers at night temperature levels of 6, 10, 14 and 18°C on movable carts. Ten plants per treatment were withdrawn 0, 7, 14 and 21 weeks for the measurement of parameters.

Data collected included: number of shoots produced, plant height, leaf length and width, shoot fresh weight, shoot dry weight, number of side shoots, root fresh weight, root dry weight, root length and number of flowers.

Salt Tolerance

Seeds were cultured on ½ MS medium containing 0, 40, 80, 120, 160 and 200 mmol/L NaCl and coded according to the NaCl level with codes beginning with the numbers 1, 2, 3, 4, 5 and 6 corresponding to 0 (Control), 40, 80, 120, 160 and 200 mmol/L NaCl. Seedlings were handled as describe above and clones developed from the

seedlings selected above were cultured on media containing 40, 80 or 120 mmol/L NaCl to test for salt tolerance. The fresh weight of the plantlets was determined before culture. Fifteen explants were used for each treatment and there were 3 explants/glass jars and the experiment was repeated twice. The culture period was 12 weeks, sub-culturing after 6 weeks. The data collected included: relative growth rate, root length, root number and root weight.

Data Analysis

Data analysis for all experiments was conducted using SPSS Windows version 8.00 (11 May 1998) SPSS Inc., 1989-1997. The mean regression of total dry weight over the night temperature was determined using the regression procedure. 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

Development of Early Flowering Clones

Clones produced from early germinated seeds reached to flower emergence and flowering significantly earlier than those clones developed from later germinating seeds and rhizome plants (Table 1). All plants flowered, however, the clones A15 and B33 from early germinating seeds produced the highest flower numbers per plant. Outgrowth and development of daughter rhizomes was only observed on in vitro derived clones, leading to plants bearing several developed side shoots (Fig. 1). Clone A15 had significantly more side shoots than the other clones. However, clone B33 had the highest mean height of side shoots. Developed side shoots of some in vitro plants flowered despite growing in relatively small pots. The plants also appeared relatively more compact than plants from rhizomes. Clone A15 were significantly shorter than the rest, while rhizome plants were tallest (leaf + petioles). Spathe and scape length were not significantly influenced by the treatments.

Development of Temperature Tolerant Clones

At the beginning of the greenhouse treatments (0 weeks), there were no significant differences in the growth parameters as influenced by clones or night temperatures. However, significant differences were observed in 7, 14 and 21 weeks. As expected, lower night temperature slowed the growth rate significantly (Table 2).

Clones selected after early germination at low temperature (clone A8 at 10°C and Clone B17 at 15°C) were significantly taller in height, greater leaf length, wider leaf diameter and increased dry weight of shoots and roots than clone C24 (20°C) at the second withdrawal 7 weeks (Table 2). Side shoot number and root length were not significantly influenced by clone type. Similar results were observed at the later observation times (data not shown).

The total dry weight after 21 weeks was significantly higher in clone B17 than clone C24. No significant difference was observed between clone C24 and clone A8 and between clone A8 and clone B17 (Table 2). The regression coefficient for total dry weight at 21 weeks was significant for night temperature, but not for clone type (Fig. 2).

Development of Salt Tolerant Clones

High NaCl levels significantly reduced the germination and also delayed germination (data not shown). High salt content significantly reduced plant growth as measured by all parameters: root weight, root number, root length and relative growth rate. Clones selected in medium containing NaCl performed better than the control (Table 3). The root length, root number and root weight was greater in clones selected in salt containing medium than in the control. Clone 5C1 (160 mmol/L NaCl) attained the highest root weight, root number and root length while the control scored lowest in all

these parameters. Clone 6B1 (200 mmol/L NaCl) recorded the highest relative growth rate. Conversely, clone 1A1 (control, 0 mmol/L NaCl) exhibited low relative growth rate equaling only clone 4A4 (120 mmol/L NaCl).

DISCUSSION

Early Flowering

The in vitro plants displayed "multibranching" habit, enabling several side shoots to develop alongside the main shoot from relatively small plants. The outgrowth of daughter rhizomes to give several developed side shoots indicates a modified behaviour of regenerated in vitro plants. This could be as a result of the plants acquiring a more compact form, thus requiring less root volume (pot size) for the development of side shoots. Hauser and Zuber (1995) also reported that in vitro plants of *Zantedeschia* 'Black-eyed Beauty', 'Goldstar' and 'Little Suzy' were more compact. The extent of the correlative inhibition exercised by the main shoot over buds on the daughter rhizomes when grown in 8 and 14 cm pots may also have declined, enabling their emergence and further growth (Ngamau, 2001a). This was possibly due to the plants accumulating more BAP over a long period during the in vitro growth, causing an internal shift in the endogenous hormone balance, thereby reducing the correlative inhibition. Similarly, D'Arth et al. (2002) observed increased bushiness in micropropagated *Zantedeschia* due to carryover effects of cytokinin.

Temperature Tolerance

The low temperature tolerance of clones selected after early germination at low temperature could not be conclusively determined since regression coefficient for clones selected from seedlings germinated at low temperature did not differ significantly when grown at different night temperatures. Zens (1988) isolated clones of *A. scherzerianum* after germination at 10°C which displayed significantly greater yield increase than seedling plants from commercial seeds within 21 weeks at a night temperature of 8°C. Similarly, Schaper (1990) categorised clones of *A. scherzerianum* according to their low temperature tolerance using their shoot dry weight and shoot fresh weight as the classification criteria. Fischer-Klüver (1994) classified 7 clones of *Sinningia* hybrids characterised by low regression coefficients over 4 temperatures tested as low temperature tolerant.

Salt Tolerance

High levels of NaCl negatively influenced the in vitro growth of the clones. No significant difference in plant growth was observed between the clones but a distinct pattern of response was observed, where clones selected after early germination on medium containing high salt levels performed better than the control. They showed improved root growth as compared to the control. Thus root growth may be important in the development of salt tolerance in 'Green Goddess', with plants having a better root system being able to survive better in salt containing medium, as was reported in in vitro selected NaCl-tolerant lines *Brassica juncea* (Kirti et al., 1991).

The selection of seeds germinating early in salt containing medium showed only marginal improvement possibly due to inadequate variability or selection pressure. Clones with probably higher salt tolerance could be developed through selection from a larger seedling population and the testing of more clones. The great difference in germination levels and the ability of 'Green Goddess' seeds to germinate even at very high NaCl concentration shows that the differences in salt tolerance may be greater at germination stage than at later growth stages. Crops are more tolerant at germination stages (Maas, 1987) and the degree of salt tolerance may be different at each of its physiological stages of development (Shannon, 1984).

In conclusion, selection of seeds germinating early under unfavourable conditions was found to lead to earlier flowering clones that had a "multibranching" growth habit.

This is important in production to reduce the production period and provide plants with multiple flowers that would fetch better prices. Differences in cold and salt tolerance were not explicit. Testing from a greater seedling population and applying greater selection pressure would probably produce a diversity of tolerance.

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Tables

Table 1. Comparison of in vitro clones of early and late germinated plants to rhizome plants.

Clone[z]	Flower no/plant	Plant height (cm)	Flower emergence (days)	Days to 1st flower	Spathe length (cm)	Scape length (cm)	Developed side shoots	Visible side shoots	Side shoot height (cm)
A15	4.2 c[y]	30.5 a	174 a	182 a	12.5	41.0	6.5 c	8.2 b	22.1 ab
B19	3.9 c	36.4 b	179 a	191 a	13.0	44.7	4.9 b	7.4 b	21.7 ab
B33	4.2 c	34.5 b	179 a	188 a	12.8	44.0	5.0 b	7.0 b	24.9 b
D3	3.5 bc	36.6 b	180 a	190 a	12.9	43.4	4.7 b	6.9 b	21.8 ab
E2	3.2 b	36.4 b	201 b	201 b	12.6	42.9	4.3 b	6.7 b	20.9 a
F2	1.3 a	38.1 b	239 c	250 c	12.4	42.9	0.0 a	3.2 a	-

[y] Means in a column having a common letter are not significantly different at $HSD_{(0,05)}$.
[z] A15, B19, B33 and D3 - Clones selected after early in vitro germination, E2 - Clone selected after late in vitro germination, F2 - Plants grown from 6 month old rhizomes. N = 20.

Table 2. Influence of night temperature on the growth of clones of *Z. aethiopica* 'Green Goddess'.

Parameter	Clone	Night temperature 6°C	10°C	14°C	18°C	HSD[c]
Plant height (cm)	C24	9.2	14.9	14.3	27.3	3.4
(7 weeks)	B17	10.2	16.3	17.3	28.7	
	A8	10.2	15.0	19.1	31.2	
Leaf length (cm)	C24	7.5	10.7	10.4	13.3	2.2
(7 weeks)	B17	6.9	10.5	11.5	15.0	
	A8	8.1	11.0	12.3	13.9	
Leaf width (cm)	C24	5.0	7.0	6.8	9.0	1.6
(7 weeks)	B17	5.9	7.5	7.9	9.8	
	A8	5.9	7.5	8.3	9.6	
Shoot dry weight (g)	C24	1.3	1.6	1.0	2.7	1.3
(7 weeks)	B17	1.1	2.1	1.6	3.3	
	A8	1.2	1.9	1.9	3.3	
Root dry weight (g)	C24	0.3	0.4	0.3	0.5	0.3
(7 weeks)	B17	0.3	0.5	0.4	0.8	
	A8	0.3	0.5	0.5	0.9	
Total dry weight (g)	C24	8.2	14.4	12.7	23.3	4.6
(21 weeks)	B17	9.8	14.9	18.0	25.7	
	A8	10.7	12.4	17.8	23.9	

Mean separation according to Tukey's $HSD_{(0,05)}$. N = 10.

Table 3. The reaction of clones of *Z. aethiopica* 'Green Goddess' on NaCl containing medium. Results of NaCl concentrations are summarised together. N=15.

Clone	Relative growth rate	Root length (cm)	Root number	Root weight
1A1 (control)[z]	0,2	1,4	0,3	26.0
2A1	0,3	2,6	0,7	44.6
3A1	0,3	2,1	0,7	46.9
4A4	0,2	1,8	0,7	35.6
5C1	0,4	3,7	0,8	71.7
6B1	0,5	2,5	0,7	47.2

[z] 1A1, 2A1, 3A1, 4A1, 5C1 and 6B1 correspond to clones developed after germination on 0 (Control), 40, 80, 120, 160 and 200 mmol/L NaCl, respectively.

Figures

Fig. 1. Left, Rhizome plants; Right, in vitro plants.

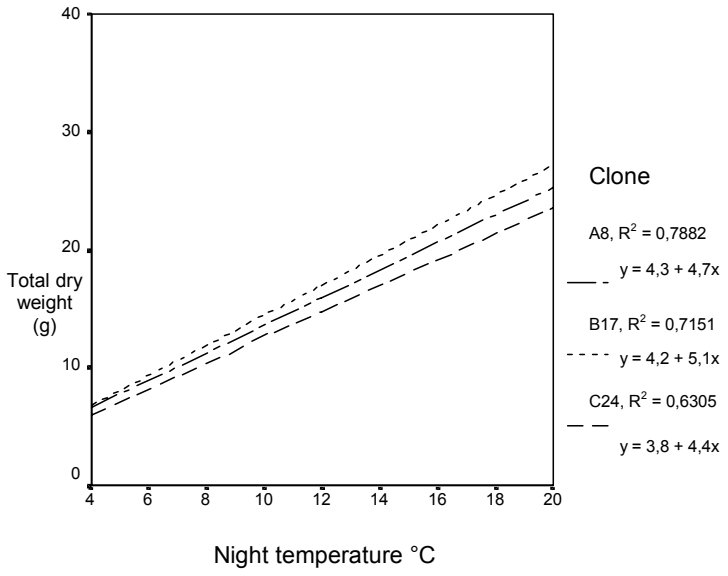

Fig. 2. Mean regression of the total dry weight over the night temperature.