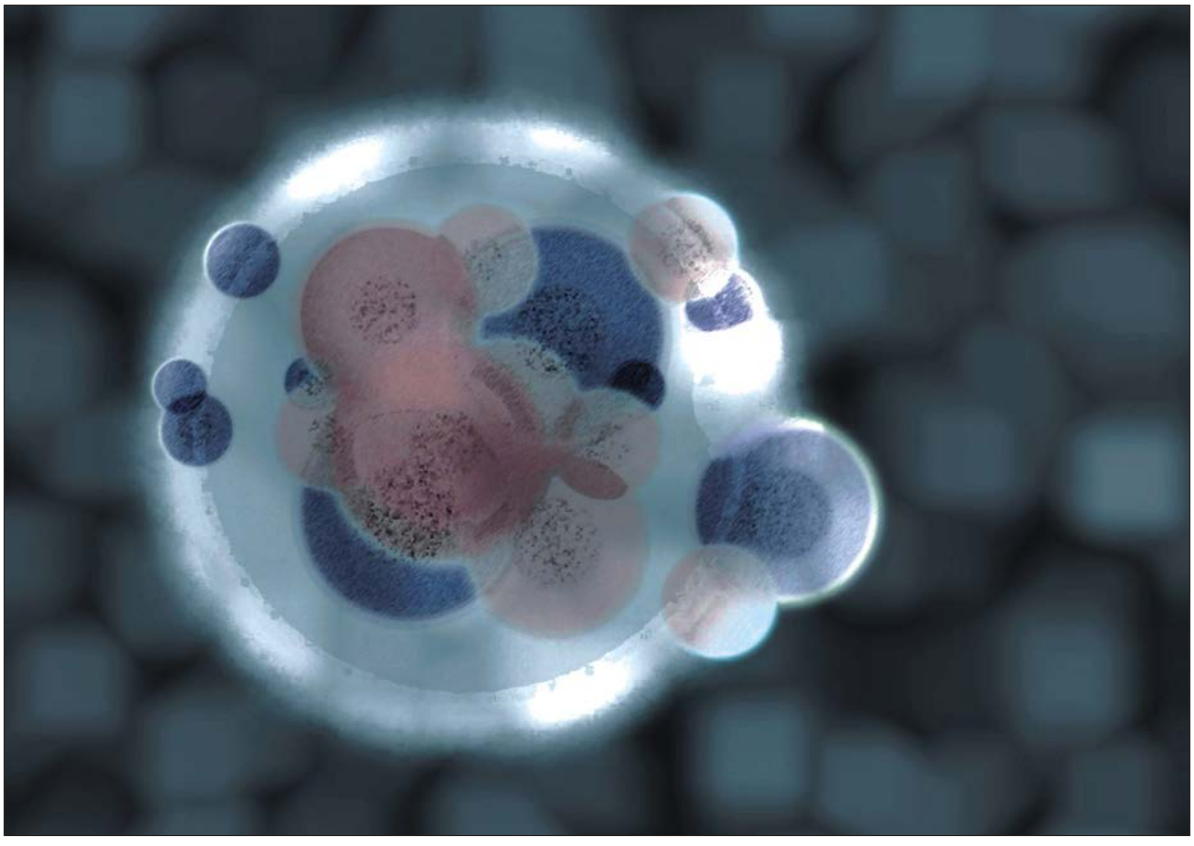

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Somaclonal Variation in *in vitro* Regenerated *Ledebouria graminifolia* (Hyacinthaceae), an Indigenous Bulb in Botswana and its Potential Exploitation as an Ornamental Plant

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Abstract: The aim of this study is to report on the occurrence of somaclonal variation in *in vitro* regenerated *Ledebouria graminifolia* an indigenous bulb in Botswana and assess its potential use as an ornamental plant. Plants that were regenerated using tissue culture were planted in a mixture of garden soil and potting soil in the greenhouse and also grown in the field under natural conditions without additional resources supplied to the plants. Morphological features and tolerance to drought were used to assess somaclonal variation among clones. *In vitro* propagated plants grew well in the greenhouse, they produced large attractive leaves and many flowered continuously. Field grown plants acclimatized well and survived periods of droughts and high temperatures. Morphological evaluation of the plants showed five visually distinct variants. Morphotype 1 consisted of prostrate plants with large, fewer, bright green leaves. Morphotype 2 consisted of prostrate plants with large, less bright green lanceolate leaves. Morphotype 3 consisted of plants with medium aristate, grayish green, curly leaves with rubbery appearance. Morphotype 4 had intermediate characteristics between 2 and 3 with grayish, lanceolate leaves. Morphotype 5 typically represented the parental phenotype of wild plants, consisted of very erect plants with curly, linear leaves with rubbery appearance. The variants also showed differences in their tolerance to drought with morphotypes 3 and 5 being most tolerant and morphotypes 1and 2 the least tolerant. The bulb could be mass propagated and exploited for ornamental purposes in Botswana to complement or as alternatives to popular exotic plants currently dominating the floriculture industry.

Key words: *In vitro* propagation, floriculture, medicinal plants, somaclonal variation, phenotypic variation, plant tissue culture, wild bulb

INTRODUCTION

In vitro propagation has great potential for propagating vegetatively reproducing plants and has been proposed to be a biotechnological tool which offers potential solution to problems of indigenous medicinal and ornamental plants over-exploitation in many parts of Africa (Afolayan and Adebola, 2004). Mass propagation using tissue culture techniques could offer cheaper alternatives to harvesting from the wild as is the common practice in many African countries and also contribute to the conservation of indigenous useful plants (McCartan and Van Staden, 1999; Entwistle *et al*., 2002; Afolayan and Adebola, 2004). Plants that have been regenerated using tissue culture methods often show variation (Sahijram *et al*., 2003; Podwyszynska, 2005). This variation, called somaclonal variation (Larkin and Scowcroft, 1981; Scowcroft and Larkin, 1988) has been found to have a genetic base and can be transmitted to the progeny through sexual reproduction or vegetative propagation. Plant tissue culture, can therefore provide additional genetic variability rapidly using simple technology and can provide an additional tool for plant improvement (Evans and Sharp, 1986; Bajaj, 1990; Karp, 1995; Jain and De Klerk, 1998; Tremblay *et al*., 1999) and a comprehensive review on propagation of ornamental plants using tissue culture is given by Rout *et al*. (2006).

Botswana is mostly a semi arid and arid country rich in diversity of indigenous plants adapted to drought conditions. The plants and their products are important in sustaining life in rural communities and many have high export potential (Taylor, 1985). Plants that are harvested from the wild are mainly leafy vegetables, fruits and medicinal plants (Mateke and Matlhare, 2000; Rositter *et al*., 1997) and sold in markets both locally and outside the country and have played a big part in supplementing income to most rural communities. In Botswana, collection and marketing of these indigenous plants has become very popular comparable to street-vended foods, especially in large towns. While there is a

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growing number of plant nursery business and street plant vendors in the country, there is little effort to promote the exploitation/use of indigenous plants as ornamental plants despite the growing awareness and interest in a green urban environment. Most of the plant species currently being exploited as ornamental plants for house and gardens are exotics and few indigenous succulents. Where, they are used, the indigenous plants are often undervalued in favour of exotics and aliens. However, there are many non-succulent plants with great potential that could be used in the floriculture industry. In addition, the plants that are currently collected from the wild have great potential for multipurpose uses. The underutilized plants could be exploited in the floriculture industry and can contribute significantly to the livelihoods of communities by providing additional income especially to rural and urban women, who are the driving force behind the booming informal economic sector in Botswana.

There are many advantages of using indigenous plants as ornamentals compared to exotic or alien species. Indigenous plants are usually well adapted to local arid conditions and increasing shortage of rains and irrigation water than most exotics. The rural communities, particularly women, are familiar with the plants and usually have potential multiple uses. Hence indigenous plants could fill in an important gap within the horticultural industry (Mander *et al*., 1996). Therefore there is a need to create awareness of these advantages and promote the exploitation of indigenous plants for ornamental purposes so as to offer an alternative source of income generation especially to rural women and to empower them with new ideas of the potential multipurpose uses and not just harvest plants for medicinal use; but also in propagation, multiplication and for biodiversity conservation.

The aim of this study is to evaluate the performance of *in vitro* regenerated *Ledebouria graminifolia* plants in the green house and their acclimatization to field conditions, so as to promote its sustainable exploitation as an ornamental plant and for the conservation of the plant. *Ledebouria graminifolia*, is an indigenous bulb in Botswana with great potential as an ornamental plant for indoors and outdoors. The bulb is extensively harvested from the wild and sold on markets for its medicinal properties (Mutanyatta *et al*., 2003; Shushu *et al*., 2005).

MATERIALS AND METHODS

This study was conducted at the University of Botswana, Department of Biological Sciences in 2004-2007. The plants used in this study were regenerated *in vitro* from scale leaves explants on Murashige and Skoog (1962) medium supplemented with BA and NAA for which results have been published elsewhere (Shushu *et al*., 2005). The bulbs were originally obtained from market vendors of medicinal plants. The plants were first transferred into commercial potting soil in 8 cm wide pots and acclimatized in the green house for three months. After establishment into the soil, the plants were transferred into larger pots in a mixture of field soil and commercial potting soil (1:1). The plants were maintained in the green house and growth and development of the plants were monitored.

Acclimatization of the plants to field conditions: In order to assess the ability of the *in vitro* regenerated plants to grow in natural field conditions, some of the plants were taken from the greenhouse and planted outside in open plots and were not given any additional supplements such as fertilizers. The plants were watered once a day only for the first one month and thereafter left to grow under natural field conditions for one year.

Determination of variants: During the growing period, the plants were regularly checked for the presence of variant phenotypes in the green house and the field and different phenotypes followed to see their stability in the field. Morphological features were used to determine somaclonal variation (Bajaj, 1990; Tremblay *et al*., 1999). The number, size of the fully expanded leaves, shape, colour and surface patterns of the leaves and flowering was determined for each plant after one year of growth in the field. The number of plants that survived and the growth habit of the plants was also determined. Analysis of variance was used to determine differences in the number and size of the leaves of variants.

RESULTS

Greenhouse plants: *In vitro* regenerated plants acclimatized successfully when transferred in the soil in the green house with 90% survival rate. The plants continued to grow very well in the green house in semi-domesticated conditions (Fig. 1). They produced large, attractive, shiny leaves and small inflorescences with purple flowers (Fig. 1e, f). The plants produced new leaves continuously when watered regularly. The plants also continued to produce inflorescences successfully, some plants produced 2-3 inflorescences at one time.

Acclimatization of the plants to field conditions: There was 100% survival of plants transferred to field conditions. The plants were watered for one month only after transfer. The plants survived periods of exposure to

Fig. 1: *Ledebouria graminifolia* plants: (a) Wild type phenotype, (b) *In vitro* regenerated plants in the green house, (c) Variants of *in vitro* regenerated *Ledebouria* in green house (wild type, far left), (d) *In vitro* propagated plants new growth after winter die off and, (e) Young inflorescence of regenerants and (f) *Ledebouria* flowers

Table 1: Variation in size and No. of leaves and flowering of variants grown in the field

Plant type	Mean leaf length (cm)	Mean leaf width (cm)	Mean No. of leaves	Flowering (No.)
Morphotype 1	18.2±0.5	5.8±0.3	5.0±00	3
Morphotype 2	17.9±0.8	5.5±0.2	8.2±0.4	2
Morphotype 3	20.7±1.4	5.7±0.2	11.6±1.2	0
Morphotype 4	22.4±1.6	6.5±1.1	11.4±0.5	0
Morphotype 5	27.9±0.6	2.3±0.3	9.5±0.5	0

Data are expressed as Mean±SE

very high temperatures (average temp. of 38°C) and drought. The plants lost all leaves during winter season (May-July). Ninety six percent of the plants resumed growth during rainy season (Oct.-April). The data presented in Table 1 below was taken from new leaves that developed during the warm wet period following the winter die-back.

Somaclonal variation: *In vitro* regenerated plants showed high levels of variation in growth habit and leaf morphology in the greenhouse and in the field (Fig. 1c, Fig. 2, Table 1). The field plants showed five visually distinct morphological types (Fig. 2). Morphotype 1 consisted of plants with large, fewer, bright green leaves with small reddish speckles (Fig. 2 T_1a and T_1b). This type showed a prostrate growth habit. Morphotype 2 consisted of plants with large, lanceolate leaves which were slightly bright green (Fig. 2, T_2). Morphotype 3 consisted of medium lanceolate, highly aristate leaves (Fig. 2, T_3). The leaves were grayish green with rubbery appearance which curled inwards. Plants were slightly erect. Morphotype 4 had characteristics intermediate between 2 and 3 with grayish, slightly lanceolate leaves (Fig. 2, T_4). Morphotype 5 consisted of very erect plants with curled, prominently linear leaves with rubbery appearance and prominent reddish speckles at the base (Fig. 2, T_5). This type typically represented the parental phenotype of wild plants. There was a significant difference in leaf length between the wild type phenotype and all variants and also between Morphotypes 2 and 3. Leaf width also differed between wild type and all variants but not among variants. There was also significant difference in the number of leaves produced by the

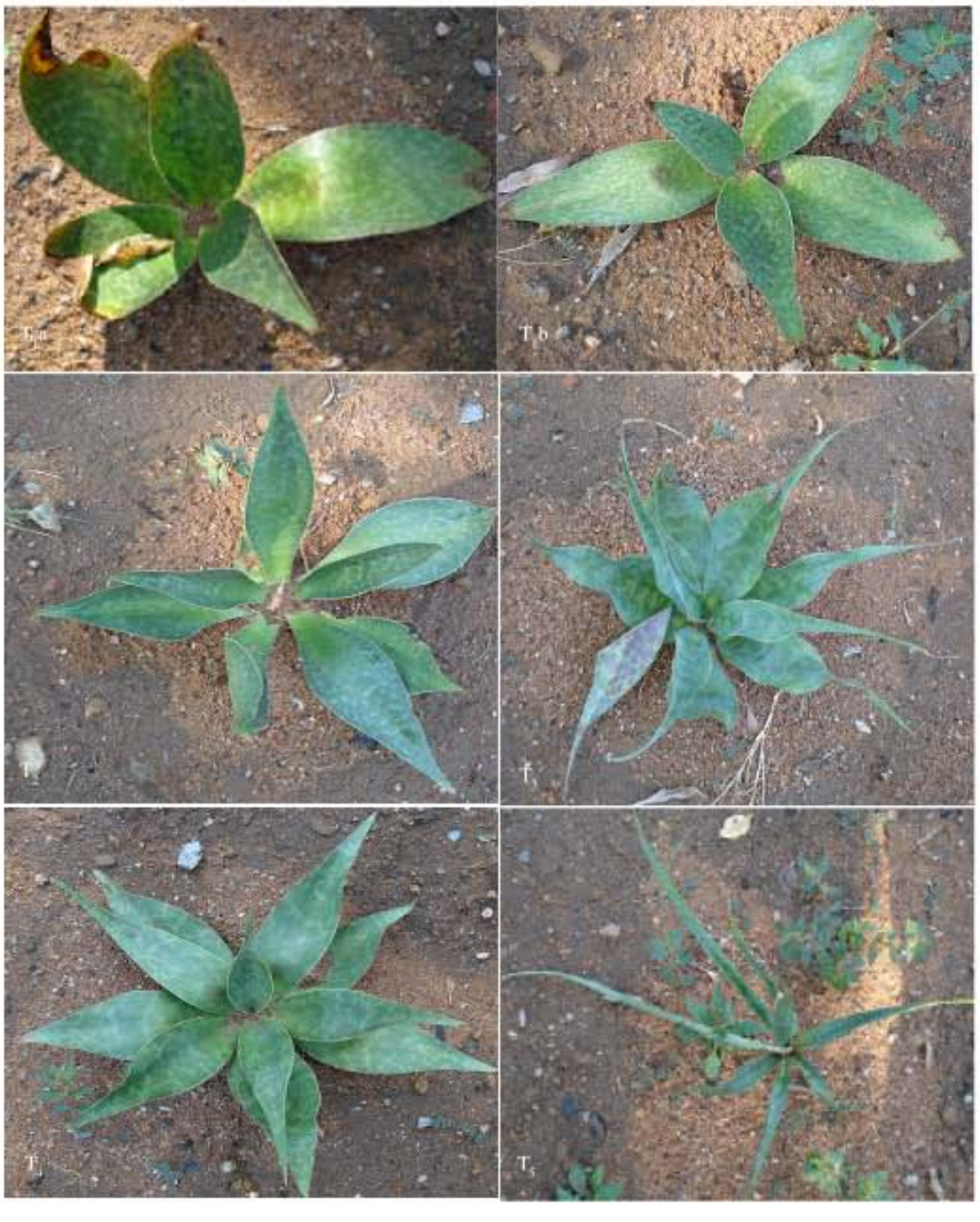

Fig. 2: Morphological variation of the *in vitro* propagated *Ledebouria* sp. in the field

variants. All types showed the prominent wavy, speckled leaves characteristic of the plant. Only Morphotype 1 and 2 produced flowers within the growing season.

There was a difference in winter die back response among the variants. Morphotype 1 was the first variant to lose the leaves, followed by Morphotype 2, 4, 5 and 3, respectively.

DISCUSSION

The present study has shown that *in vitro* regenerated indigenous bulb *Ledebouria graminifolia* acclimatized very well both to greenhouse and field conditions. The survival rate in the green house was 90%. Under a semi-domesticated environment, *Ledebouria* produced bigger attractive leaves than wild plants when provided with water and good growth medium such as a mixture of soil and potting soil. Although the bulb produced small and not-so attractive inflorescences (Fig. 1c, e). *Ledebouria* can be grown as a potted ornamental plant for its large attractive speckled leaves, features that are desirable in the industry. *In vitro* regenerated *Ledebouria* plants showed remarkable tolerance and resilience to extreme environmental conditions when grown in the field. The plants acclimatized well after transfer to the field and exposed to natural conditions with minimum care. There was 96% survival rate in the field. The plants went through a winter die back (May-July) and were able to resume growth thereafter. Botswana has very low levels of precipitation of between 200 and 600 mm per year and temperatures can reach as high as 40°C. The ability of *in vitro*

propagated plants to withstand the dry conditions makes *Ledebouria* a suitable plant to be used to fill an important gap in the flourishing and growing floriculture industry in Botswana that is currently being dominated by exotic plants which usually require high resource in-put, especially water. *Ledebouria* is also an important medicinal bulb in Botswana hence extensively harvested from the wild (Mutanyatta *et al*., 2003). Tissue culture could be used to mass propagate the plant (Shushu *et al*., 2005) for distribution to communities for use as an indoor or outdoor ornamental plant as well hence contribute to the conservation of the plant.

Somaclonal variation: *In vitro* regenerated plants showed a wide range of variation for leaf morphology such as leaf size, shape, colour and growth habit in the greenhouse and in the field especially considering that all these plants were regenerated from a single bulb. Somaclonal variation is the variation observed among plants regenerated by tissue culture (Larkin and Scowcroft, 1981). There a number of ways of assessing somaclonal variation, variation in phenotype and response to environmental conditions was used to assess variation in this study. Wild *Ledebouria* plants consist of linear leaves with grey and dark green speckles. The leaf bases are decorated with bright purple speckles. Morphological evaluation of the *in vitro* regenerated plants after one year's growth showed five distinct types of variations. Morphotype 1 had prostrate growth habit; had the least number but largest, attractive leaves. This type however was not very well adapted to field conditions as shown by early drying of leaves which started from the tips. Morphotype 2 had more leaves than type 1. However, these were the only variants that produced flowers. Morphotypes 3 had semi-erect habit. This type produced more leaves that were hardy with leathery appearance. These plants were more tolerant to drought than types 1 and 2 as they showed less signs of stress even under very hot and dry conditions. The leaves of Morphotype 3 tended to curl inwards probably reducing the exposed leaf lamina surface as an adaptation to drought. Morphotype 5 consisted of long, linear leaves that curled inwards as well. As mentioned earlier, this represented the phenotype naturally found in the wild. Morphotype 4 had characteristics intermediate between 2 and 3 (Fig. 2). It was noted in this study that variations in response to environmental conditions appeared to relate to morphological and growth habit variation indicated above. That is, Morphotype 1 and 2 plants (with large, greener leaves and a prostrate habit) were prone to desiccation more than other types. Leaf size could not have accounted for this since there was no significant difference among variants except type 5. The lack of tolerance to drought could have been due to the prostate growth habit and fully exposed leaf lamina that were very close to the heated soil which may have led to high exposed surface area for increased water loss. Morphotype 3, 4 and 5 were more tolerant to drought and heat; also they were the last plants to lose leaves as winter season set in probably due to curling of leaves and semi erect growth habit which reduced water loss from the leaves. Other factors such as differences in leaf anatomical features may have been involved , this aspect was not investigated in this study.

There are many factors possibly causing somaclonal variation. Genetic disorders in the cells of initial explants, explant source, time in culture and growth hormone have been cited to have an effect on the levels of somaclonal variation (Karp, 1995). The variation seen in these plants could have been due to the effects of growth substances. All the plants in this study came from a single bulb as the source of explants and duration of the plants in culture was 12 weeks. It is less likely that genotype and duration in culture could have been the major source of the high variation seen in this study although we can not completely discount it. Variation found in *in vitro* regenerated plants may not be stable due to physiological disturbances and epigenetic influences (Bajaj, 1990). The variation observed in these plants, seemed to be stable variation as the phenotypes presented in this study were obtained from second generation set of leaves that developed after a winter die-back which was similar to phenotypes of plants that were transferred to the field. Somaclonal variation has been found to be a useful alternative for new product development. It offers an opportunity to uncover the natural variability in plants especially for vegetative propagated plants (Larkin and Snowcroft, 1981; Bajaj, 1990; Sahijram *et al*., 2003). The high level of variation in phenotype unfolding in this study was considered to be a good and valuable source of desired phenotypes that can be commercially exploited in floriculture to meet the different consumer market preferences in the industry.

The use of indigenous plants in the ornamental plant industry has really not been given much attention although many of these plants can offer better alternative to exotic species that are currently widely used in this industry. There are very few countries in Africa that have directed effort towards popularizing indigenous plants for ornamental use (Reinten and Coetzee, 2002) despite the

growing interest in a green urban environment. Many of the plants used for medicinal purposes can also be used as ornamental plants. Research and commercial cultivation of indigenous bulbs for medicinal and ornamental markets has made progress in other countries such as South Africa (McCartan and Van Staden, 1999; Reinten and Coetzee, 2002) and Turkey (Entwistle *et al*., 2002; Acar *et al*., 2007). Botswana has a rich diversity of indigenous bulbs such as *Ledebouria* sp. that are suitable for the ornamental plant industry which could complement or offer ideal alternatives to exotic plants currently dominating town landscapes and private house gardens. *Ledebouria* could be mass propagated and distributed for planting therefore contributing to urban biodiversity, sustainable utilization and conservation. Research efforts should be directed towards efficient mass propagation methods, growth requirements for improving their agronomic characteristics and markets.

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REFERENCES

Acar, C., H. Acar and E. Eroglu, 2007. Evaluation of ornamental plant resources to urban biodiversity and cultural changing: A case study of residential landscapes in Trabzon city (Turkey). Build. Environ., 42: 218-229.

Afolayan, A.J. and P.O. Adebola, 2004. *In vitro* propagation: A biotechnological tool capable of solving the problem of medicinal plants decimation on South Africa. AJB, 3: 683-687.

Bajaj, Y.P.S., 1990. Somaclonal Variation-Origin, Induction, Cryopreservation and Implications in Plant Breeding. In: Somaclonal Variation in Crop Improvement, Bajaj, Y.P.S. (Ed.). Springer-Verlag, Berlin, ISBN: 3 540 507 85-x.

Entwistle, A., S. Atay, A. Byfield and S. Oldfield, 2002. Alternatives for the bulb trade from Turkey: A case study of indigenous bulb propagation. Oryx, 36: 333-341.

Jain, S.M. and G.J. De Klerk, 1998. Somaclonal variation in breeding and propagation of ornamental crops. Plant Tissue Cult. Biotech., 4: 63-75.

Karp, A., 1995. Somaclonal variation as a tool for crop improvement. Euphytica, 85: 295-302.

Larkin, P.J. and W.R. Scowcroft, 1981. Somaclonal variation- a novel source of variability from cell cultures for plant improvement. Theor. Applied Gent., 60: 197-214.

Mander, M., J. Mander and C. Breen, 1996. Promoting the Cultivation of Indigenous Plants for Markets: Experience from Kwazulu-Natal, South Africa. In: Domestication and Commercialization of Non-Timber Forest Products in Agroforestry Systems, Van Honten, H. and K. Kebaara (Eds.). FAO Technical Publication, Italy, Rome, ISBN: 9251037019.

Mateke, S. and T. Matlhare, 2000. Under-utilized plants-their conservation and sustainable use by rural communities in arid, semiarid ecosystems of Botswana. Proceedings of the 2nd Botswana Workshop on Plant Genetic Resources: Plant Genetic Resources-A Heritage for the Future June 20-23, Printing and Publishing House, Gaborone, Botswana, pp: 18-31.

Mc Cartan, S.A. and J. Van Staden, 1999. Micropropagation of members of Hyacinthaceae with medicinal and ornamental potential. Rev. S. Afr. J. Bot., 65: 361-369.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Plant Physiol., 15: 473-497.

Mutanyatta, J., Matapa, B.G. Shushu and D.D. Abegaz, 2003. Homoisoflavonoids and xanthones from the bulbs of wild and *in vitro* regenerated *Ledebouria graminifolia* and cytotoxic activities of some of the homoisoflavonids. Phytochemistry, 62: 797-804.

Podwyszynska, M., 2005. Somaclonal variation in micropropagated tulips based on phenotype observation. J. Fruit Ornam. Plant Res., 13: 109-122.

Reinten, E. and J.H. Coetzee, 2002. Commercialization of South African Indigenous Crops: Aspects of Research and Cultivation of Products. In: Trends in New Crops and New Uses, Janick, J. and A. Whipkey (Eds.). ASHS Press, Alexandria, ISBN: 097075655.

Rositter, S., S. Pellegrin, K. Hill, A. Bunting, S. Robinson, T. Mason, T. Boitumelo and A. Madiba, 1997. The Present Importance and Future Potential of Veld Products in the Livelihoods of Villagers from the Tswapong Hills. 1st Edn., Botswana Society, Gaborone.

Rout, G.R., A. Mohapatra and S.M. Jain, 2006. Tissue culture of ornamental pot plant: A critical review on present scenario and future prospects. Biotech. Adv., 24: 531-556.

Sahijram, L., J.R. Soneji and K.T. Bollamma, 2003. Analyzing somaclonal variation in micropropagated banana (*Musa* spp.). *In vitro* Cell Dev. Biol. Plant, 39: 551-556.

Scowcroft, W.R. and P.J. Larkin, 1988. Somaclonal Variation. In: Applications of Plant Cell and Tissue Culture, Bock, G. and L. March (Eds.). CIBA Foundation Symp. Wiley, Chichester.

Shushu, D.D., J. Mutanyatta and B.M. Abegaz, 2005. *In vitro* propagation and phytochemical investigation of *Ledebouria graminifolia*, an important medicinal plant in Southern Africa. J. Herbs Spices Medicinal Plants, 11: 97-107.

Taylor, F.W., 1985. The Potential for Utilization of Indigenous Plants in Botswana. In: Plants for Arid Lands, Wickens, G.E., J.R. Gooding and D.V. Field (Eds.). George Allen and Unwin, London.

Tremblay, L., C. Levasseur and F.M. Tremblay, 1999. Frequency of somaclonal variation in plants of black spruce (*Picea mariana*, Pinaceae) and white spruce (*P. glauca*, Pinaceae) derived from somatic embryogenesis and identification of some factors in genetic instability. Am. J. Bot., 86: 1373-1381.