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The Effects of Different Hormones on Regeneration of Gazania (*Gazania rigens*)

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Abstract

Recent advances in the modern techniques of propagation could help growers to meet the demand of the ornamental industry. New ornamental plant varieties are being created by breeders in response to consumer demand for new products. In this study, different concentrations of BA (0.0, 0.2, 0.5, 1.0 mg/l) and IAA (0.0, 0.5, 1.0 mg/l) were investigated to optimize regeneration of Gazania rigens. The best callus formation was found from the media containing 0.2 mg/l BA-1.0 mg/l IAA (100 %), 0.5 mg/l BA-1.0 mg/l IAA (98 %), 0.5 mg/l BA-0.5 mg/l IAA (97 %), 1 mg/l BA -1 mg/l IAA (97 %), 1.0 mg/l BA-0.5 mg/l IAA (98 %), 0.5 mg/l BA (97 %). The most promising regeneration and growth were obtained from the media with 0.2 mg/l BA-1.0 mg/l IAA (30 %), 1.0 mg/l BA-0.5 mg/l IAA (22 %) and 1.0 mg/l BA (18 %). The regenerated plantlets were rooted on the media containing 0.5 and 1.0 mg/l IAA. The best rooting percentage was observed on the media containing 1.0 mg/l IAA (75 %).

Keywords: Gazania, *Gazania rigens,* Regeneration, Cytokinin, Auxin

Introduction

The economic importance of ornamentals worldwide suggests a bright future for ornamental breeding. Floriculture specifically includes most herbaceous ornamental plant species, such as cut flowers (rose, carnation, chrysanthemum, gladiolus, freesia, gerbera, orchid…etc.), pot plants (African violet, kalanchoe, azalea, poinsettia, cyclamen) and bedding plants (gazania, geranium, pansy, fuchsia, petunia, impatients, begonia). New ornamental plant varieties are being created by breeders in response to consumer demand for new products (3).

Traditionally, classical breeding has been used to introduce new traits and create new varieties in ornamentals. However, it is a tedious process, based on crosses between related species or within the same species, and on the selection of offspring with promising characteristics. With classical breeding, the available gene pool for new traits is limited to the genetic background of the parents. Moreover, many varieties of ornamental plants are sterile.

Intensive research into the micropropagation of ornamentals has led to numerous reports on regeneration procedures from various explants of these species, and has been

subject of several reviews (1, 6). The plant cell, tissue or organ culture of many ornamental species and their regeneration are essential for providing the material and systems for their genetic manipulation, and this is therefore the first requirement of genetic engineering (11).

Gazania is a genus of flowering plant in the family Asteraceae, native to Southern Africa. It is often planted as drought-tolerant ground cover. The genus occurs in South Africa, Swaziland, Mozambique, Tanzania and Angola. Additionally, species are naturalized in Australia, New Zealand and California. They are widely cultivated as ornamental garden plants. Gazanias are grown for the brilliant color of their flower which appears in the late spring and early summer. They prefer a sunny position and are tolerant of dryness and poor soils. A commonly grown variety is the Trailing Gazania (*Gazania rigens* var. *leucolaena*). They are commonly used as groundcovers and can be planted to cover large areas or embankments, assisted by their fast growth rate. Another popular cultivated variety is the Clumping Gazania (*Gazania rigens*) which has a number of named cultivars including 'Aztec', 'Burgundy', 'Copper King', 'Fiesta Red', 'Goldrush' and 'Moonglow' (2).

Gazania rigens is a native of South Africa that is easy to grow. Flowers bloom in solid colors from bright yellow to orange, red, pink and white, or in wild color combinations with splashy stripes or rings of contrasting colors. *Gazania* is considered an annual in the north but is a short-lived perennial here in the south. Although it is very popular bedding plant, there is no report on micropropagation and regeneration of *Gazania*. Therefore, this will be first report on regeneration of *Gazania rigens.* In this research, regeneration process and rooting as well as the effect of different hormones and their concentrations were investigated.

Material and Method

Gazania seeds were used as starting material. The seeds were surface disinfested in 70 % ethanol for 5 minutes followed by 5 % NaOCl solution containing few drops of Tween 20 for 10 minutes. They were rinsed three times in sterilized distilled water and placed into 100x15mm petri dishes containing MS (4) basal medium with 3% sucrose. The pH of the medium was adjusted to 5.7 prior to add gelling agents. The media were sterilized by autoclaving at 121^{o}C for 20 minutes.

In vitro grown seedling explants were transferred in to regeneration medium containing different concentration of BA (0.0, 0.2, 0.5, 1.0 mg/l) - IAA (0.0, 0.5, 1.0 mg/l) for direct organogenesis. Explants (cotyledon) were incubated at 25 $\pm$ 2^{o}C under 16-h photoperiod provided by cool white fluorescent lamps. Plantlets were rooted in rooting medium containing IAA (0.0, 0.5, 1.0 mg/l).

Result and Discussion

Different BA (0.0, 0.2, 0.5, 1.0 mg/l) and IAA (0.0, 0.5, 1.0 mg/l) concentrations and combinations were investigated to optimize regeneration of *Gazania rigens.*

There were considerably differences in responses according to the hormone concentrations. The best callus formation ratios were found from the media containing 0.2 mg/l BA-1.0 mg/l IAA (100%), 0.5 mg/l BA-1.0 mg/l IAA (98%), 0.5 mg/l BA-0.5 mg/l IAA (97%), 1 mg/l BA -1 mg/l IAA (97%), 1.0 mg/l BA-0.5 mg/l IAA (98%), 0.5 mg/l BA (97%), respectively (Table 1).

The response of explants cultivated on MS medium with BA (0.0, 0.2, 0.5, 1.0 mg/l) and IAA (0.0, 0.5, 1.0 mg/l) was as follows. The best regeneration was obtained from the media containing 0.2 mg/l BA-1.0 mg/l IAA (30%). This is followed by the media with 1.0

mg/l BA-0.5 mg/l IAA (22%) and 1.0 mg/l BA (18%). There was no shoot regeneration from the media containing 0.5 and 1.0 mg/l IAA, but root formation. The best rooting percentage was observed on the media containing 1.0 mg/l IAA (75%) (Table 1).

Comparison of the media containing 1.0 mg/l BA, 1.0 BA-0.5 mg/l IAA and 1 mg/l BA-1.0 mg/l IAA showed that increasing auxin concentration decreased the regeneration ratio although the cytokinin concentration was same for each of them (Table 1) (Figure1). PIERIK, (1997) (6) also mentioned that auxins generally cause cell elongation, swelling of tissues, callus formation and especially inhibition of adventitious and axillary shoot formation.

Same situation was shown for BA and IAA combination if the media containing 0.5 mg l^{-1} BA - 0.5 mg l^{-1} IAA (43 %) and 0.5 mg l^{-1} BA - 1 mg l^{-1} IAA (23 %) were compared (9). Higher auxin caused lower regeneration whenever cytokinin concentration was kept constant in the media. Higher BA concentration with IAA (2.0 mg l^{-1} BA – 1.0 mg l^{-1} IAA) showed very low regeneration as 3.0 % ratio. These results are similar with the results obtained by NHUT et al., 2005 (5). The highest shoot formation (56.67 %) obtained from caulogenesis was recorded in the presence of 1.0 mg l^{-1} BA alone. When both BA and auxin were used at low concentrations, shoot formation was over 75%. In combination, shoot formation rate was recorded to decrease as BA concentration was increased.

Table 1. Callus formation, regeneration and rooting percentages of explants on the medium containing different concentration of BA (0.0, 0.2, 0.5, 1.0mg/l) and IAA (0.0, 0.5, 1.0 mg/l).

Treatment	Callus (%)	Regeneration(%)	Rooting (%)
Control	13	0	10
IAA 0.5	60	0	3
IAA 1.0	87	2	75
BA 0.2	78	0	0
BA 0.5	97	0	0
BA 1.0	90	18	0
IAA 0.5; BA 0.2	95	3	0
IAA 0.5; BA 0.5	97	15	0
IAA 0.5; BA 1.0	97	22	0
IAA 1.0; BA 0.2	100	30	5
IAA 1.0; BA 0.5	98	3	0
IAA 1.0; BA 1.0	97	3	0

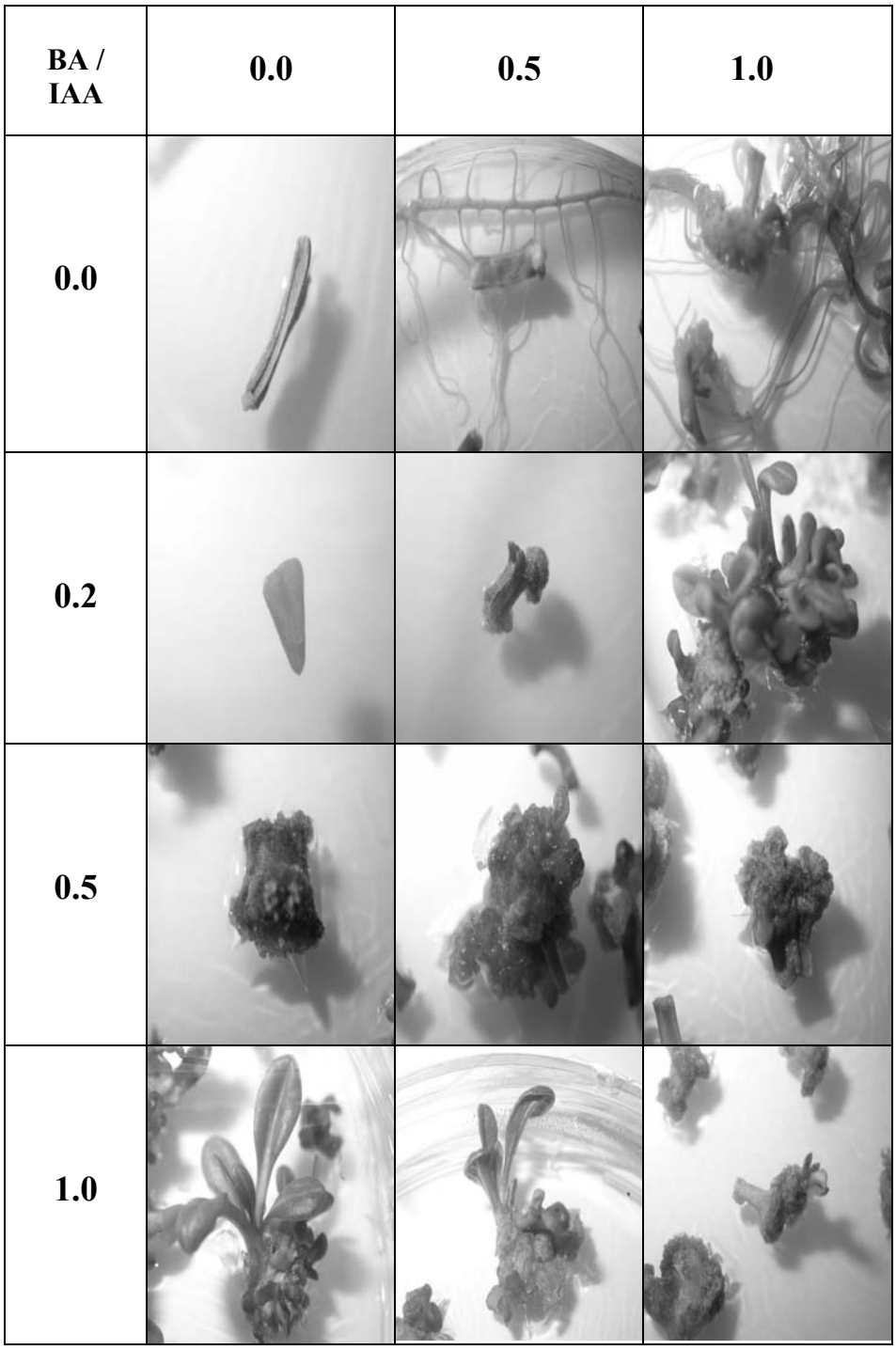

Figure 1. Regeneration of explants on the medium containing different concentration of BA (0.0, 0.2, 0.5, 1.0mg/l) and IAA (0.0, 0.5, 1.0 mg/l).

Conclusion

Tissue and organ culture of many ornamental species and their regeneration are essential for providing the material and systems for their genetic manipulation. Plant regeneration system via direct organogenesis was established from cotyledon explants for *Gazania rigens* in this study. BA-IAA combination in the media showed better regeneration

than BA alone. In addition to these hormones, different hormones (Kinetin, Zeatin, IBA, NAA) and concentrations, different explant types (hypocotyls, leaves, root), explant age, dark and light treatments could be tested to improve regeneration ability in *Gazania rigens.*

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