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EFFECT OF GRAVITATIONAL STIMULI ON LEAF-PLANTLET FORMATION AND ASEXUAL REPRODUCTION OF *KALANCHOË DAIGREMONTIANA*

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1. INTRODUCTION

The aim of our project was to develop a plant model system with *Kalanchoë daigremontiana* (*Crassulaceae*) to study the effects of gravitational stimuli at the first division in leaf-plantlet formation, on leaf cell fate, plant development and asexual reproduction, for subsequent plant generations. The main reasons for selecting *Kalanchoë* were: (1) it reproduces asexually, *in vivo* and *in vitro*, by forming plantlets in precise locations of the leaf; (2) leaf-plantlet formation occurs in light and in darkness; (3) sexual reproduction can be ruled out due to early inflorescence death; (4) a life cycle is completed in 8 weeks, *in vitro*. Here, we describe the effect of acute and short-term hypergravity and simulated hypogravity on leaf-plantlet formation and asexual reproduction of *K. daigremontiana*.

2. MATERIAL AND METHODS

2.1 Plant material

Clonal leaves and leaf-plantlets isolated from shoots cultured *in vitro* [1] were used as experimental systems. Cotyledons were also used in same experiments.

2.2 Hypergravity and simulated hypogravity experiments

Leaf and leaf-plantlet cultures were exposed to hypergravity (20 xg to \leq 600 xg; in darkness, for 5 min to 15 days) and simulated hypogravity (clinostats, 1 rpm; in light and darkness, for 15 to 30 days). Cultures were then transferred to unit gravity (16-h photoperiod). An ethylene free environment was provided by Purafil beads.

2.3 Data analysis

Leaf-plantlet formation and asexual reproduction rate were determined for the current and subsequent generation. Experiments were repeated at least three times with a minimum of 60 explants per experiment. Simple analysis of variance (ANOVA) was performed to test the hypothesis that means from 2 or more populations were equal; the significance level entered was $P < 0.05$ and $P < 0.01$.

3. RESULTS

Plantlet formation from isolated leaves *in vitro* was identical to that obtained from leaves remaining attached to shoots in all treatments. Leaf-plantlet formation was not significantly affected by leaf orientation on the culture medium ($P < 0.05$). Plantlet production from cotyledons and young leaves was significantly lower ($P < 0.01$) than for fully differentiated leaves. Earlier stages of plantlet formation at leaf indentations were negatively affected by gravitational changes.

Leaf-plantlet formation and asexual reproduction from clonal explants were significantly affected ($P < 0.05$) by gravitational treatments. Acute hypergravity (< 60 min) significantly increased ($P < 0.01$) leaf-plantlet formation and asexual reproduction compared to cultures at unit gravity. The increase was directly related to the centrifugation force and duration of the treatment. Long-term (15 days) exposure of leaves to hypergravity (up to 150 xg), significantly increased leaf-plantlet formation and asexual reproduction, when compared to unit gravity (Fig. 1). Asexual reproduction decreased with the increase of hypergravity (from 20 xg to 200 xg), increasing again at 300 xg. At 300 xg, it was identical to that at 20 xg and 3.2-fold higher than at unit gravity (Fig. 1). Leaf-plantlet formation also decreased between 150 xg and 200 xg (2.2-fold for the 1st and 2nd generations), increasing again at 300 xg (1.5-fold). Identical results were obtained for leaf-plantlets.

Simulated hypogravity, in light and darkness, significantly decreased ($P < 0.05$) leaf-plantlet formation and asexual reproduction, when compared to unit gravity (Fig. 2). Leaf-plantlet formation and asexual reproduction, in simulated hypogravity in darkness, were significantly lower than for cultures in light (Fig. 2).

Ethylene release was not detected either in the equipment or culture vessels.

Compared to unit gravity, aging and morphological aberrations increased in plantlets formed in hypergravity and simulated hypogravity.

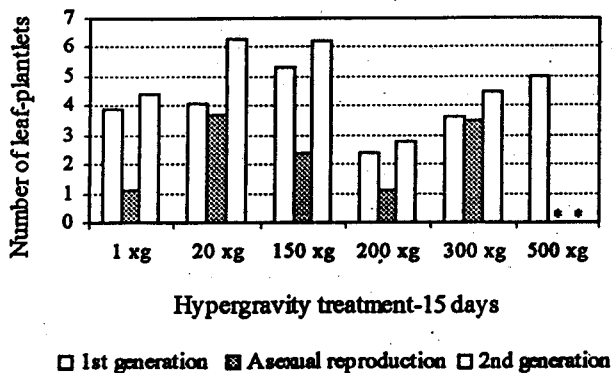


Fig. 1. Comparison between leaf culture responses, for leaf-plantlet production (1st and 2nd generation) and asexual reproduction (AS), under control conditions (1 xg) and under hypergravity (20 to 500 xg). Values (mean \pm SD) were recorded 2 (1st generation) and 8 weeks after culture initiation. Populations, except for AS at 20 and 300 xg, are significantly different at $P < 0.05$. (*), not determined.

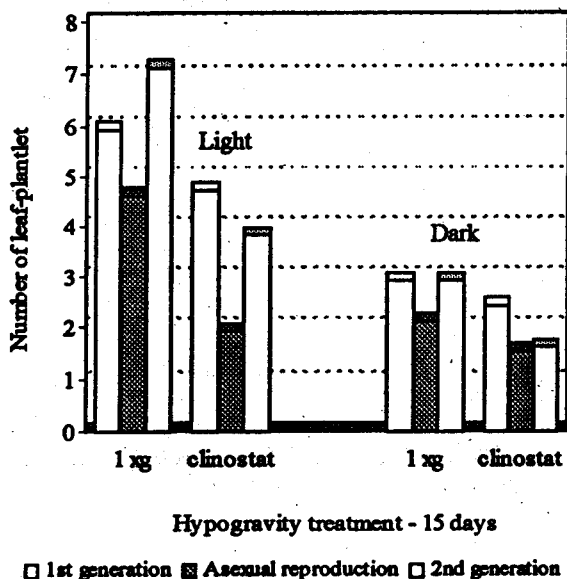


Fig. 2. Comparison between leaf culture responses, for leaf-plantlet production (1st and 2nd generation) and asexual reproduction, under control conditions (1 xg) and under simulated hypogravity. Values (mean \pm SD) were recorded 2 (1st generation) and 8 weeks after culture initiation. Populations are significantly different at $P < 0.05$.

4. DISCUSSION

The results have shown that short-term exposures to gravitational stimuli affected leaf-plantlet formation, development and asexual reproduction. Trendlines showed that hypergravity increased leaf-plantlet formation and asexual reproduction, while simulated hypogravity had the opposite effect. Also, explants at early stages of developmental organization were more sensitive to gravity changes than more differentiated ones. The sinusoidal pattern for leaf-plantlet production and asexual reproduction recorded for acute and short-term hypergravity exposures (at 150 xg to 300 xg) was related with changes in TUNEL reactivity [2]. The possibility of overproduction of secondary metabolites which interfered with the cell cycle has not been ruled out. The presence of aberrant leaf shapes, root formation and abscission-like wounds under gravitational stressful environments, revealed the importance of studying the effects of acute hypergravity treatments and simulated hypogravity at first divisional stages of leaf-plantlet formation, at cellular and ultrastructural levels, and on subsequent generations.

K. daigremontiana leaves provided a model system for studying the differential effects of g forces on cell fate, plantlet development and asexual reproduction throughout the plant generations, as well as, the study of adaptative responses coupled to cell death.

5. REFERENCES AND ACKNOWLEDGMENTS

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