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Ethanol treatment induces compact growth in *Kalanchoë*

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ABSTRACT

Compact growth is a major quality characteristic for the attractiveness and transportation of *Kalanchoë*, one of most economically important potted plants in Europe. In the present experiments, we examined the possibility of using ethanol as an alternative plant growth regulator. We compared using either an ethanol spray or an ethanol watering treatment during standard cultivation of several *Kalanchoë* species and varieties. The results of the present study demonstrated that watering the plants with an ethanol solution was more effective than the ethanol spray treatment. All tested genotypes showed a correlation between the ethanol concentrations used for watering and the internode lengths internode after ethanol watering. However, high ethanol concentrations (more than 2%) led to leaf damage and delayed flower development in some genotypes. The use of ethanol as a growth regulator for ornamentals has several advantages. Ethanol is a biodegradable molecule that is inexpensive, easy and safe to apply, and non-toxic in the concentrations required.

1. Introduction

Kalanchoë blossfeldiana is one of the most economically important, flowering, potted plant species in Europe, with a production of more than 150 million plants per year. In 2012, *Kalanchoë* placed second on the list of the top 25 indoor plants traded at the world's largest flower auction, FloraHolland in The Netherlands, with a turnover of 55 million € and 77 million units sold (FloraHolland, 2012).

The quality of produced ornamental potted plants has become increasingly important. One of the most important quality characteristics for the attractiveness and transportation of potted and bedding ornamentals is compact growth. Accordingly, influencing the plant habit using chemical treatments has become an essential part of ornamental plant production. The primary goal of these treatments is to reduce the plant size in a desired way, without extending the production time or being phytotoxic (Rademacher, 2000).

During the last several decades, it has become clear that several chemicals have environmental and health risks and that a reduction in the use of these chemicals would be welcomed by both plant producers and consumers (Andersen et al., 2002).

Therefore, several alternatives to synthetic growth regulators have been developed and investigated. Within the last 20 years, different temperature and light strategies (e.g., DIF or cool morning), abiotic stress treatments (e.g., drought and mechanical stimuli, such as touch and nutrient deficiencies) and the breeding of new cultivars that are genetically inclined towards compactness have been investigated. The main goal of these developments was the production of high quality, compact ornamentals, without the use of potentially environmentally harmful substances.

Another alternative strategy, utilized in place of growth regulators, is the use of genetic engineering in the production of drafted genotypes. For example, *rol*-genes (root loci genes) were transferred to *K. blossfeldiana.* The achieved Ri-lines had shorter internodes, resulting in a growth habit that was more compact compared to control plants (Christensen et al., 2008).

Elongation growth in plants is primarily controlled by gibberellic acid (GA) (Lange and Lange, 2006). The genetic modifications to ornamentals and the use of synthetic growth regulators have mainly been aimed at the reduction of GA content by blocking GA synthesis-related enzymes. To reduce the GA concentration in transgenic *Kalanchoë*, an alcohol inducible promoter system was used to control the silencing of GA activating enzymes (*GA20ox*). The ethanol treated plants had reduced height, but otherwise appeared normal. The flowering was delayed, but with large variations in time among different transgenic lines (Topp et al., 2008). However, genetically manipulated organisms (GMOs) are not fully accepted by consumers, and transgenic varieties are difficult to register for commercial use, especially in Europe. Consequently, it is necessary to develop and establish new non-toxic methods for growth regulation during plant production. The results of Topp et al. (2008) demonstrated that the ethanol treatment of non-transgenic *Kalanchoë* led to a growth reduction of 66% compared to the non-ethanol-treated control. In *Narcissus tazetta* variety 'Ziva' ethanol

Abbreviations: GA, gibberellic acid; LD, long day; SD, short day.

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concentrations of 1% to 5% in the root-zone reduced plant height without causing a visible phytotoxicity to the roots (Miller and Finan, 2006).

Accordingly, in the present experiments, we examined the possibility of using ethanol as a growth regulator in *Kalanchoë*. We compared ethanol spray treatment and ethanol watering in several *K. blossfeldiana* varieties and other *Kalanchoë* species during the standard cultivation time.

2. Materials and methods

2.1. Plant materials

The *K. blossfeldiana* varieties 'Molly', '1998-469', and 'African Pearl', as well as two species, *K. pubescens* and *K. campanulata*, were obtained from Knud Jepsen A/S (Hinnerup-Denmark), while *K. blossfeldiana* 'Sylt' was obtained from Dehne Topfpflanzen GmbH & Co. KG (Wismoor-Germany).

Vegetative propagation was performed in an experimental greenhouse at the Leibniz University, Hannover, in November (week 45). One hundred fifty cuttings per genotype were rooted for two weeks in 10 cm-diameter pots with a commercially produced potting substrate with 60% of white peat (Einheitserde) under the following conditions: a temperature of 22 °C/20 °C (day/night) and an 80% relative humidity (RH). Day length was extended to 16 h by SON-T lamps (Osram, 400 W, Philips Co., The Netherlands) that supplied 100 μmol m^{-2} s^{-1} of supplementary assimilation light.

2.2. Ethanol treatments

Spray treatment: Water solutions containing 0%, 2%, 4%, 8% or 20% (v/v) ethyl alcohol that was denatured with 1% petroleum (Fa. Sonnenberg GmbH & Co KG, Braunschweig, Germany) and 0.1% "ProNet-Alfa" wetting agent (PROAgro GmbH, Germany) were prepared. For all treatments, a spray flask was used. Spray applications of 10–15 ml per plant started after the two week rooting period, with spraying being performed once weekly for 8 weeks.

Watering treatment: Water solutions containing 0%, 0.5%, 1%, 2% or 4% (v/v) ethyl alcohol (as above) that was denatured with 1% petroleum were prepared. Two-week-old rooted cuttings received 50 ml of the ethanol solution per plant once a week for 16 weeks.

2.3. Cultivation

The experimental plants were cultivated under the following conditions: a temperature of 20 °C/18 °C (day/night), a 60% relative humidity (RH) and 100 μmol m^{-2} s^{-1} supplementary assimilation light. To investigate the vegetative growth, all plants were cultivated under long day (LD) conditions (16 h light) for 6 weeks. Plants that received the spray treatment were grown in the experimental greenhouse at the University of Hannover between December and January, and plants that received the watering treatment were grown between October and March. For flower induction, initiation and development, the plants that received watering applications were transferred to short day (SD) conditions with 8 h of light for an additional 19 weeks.

2.4. Evaluation and statistical analyses

The experiments were conducted in a completely randomized design, using fifteen plants replications per treatment of each genotype. The plant height from the soil surface to the apical meristem was measured weekly. Additionally, physiological reactions, such as chlorotic or necrotic spots, leaf deformations and dead plants, were evaluated. The final flower initiation time was defined as the time from the start of the short day (SD) treatment until a visible change in the meristem was observed (development of the inflorescence). The flowering time was defined as the time from the start of the SD treatment until the first open flower (anthesis) appeared. The experiment using the spray application ended after 8 weeks under the long day (LD) condition, while the plants that received the watering treatment were cultivated and evaluated for 16 weeks (4 weeks of LD + 12 weeks of SD). For detailed statistical analyses, the plant height and the number of internodes were recorded after 4 and 16 weeks cultivation time (LD and SD). Additionally, the data regarding the flower initiation time, time to first flower and the lengths of the inflorescences were collected during the entire SD period. To collect all data for the opening of the first flower (anthesis), plants were cultivated for additional 8 weeks under SD conditions without ethanol treatment.

The node number and the total length of the plants were analysed by linear mixed models. After fitting the model, multiple comparison procedures (Tukey's multiple comparison test modified by Hothorn et al. (2008)) were used to compare the means of the internode lengths, inflorescence length and time of anthesis after different ethanol treatments for each genotype. The statistical analysis was performed using the programme R 2.12.1 (R Development Core Team, 2010), Vienna, Austria.

3. Results

3.1. Growth control with ethanol sprays

Ethanol sprays at concentrations up to 8% ethanol had very little effect on internode length in the genotypes tested (Fig. 1). Spray treatments with 20% ethanol resulted in significant reductions in internode lengths for all of the treated varieties and species (Fig. 1). Unfortunately, the plants were weak and had smaller leaves. 'Molly' showed wilting leaves with ethanol concentration above 8%. After 8 weeks of treatment with the 20% ethanol spray, 60% of 'Molly' and 70% of *K. pubescens* plants were dead (data not shown). All other investigated varieties survived treatment with the 20% ethanol spray.

To reduce the internode length using ethanol spray treatment, high ethanol concentrations were necessary for all tested varieties. However, after treatment with such high ethanol concentrations, most of the experimental plants began to wilt and collapse. Consequently, the experiment was ended after 8 weeks.

3.2. Growth control by watering with different ethanol concentrations

Weekly watering with 50 ml of a water solution containing ethanol at concentrations ranging from 0.5% to 2% did not result in any damage to the leaves or roots in any of the investigated varieties or species (data not shown). *K. pubescens* and *K. campanulata* had adverse reactions to the highest concentration of 4% ethanol, as evidenced by the smaller and deformed leaves and weak stems (data not shown). All of the investigated varieties of *K. blossfeldiana* were able to tolerate the weekly 4% ethanol treatments without any observed symptoms in the leaves, roots or stems (data not shown).

The application of 0.5% ethanol over six weeks had no significant effects on the growth reduction of the 'Molly', '1998-469', and 'Sylt' varieties or *K. pubescens*. However, 'African Pearl' and *K. campanulata* exhibited significantly reduced internode lengths after treatment with ethanol concentrations of 0.5% or higher (Fig. 2). Concentrations higher than 0.5% (up to 4%) had no significant influence on the internode lengths of the 'African Pearl' variety. Significant correlations were observed between the ethanol