Biomass Distribution in Kalanchoe blossfeldiana Transformed with rol-genes of Agrobacterium rhizogenes

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Additional index words. dwarfism, hairy roots, ornamentals, Ri plasmid

Abstract. Kalanchoe blossfeldiana transformed with Agrobacterium rhizogenes exhibited marked alterations in morphology and biomass distribution. Plants termed root-inducing (Ri) lines were regenerated from hairy roots produced by inoculating leaf explants with Agrobacterium rhizogenes wild-type strain ATCC15834. Six Ri lines were characterized in a greenhouse trial and all Ri lines had reduced dry weights of main shoot, lateral shoots, leaves, and flowers compared with control plants. The reduction in dry weights of these organs correlated with reduced plant height, shoot length, leaf area, and number of flowers per plant. Furthermore, an altered distribution of dry matter was evident in the Ri plants, where the greater part of dry matter was allocated into leaves and secondly into flowers, whereas the majority of dry matter in control plants was allocated into flowers and secondly into leaves. Furthermore, a higher percentage of dry matter was allocated into the main shoot of the Ri lines in comparison with that of control plants. Increased dry matter in leaves and in the main shoot in the Ri lines appeared to be at the expense of dry matter allocated into flowers. Moreover, an increased number of vegetative lateral shoots was recorded in the Ri lines, whereas the number of reproductive lateral shoots was decreased. Possible mechanisms behind the altered resource distribution are discussed.

Transforming crop plants with the root loci genes, termed rol-genes of Agrobacterium rhizogenes, have been used to create new genotypes with desired characteristics (Christensen et al., 2008; Handa, 1992; Hosokawa et al., 1997; Pellegrineschi and Davolio-Mariani, 1996). Plants transformed with these genes exhibit varying degrees of altered phenotype termed the Ri (root-inducing) phenotype. The typical Ri phenotype exhibits reduced plant height and apical dominance giving the plant a compact and bushy appearance (Handa, 1992; Hosokawa et al., 1997; Pellegrineschi and Davolio-Mariani, 1996). Furthermore, the rol genes have been shown to improve postharvest performance (Christensen and Müller, 2009a). These characteristics are very valuable in ornamental plants. The aim of the present study was to further characterize plants obtained in an earlier study (Christensen et al., 2008). The focus of the investigation was to elucidate alteration in biomass distribution between vegetative and reproductive shoots, because changes in resource allocation in flowering plants potentially influence ornamental value.

The source of the rol genes is the soil-borne bacterium A. rhizogenes, and this bacterium is the causative agent of the hairy root disease characterized by the formation of a large number of roots at the site of infection. During disease inception, a piece of DNA, the T-DNA, which is situated at the large Ri plasmid, is transferred from the bacteria to the plant cell nucleus and integrated into the plant nuclear genome (Chilton et al., 1982). Expression of T-DNA genes, especially rol genes, is responsible for the disease symptoms (White et al., 1985). Plants containing Ri T-DNA can be generated from the hairy roots and these plants exhibit the typical Ri phenotype (Christensen et al., 2008; Handa, 1992; Hosokawa et al., 1997; Pellegrineschi and Davolio-Mariani, 1996). The rol genes are the main determinants of the Ri phenotype (Slighotom et al., 1986; White et al., 1985).

The wild-type A. rhizogenes strain ATCC15834 has been used in molecular breeding studies to produce Ri plants (Christensen et al., 2008; Jaziri et al., 1994; Pellegrineschi et al., 1994) and this strain contains the agropine-type plasmid pR15834. This plasmid contains two T-DNA regions, termed T1-DNA and T2-DNA (Jouanin, 1984; Meyer et al., 2000), which are separated from each other by approximately 24 Kb of nontransferred DNA and transferred independently into the plant (Durand-Tardif et al., 1985). The T1-DNA has a length of approximately 20 Kb and it contains at least 18 open reading frames (ORF) and ORF 10, 11, 12, and 15 coincided with rolA, rolB, rolC, and rolD, respectively (Slighotom et al., 1986; White et al., 1985). The rol genes can be transferred to plants either as single genes by recombinant DNA technology or together with the whole T-DNA by natural transformation using wild-type A. rhizogenes strains (Schmulling et al., 1988). The advantage of using wild-type strains is that transformed plants can be produced without the use of recombinant DNA technology and the natural transformation processes do not fall directly under the definition of genetically modified organism by the European Union (European Union, 2001). Furthermore, by using wild-type strains, no marker genes are needed for selection, because transformed plants can be selected based on the hairy root morphology (Christensen et al., 2008; Giovannini et al., 1997).

Expression of rolA in transgenic plants results in an aberrant phenotype characterized by dwarf or semidwarf plants with reduced internodes, whereas the rolB and the rolC gene seem to be important in hairy root formation. Plants transformed with rolB alone exhibit often an altered morphology such as reduced apical dominance and wrinkled leaves. rolC has a cytokinin-like effect in plants, and plants transformed with rolC alone show reduced height and apical dominance, increased number of lateral shoots, earlier flowering, reduced flower size, and reduced pollen production. Little is known about the function of rolD, although some reports on early flowering are available (Christensen and Müller, 2009b). Despite the fact that many reports on Ri plants exist, the function of the rol genes is far from elucidated and it has been implicated that rol genes are altering hormone homeostasis of Ri plants (Prinsen et al., 1994). Because Ri plants have changed morphology, physiology, and hormone homeostasis, it can be hypothesized that rol genes have an impact on biomass distribution. Therefore, the objective of this study was to analyze the influence of rol genes on resource distribution in Ri lines of Kalanchoe blossfeldiana.
transformed with \textit{A. rhizogenes} strain ATCC15834.

\textbf{Materials and Methods}

\textit{Plant material.} \textit{Kalanchoe blossfeldiana} ‘Molly’ control plants and Ri lines 306, 312, 317, 319, 324, and 331 with varying degrees of Ri phenotypic expression developed from transformation with \textit{rol} genes of wild-type \textit{A. rhizogenes} strain ATCC15834 (Christensen et al., 2008) were used. The plants were propagated by cuttings, which were rooted in peat (Pindstrup II; Pindstrup Mosebrug A/S, Ryomgaard, Denmark) under clear plastic in a propagation room at 20 °C. Light was provided by SON-T high-pressure sodium lamps (Philips, Amsterdam, The Netherlands) for a 16-h photoperiod and with an intensity of 145 \textmu mol m^{-2} s^{-1} (photosynthetically active radiation) at the plant surface. After 14 d, 32 rooted cuttings of each Ri line and control plants were transplanted into plastic pots (11 cm) with peat (Pindstrup II) and transferred to the greenhouse. Four weeks after transferring to the greenhouse, flower induction was started by short-day treatment (8 h light) at 22/18 °C day/night. The plants were irrigated twice a week with standard fertilizer (Brun Komplet; Garta A/S, Copenhagen, Denmark) with an electrical conductivity of 1.2 mS cm^{-1}.

The experiment was evaluated 100 d after the start of the short-day treatment when all plants flowered. Data on number and length of vegetative and reproductive lateral shoots and number of flowers were recorded; leaf area was measured using a leaf area meter (LI-3100 Area Meter; LI-Cor Biosciences, Lincoln, NE). Flowers, main shoot, lateral shoots, and leaves were dried separately at 85 °C for 5 d for dry weight measurements and the leaf area ratio \((\text{cm}^2 \cdot \text{g}^{-1} \text{ plant dry weight})\) (Poorter and Remkes, 1990) and specific leaf area (SLA) \((\text{cm}^2 \cdot \text{g}^{-1} \text{ leaf dry weight})\) (Garnier et al., 2001) were calculated. The experiment was carried out as a block experiment consisting...
of eight blocks with one replicate per block and the experiment was repeated four times.

Statistical analysis. The data obtained were subjected to analysis of variance using the general linear models (Mardia et al., 1980) (PROC GLM) procedure in the Statistical Analysis System (SAS Institute, Cary, NC). Multiple comparisons among means were performed using Duncan’s multiple range test with the level of significance at $P = 0.05$ (Duncan, 1955).

Results

The mean length of vegetative shoots was significantly increased ($P < 0.001$) in the Ri lines except in Ri line 319 (Figs. 1 and 2A), whereas the mean shoot length of reproductive shoots was significantly reduced ($P < 0.001$) in Ri lines when compared with the values of control plants (Fig. 2B).

The total number of lateral shoots per plant in the Ri lines compared with control plants was either at the same level as in the case of Ri line 312, significantly increased ($P < 0.001$) as in Ri line 331 or reduced as in the other Ri lines (Fig. 2C). However, comparing the number of reproductive and vegetative shoots instead of the total number of shoots gave another picture of shoot development in the Ri lines. In comparison with control plants, the number of vegetative shoots per plant in the Ri lines was significantly increased ($P < 0.001$) except in Ri line 319 (Fig. 2D), whereas the number of reproductive shoots was significantly reduced ($P < 0.001$) except in Ri line 331 (Fig. 2E). The modification in the number of vegetative and reproductive shoots in the Ri lines resulted in a significant change ($P < 0.001$) in the distribution of reproductive and vegetative shoots (Fig. 3A). All Ri lines produced a higher percentage of vegetative shoots compared with the control plants and the percentage of vegetative shoots was 0.5% in control plants and varied from 15.3% in Ri line 317 to 32.8% in Ri line 324.

The total leaf area per plant was significantly reduced ($P < 0.001$) in the Ri lines compared with control plants (Table 1). The leaf area ratio was significantly increased ($P < 0.001$) in the Ri lines compared with that of control plants (Table 1). Also, the mean dry matter of flowers, lateral shoots, main shoot, and leaves was reduced significantly ($P < 0.001$) in the Ri lines compared with control plants (Table 2). The marked decrease in the amount of dry matter recorded for these organs resulted in a significant reduction ($P < 0.001$) of the total dry matter per plant of the Ri lines (Table 2). The distribution of dry matter of flowers, lateral shoots, main shoot, and leaves relative to the total plant dry weight changed significantly. A significant amount of dry matter ($P < 0.001$) was allocated into the leaves of the Ri lines and the dry matter allocation into leaves in control plants was 34.7%, but it increased to 43.9% in Ri line 306, which had the lowest percentage dry matter distributed into leaves of the Ri lines (Fig. 3B). Ri line 312 had the highest percentage dry matter (55.9%) allocated into leaves. In the Ri lines, the percentage of dry matter allocated into flowers was significantly reduced ($P < 0.001$), whereas the percentage of dry matter distributed into the main shoot was significantly increased ($P < 0.001$) compared with control plants. The percentage of dry matter allocated into flowers of the control plants was 44.3% and varied from 22.3% to 39.6% in the Ri lines. The percentage of dry matter allocated into the main shoot was 8.8% in control plants and varied from 10.0% to 11.1% in

![Fig. 3. Lateral shoot and dry matter distribution of K. blossfeldiana ‘Molly’ control plants and root-inducing lines transformed with A. rhizogenes strain ATCC15834.](image)

**Table 1. Mean leaf area, leaf area ratio, specific leaf area, number of flowers, dry weight per flower, flower diameter, and flower dry weight ratio of K. blossfeldiana ‘Molly’ control plants and root-inducing lines transformed with A. rhizogenes strain ATCC15834.**

| Line | Leaf area per plant (cm$^2$) | Leaf area ratio (cm$^2$ g$^{-1}$ plant dry wt) | Specific leaf area (cm$^2$ g$^{-1}$ leaf dry wt) | Number of flowers per plant | Flower diam (mm) | Dry wt per flower (mg) | Flower diam and flower dry wt ratio (mm g$^{-1}$ flower dry wt) |
|------|-----------------------------|---------------------------------------------|-----------------------------------------------|-----------------------------|----------------|----------------------|---------------------------------------------------------------|
| Control | 1108 a$^*$ | 86 c | 253 b | 858 a | 17.9 a | 6.9 a | 2.7 d |
| 306 | 448 d | 118 c | 273 ab | 399 b | 14.1 c | 4.8 c | 3.7 b |
| 312 | 515 c | 150 a | 269 ab | 281 cd | 12.4 e | 2.8 d | 4.5 a |
| 317 | 337 e | 112 c | 256 b | 323 c | 13.9 c | 3.8 c | 3.7 b |
| 319 | 275 e | 97 d | 201 c | 235 d | 13.2 d | 4.1 c | 3.4 b |
| 324 | 515 c | 148 a | 270 ab | 277 cd | 12.5 e | 2.8 d | 4.5 a |
| 331 | 739 b | 130 b | 291 a | 430 b | 15.2 b | 4.8 b | 3.3 c |

$^*$The numbers in a column followed by different letters (a, b, c, d, e) are significantly different at $P \leq 0.05$ by Duncan’s multiple range test. Mean $\pm SD$ (n = 32).
Table 2. Mean dry matter of flowers, leaves, lateral shoots and main shoot of K. blossfeldiana 'Molly' control plants and root-inducing lines transformed with A. rhizogenes strain ATCC15834.

| Line   | Flowers (g) | Lateral shoots (g) | Main shoot (g) | Leaves (g) | Total (g) |
|--------|-------------|--------------------|----------------|------------|-----------|
| Control | 5.70 (± 0.45) a | 1.59 (± 0.15) a | 1.13 (± 0.10) a | 4.47 (± 0.39) a | 12.88 (± 0.94) a |
| 306    | 1.51 (± 0.17) c | 0.26 (± 0.04) d | 0.34 (± 0.06) de | 1.92 (± 0.24) c | 3.48 (± 0.48) cd |
| 312    | 0.79 (± 0.17) e | 0.42 (± 0.10) c | 0.33 (± 0.04) e | 1.33 (± 0.15) c | 3.06 (± 0.36) de |
| 317    | 1.22 (± 0.16) d | 0.33 (± 0.02) d | 0.33 (± 0.04) d | 1.64 (± 0.11) c | 3.83 (± 0.32) cd |
| 319    | 0.96 (± 0.13) e | 0.39 (± 0.05) e | 0.38 (± 0.06) c | 1.93 (± 0.19) c | 3.52 (± 0.32) cd |
| 324    | 0.80 (± 0.14) e | 0.41 (± 0.08) c | 0.38 (± 0.06) c | 1.93 (± 0.19) c | 3.52 (± 0.32) cd |
| 331    | 1.95 (± 0.20) b | 0.63 (± 0.08) b | 2.56 (± 0.15) b | 5.73 (± 0.42) b |

*aThe numbers in a column followed by different letters (a, b, c, d, e) are significantly different at P < 0.01 by Duncan’s multiple range test. Mean ± s (n = 32).
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