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Quantitative and qualitative characterization of novel features of *Kalanchoë* interspecific hybrids

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Abstract *Kalanchoë blossfeldiana* and its cultivars are important worldwide as potted plants. Their improvement relies mainly on cross-breeding and interspecific hybridization, although the use of the resource of wild species was limited. The present study aimed to characterize novel features of interspecific hybrids that can increase attractiveness of these commercially important ornamental plants. We investigated the new traits of selected hybrids resulting from hybridization between *K. blossfeldiana* and three wild *Kalanchoë* species, namely *K. nyikae*, *K. pubescens* and *K. marnieriana*. The selected qualitative and quantitative traits of plant growth and flowering were analyzed in hybrids and parental species. The analysis demonstrated generally intermediate character of hybrids. Following principal component analysis, clear separation among plants forming three distinct clusters was observed, where hybrids were situated between the two parental species. The plants were clearly grouped according to flower characteristics, plant height and timing of flowering in all investigated groups. Quantitative analysis also revealed significant differences among parental plants and hybrids for all examined characteristics. Transgressive segregation and heterosis were observed in relation to specific traits such as days until first open flower, plant height, number of inflorescences, flower diameter and number of flowers. Pollen analysis of the interspecific hybrids revealed low viability of pollen or complete sterility. The interspecific hybrids exhibited novel features valuable for breeders. Thus, wide hybridization can contribute to the development of new cultivars and this study clearly demonstrates the usefulness of interspecific crosses in the improvement of *Kalanchoë* cultivars.

Keywords Heterosis · Hybrid sterility · Intermediate phenotype · Interspecific hybrid · *Kalanchoë* · Transgressive trait

Introduction

In the improvement of ornamental plants, distant hybridization is still a leading strategy to increase genetic variability in commercial cultivars. The *Kalanchoë* genus consists of around 140 species native to Madagascar, Southern and Eastern Africa,

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and to some extent, tropical Africa, the Arabian Peninsula, and Southern Asia. Several *Kalanchoë* species possess characteristics that can be of commercial value. Bell-shape pendant flowers are a common feature of the *Bryophyllum* section that can be interesting in breeding cultivars with new flower shapes. Epiphytic species such as *K. gracilipes*, *K. ndotoensis* and *K. porphyrocalyx* have potential for breeding of creeping and hanging cultivars. Plant fragrance can be obtained by hybridization with *K. aromatica* that has glandular-hairy aromatic indumentum as well as *K. thyrsiflora*, *K. petitiana* or *K.* × *ena*, which have scented flowers (Descoings 2003; Currey and Erwin 2011).

Kalanchoë blossfeldiana and its interspecific hybrids are popular potted indoor plants and garden plants mainly due to abundant flowering and low demand of water and nutrients. *K. blossfeldiana*-derived cultivars represent one of the economically most important potted plants in Europe with an annual production of 41 million plants in Denmark (Floradania 2014) and 83 million plants sold on auctions in The Netherlands (FloraHolland 2014) in 2013. The commercial value of these ornamental plants leads to continuous development of new cultivars that are more attractive for consumers and have reduced production costs (Lütken et al. 2012).

Kalanchoë blossfeldiana was introduced to Europe from Madagascar in 1924. After cultivation in botanical gardens, breeding of new cultivars was initiated in the 1930s. The new plants, however, resulted from a selection within the progeny of a single plant. Distant hybridization was initiated in 1939, nevertheless the use of resources of wild plants was limited and breeding goals focused essentially on flower characteristics and dwarf growth habit. Some of the *Kalanchoë* cultivars were developed using naturally occurring mutants, especially in respect to flower color (Voorst and Arends 1982). Mutation breeding did not play an important role in development of new commercial varieties (Descoings 2003). In recent years, several interesting traits have been introduced into *K. blossfeldiana* using genetic engineering resulting in production of compact and dwarf plants, plants with reduced ethylene-sensitivity, and male-sterile plants (Christensen et al. 2008; Garcia-Sogo et al. 2010; Lütken et al. 2010, 2011).

Interspecific crosses among two cultivars of *K. blossfeldiana* and species belonging to the *Kalanchoë* genus resulted in production of several interspecific hybrids (Kuligowska et al. 2015). The present study aimed to evaluate novel features of the obtained hybrids, morphological traits and their ornamental values as well as assessment of flowering characteristics and overall usefulness of the hybrids as material in further breeding programs. We also investigated the consequences of interspecific hybridization with relevance for commercial production of new cultivars such as plant vigor and occurrence of post-fertilization barriers.

Materials and methods

Plant material

Five genotypes belonging to four *Kalanchoë* species used as parents in interspecific hybridization (obtained from the nursery Knud Jepsen A/S, Hinnerup, Denmark) and 34 genotypes of hybrids obtained from six cross-combinations, resulting from interspecific hybridization (Kuligowska et al. 2015), were used in the experiment. A specific overview of the plant material is shown in Table 1.

Kalanchoë species and hybrids were established from stem cuttings of 2–3 leaf pairs in 11 cm pots with peat (Pindstrup Substrate no. 1, Pindstrup Mosebrug A/S, Kongerslev, Denmark). The potted plants were maintained in the greenhouse under 16/8 h photoperiod and 22/18 °C ± 4 °C, day/night with additional light 180 μmol s^{-1} m^{-2} (Philips Master SON-T PIA Green Power 400 W, Eindhoven, The Netherlands). The plants were irrigated weekly with fertilizer (Pioner NPK Makro 14-3-23, Tilst, Denmark) with an electrical conductivity of 1.3 mS cm^{-1}. After 4 weeks of rooting, plants were transferred to short day conditions (8/16 h, day/night) in 22/18 °C ± 2 °C, day/night and irrigated every third day with fertilizer (Pioner NPK Makro 14-3-23) with an electrical conductivity of 1.3 mS cm^{-1}. The experiment was carried out from January until July 2014.

Data collection

Data were collected in terms of number of days until first open flower, number of days until first wilted flower, flower longevity, plant height, broadest plant diameter, number of inflorescences, diameter of the

Table 1 Plant material used in the experiment

	Section	Number of genotypes
Parental plants		
K. blossfeldiana ‘0089A’	*Kalanchoë*	1
K. blossfeldiana ‘Jackie’	*Kalanchoë*	1
K. nyikae	*Kalanchoë*	1
K. pubescens	*Bryophyllum*	1
K. marnieriana	*Bryophyllum*	1
Hybrids		
K. blossfeldiana ‘0089A’ × *K. nyikae*	Intra-sectional	6
K. nyikae × *K. blossfeldiana* ‘0089A’	Intra-sectional	6
K. blossfeldiana ‘0089A’ × *K. pubescens*	Inter-sectional	6
K. blossfeldiana ‘0089A’ × *K. marnieriana*	Inter-sectional	6
K. blossfeldiana ‘Jackie’ × *K. nyikae*	Intra-sectional	4
K. blossfeldiana ‘Jackie’ × *K. pubescens*	Inter-sectional	6

Hybrids resulting from interspecific hybridization (Kuligowska et al. 2015)

flower, length of the flower, length of the style, and number of flowers.

Days to first open and wilted flower were calculated by subtracting the date of placement of plants into short day condition from the date of first flower opening/wilting. Flower longevity was determined as the difference between first open and first wilted flower. Plant height, broadest plant diameter were measured on the day when first flower was open. Flower diameter, flower length and style length were determined at the time point of stigma receptivity in the “sticky stage” (Traoré et al. 2014). The number of flowers was determined on the day when first wilted flower was spotted. Total flower number was calculated by adding the terminal and axillary inflorescence flower numbers.

Additionally, information about direction of flower, corolla tube and limb, leaf arrangement and morphology were collected. The flower color was determined by using the Royal Horticultural Society Colour Chart, London, 2005. Data collection was terminated when the first wilted flower appeared for each plant.

Pollen viability

Pollen was collected at the point of anther dehiscence i.e. in the day of flower opening before noon. Pollen of three flowers was immersed in the drop of 1 % (w/v) acetocarmine solution. Pollen was examined under a light microscope (Leica DM2000 LED, Leica, Germany) and pollen grains were scored (stained red as viable and unstained as unviable) (Singh 2002). At least 100 pollen grains were analyzed per plant.

Data analysis

The experiment was designed in a randomized block design and replicated twice in time (displaced 3 weeks) with eight plants per replicate. The mean value (±SE) for each character was calculated as the average for the 16 plants and used for the statistical analysis.

An overview of the variation among parental plants and hybrids in terms of quantitative traits was obtained using principal component analysis (PCA) in the SPSS 22.0 for windows statistical software package (SPSS Inc., Chicago, IL, USA).

The significance of differences between genotypes was determined using one-way analysis of variance (ANOVA) followed by Tukey’s honestly significant difference test in the SPSS 22.0 for windows statistical software package (SPSS Inc., Chicago, IL, USA).

Results

Qualitative assessment

The morphological features of maternal genotypes and selected hybrids were assessed (Figs. 1, 2, 3). All the hybrids showed intermediate phenotypes between both parental genotypes, but also features of one-parent origin and new hybrid features were present. Tables 2 and 3 summarize findings of qualitative assessment of vegetative and generative characteristics.

Growth habit (Fig. 1) and flower features (Fig. 2) had a clearly intermediate character. The flowers of all hybrids had intermediate shape and color between

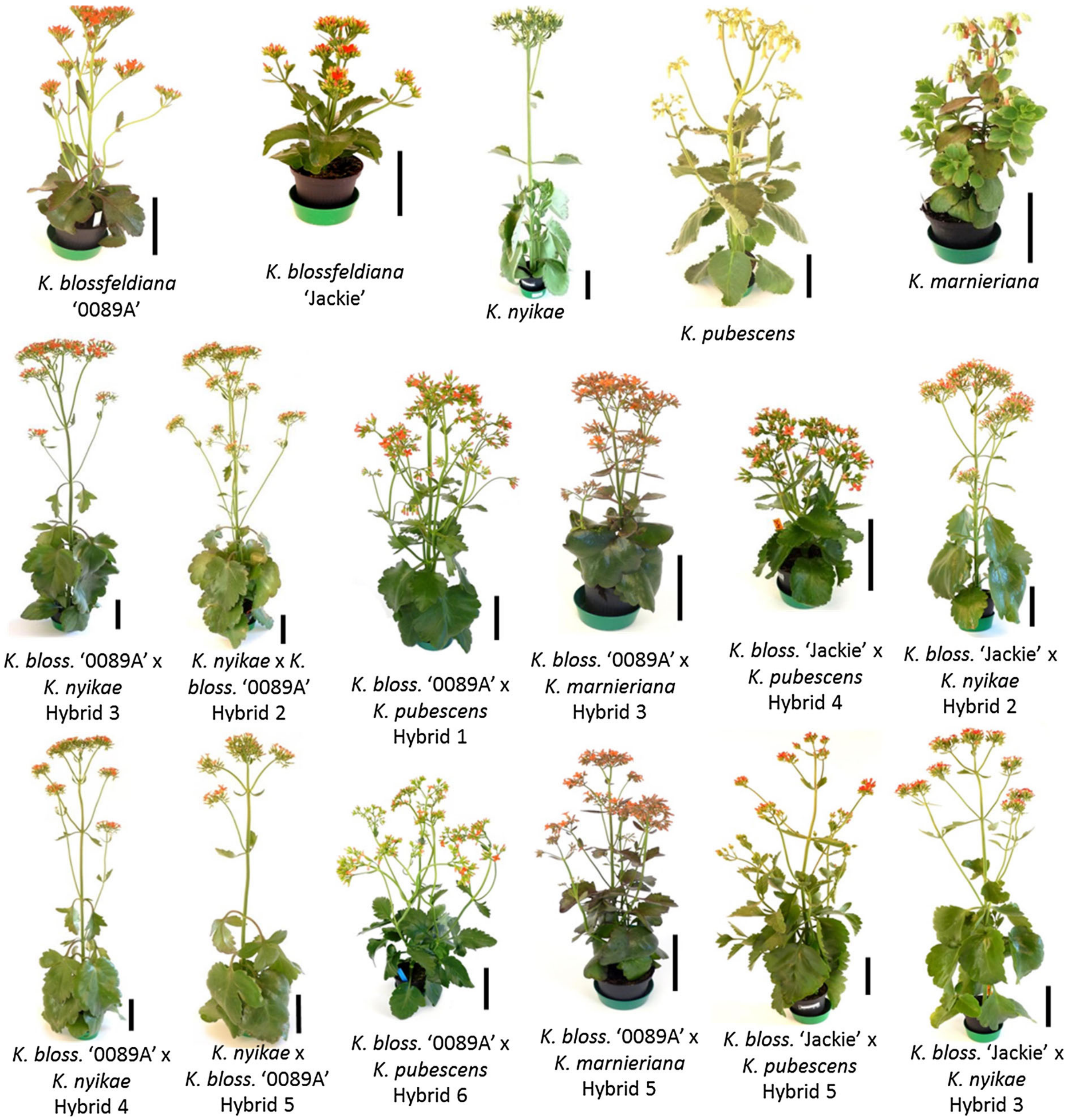


Fig. 1 Flowering plants of *Kalanchoë* species and interspecific *Kalanchoë* hybrids, *Scale bars:* 10 cm

both parental genotypes, with the exception for the occurrence of pink flowers resulting from the cross between red *K. blossfeldiana* 'Jackie' and yellow *K. nyikae* (Fig. 2a, b). Hybrids resulting from intersectional crosses had filaments fused with the corolla in the middle part of the corolla tube, while members of different sections had them attached above or below the middle of the corolla tube (Fig. 2c).

The flowers of the hybrids resulting from the crosses between *K. blossfeldiana* '0089A' and *K. marnieriana* exhibited altered flower angle according to the developmental stage i.e. pendant to horizontal in bud and upwards in flowering (Table 3). The hybrids between *K. blossfeldiana* and *K. pubescens* showed formation of new types of flowers with alternated number of petals (Fig. 2d).

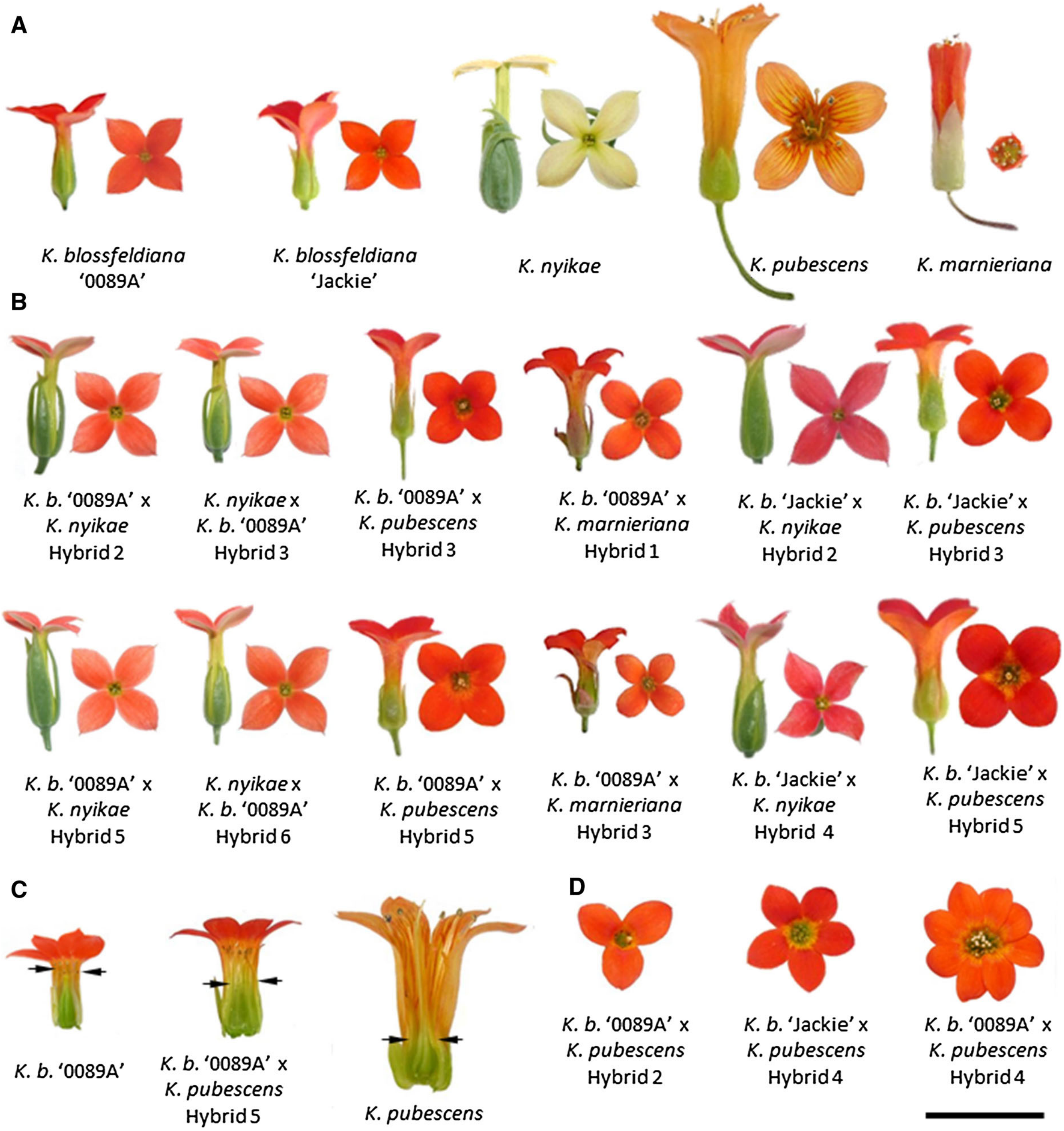
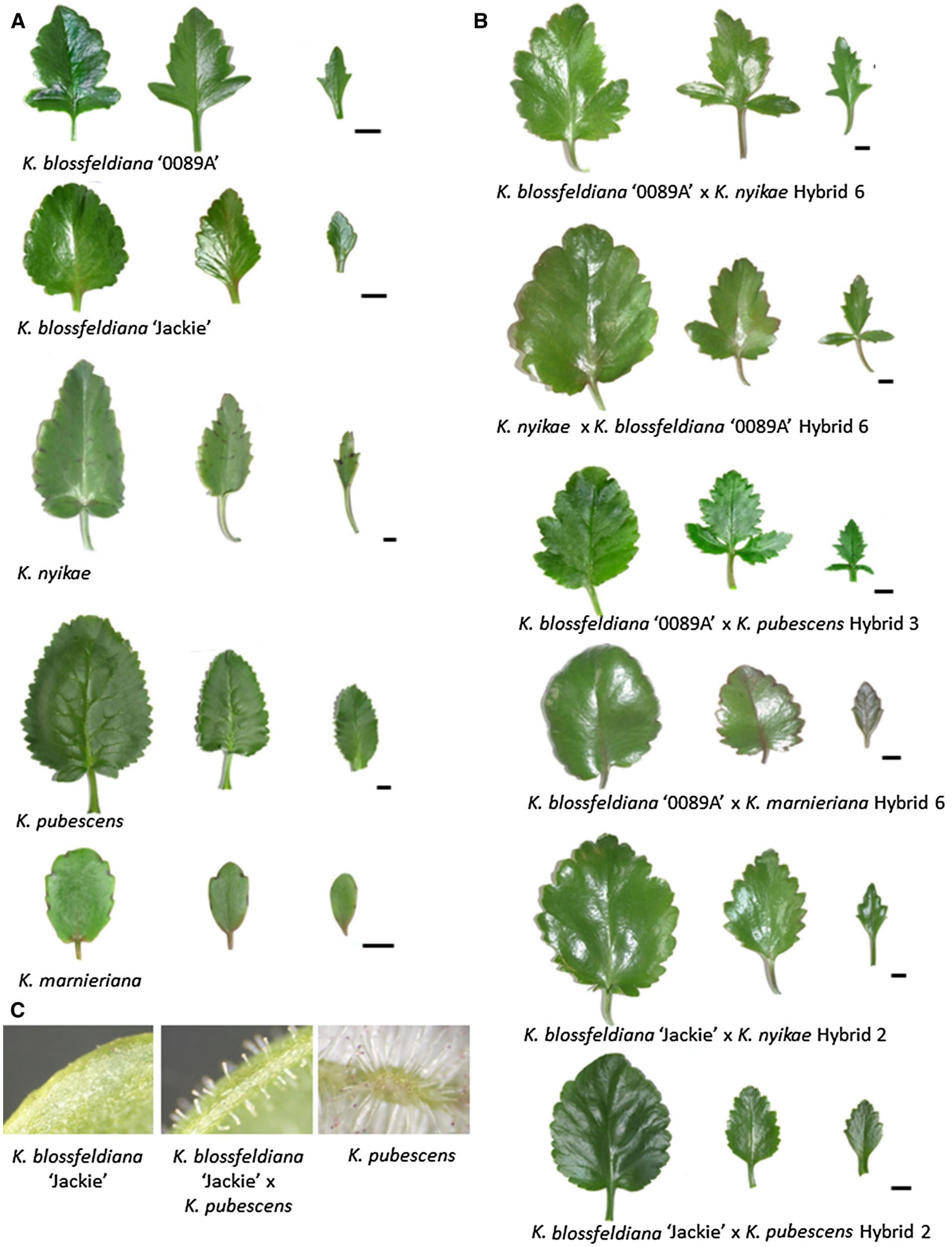


Fig. 2 Flower characteristics of selected interspecific *Kalanchoë* hybrids and their parents **a** *Side* and *top view* of the flowers of parental plants; **b** *Side* and *top view* of the selected interspecific hybrids; **c** Longitudinal inside view of the flower of *K. blossfeldiana* '0089A' (*left*), interspecific hybrid *K. blossfeldiana* '0089A' × *K. pubescens* (*middle*) and *K. pubescens* (*right*). The *arrows* indicate the place of the attachment of filaments to the corolla tube; **d** Variation in the petal number of the interspecific hybrids between *K. blossfeldiana* and *K. pubescens; Scale bars:* 2 cm

The morphologies of leaves from the interspecific hybrids exhibited shapes intermediate to parental genotypes (Fig. 3). In the hybrids originating from the crosses where *K. blossfeldiana* was one of the parents, the lobed leaf margin was more pronounced. Thus, in these hybrids the leaf margin was strongly


A
K. blossfeldiana '0089A'
K. blossfeldiana 'Jackie'
K. nyikae
K. pubescens
K. marnieriana
B
K. blossfeldiana '0089A' x K. nyikae Hybrid 6
K. nyikae x K. blossfeldiana '0089A' Hybrid 6
K. blossfeldiana '0089A' x K. pubescens Hybrid 3
K. blossfeldiana '0089A' x K. marnieriana Hybrid 6
K. blossfeldiana 'Jackie' x K. nyikae Hybrid 2
K. blossfeldiana 'Jackie' x K. pubescens Hybrid 2
C
K. blossfeldiana 'Jackie'
K. blossfeldiana 'Jackie' x K. pubescens
K. pubescens

◀**Fig. 3** Leaf characteristics of selected interspecific *Kalanchoë* hybrids and their parents; **a** Leaves of parental plants; **b** Leaves of hybrid plants; **c** Close up to the leaf surface of *K. blossfeldiana* 'Jackie' (*left*), interspecific hybrid *K. blossfeldiana* 'Jackie' × *K. pubescens* (*middle*) and *K. pubescens* (*right*); *Scale bars:* 2 cm

lobed and some of the leaves could be classified as divided (Fig. 3a, b). The hybrids between *K. blossfeldiana* '0089A' and *K. pubescens* exhibited formation of short glandular hairs on the surface of leaves, stems and flowers. This trait was inherited from *K. pubescens,* that however had longer and more dense hairs (Fig. 3c). *K. marnieriana* has distinct purple spots on the surface of the leaves at the base of crenations. This trait was successfully transferred to interspecific hybrids (data not shown).

Principal component analysis

Clear separation among plants forming three distinct groups was observed, where hybrids were situated between the two parental species (Fig. 4). The first two principal components explained from 69.1 % of total variance observed in the analysis of *K. blossfeldiana* '0089A' × *K. marnieriana* hybrids and parental species (Fig. 4d) to 78.3 % for *K. blossfeldiana* '0089A' × *K. nyikae* hybrids and parental species (Fig. 4a). The first components explained from 44.5 % total variation (*K. blossfeldiana* '0089A' × *K. pubescens* hybrids and parental species—Fig. 4c) to 67.5 % (*K. blossfeldiana* 'Jackie' × *K. nyikae* hybrids and parental species—Fig. 4e). Based on the first components the plants were clearly grouped according

Table 2 Morphological characteristics of parental plants and interspecific hybrids—vegetative traits

Genotype	Vivipary	Leaf arrangement	Leave shape/margin	Leaf/margin coloration	Leaf surface
K. blossfeldiana '0089A'	No	Opposite	Ovate, lobed	Sometimes red to purple coloration of margin	Smooth
K. blossfeldiana 'Jackie'	No	Opposite	Ovate, sinuate-crenate	Sometimes red to purple coloration of margin	Smooth
K. nyikae	No	Opposite	Oval to cordate, crenate-dentate	Sometimes red coloration of margin, purple spots	Smooth
K. pubescens	Yes	Opposite	Elliptic to ovate, crenate-dentate	Uniformly green	Glandular hairs
K. marnieriana	Yes	Opposite	Oblong to ovate, subentire, sometimes a few crenations in the upper part	Red to purple coloration of margin, purple spots	Smooth
K. bloss. '0089A' × *K. nyikae* hybrids	No	Opposite	Ovate, lobed to parted	Uniformly green	Smooth
K. nyikae × *K. bloss.* '0089A' hybrids	No	Opposite	Ovate, lobed to parted	Uniformly green	Smooth
K. bloss. '0089A' × *K. pubescens* hybrids	No	Opposite (hybrids 1–4), opposite, alternate or sometimes whorled (hybrids 5 and 6)	Ovate, lobed to parted	Sometimes purple spots	Short glandular hairs
K. bloss. '0089A' × *K. marnieriana* hybrids	No	Opposite	Orbicular, to ovate, crenate-dentate	Purple coloration of margin, sometimes purple spots	Smooth
K. bloss. 'Jackie' × *K. nyikae* hybrids	No	Opposite	Orbicular to ovate, crenate-dentate	Uniformly green	Smooth
K. bloss. 'Jackie' × *K. pubescens* hybrids	No	Opposite	Orbicular, ovate to oval, crenate-dentate	Sometimes purple spots	Short glandular hairs

to flower characteristics, plant height and timing of flowering in all investigated plant groups. The *K. blossfeldiana* cultivars representing parental plants were mainly separated due to number of days until first open flower, first wilted flower and flower longevity. Wild species of *Kalanchoë* were mainly separated having larger flower diameter and longer flowers and styles (Fig. 4).

Quantitative trait analysis

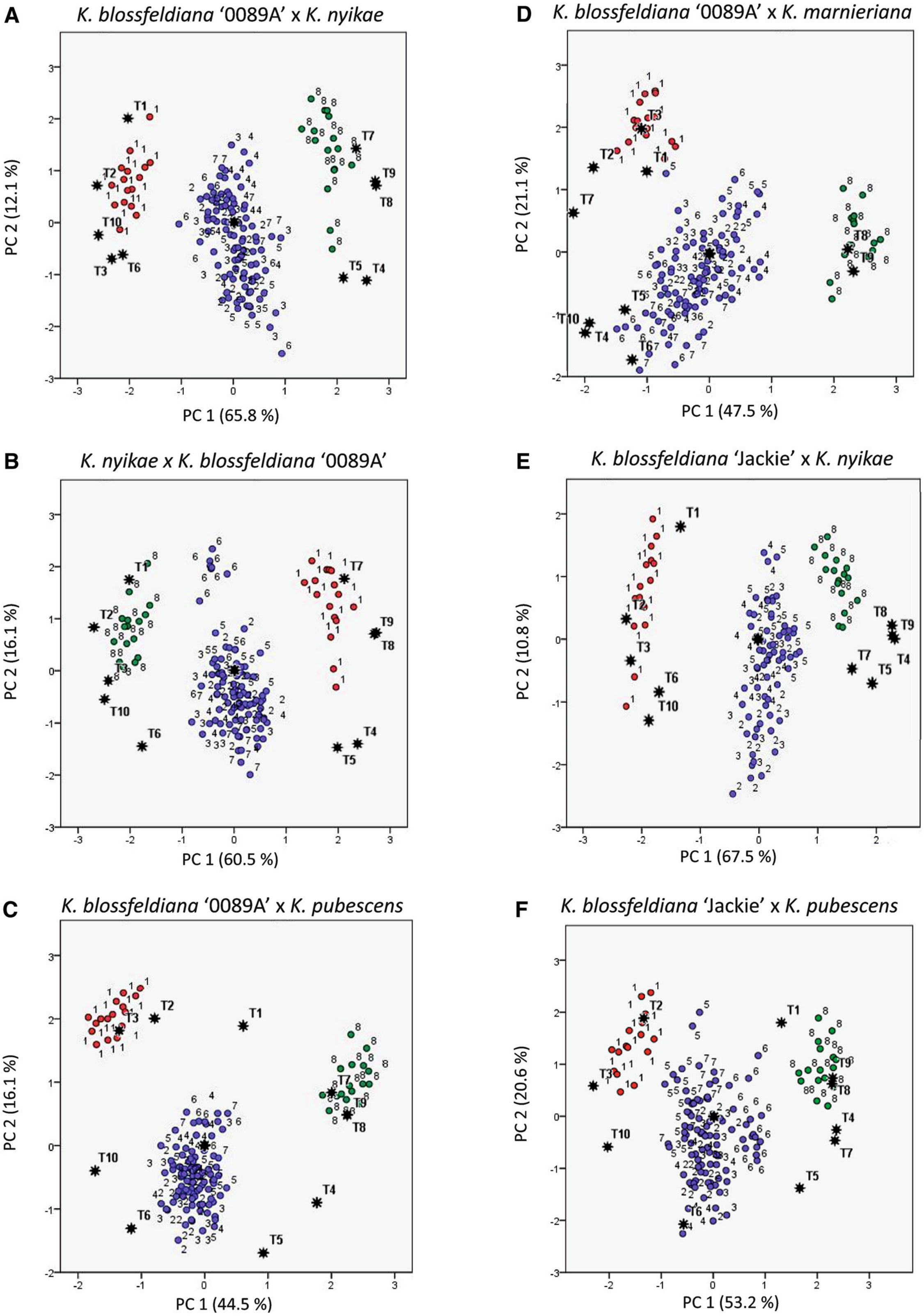
Of the evaluated traits, significant variation between both parental species was observed for all examined traits except for days until first open flower (comparison between *K. blossfeldiana* ‘0089A’—82.1 ± 1.0 days and *K. pubescens*—83.2 ± 0.7 days), plant diameter (*K. blossfeldiana* ‘0089A’—23.8 ± 0.6 days vs. *K. marnieriana*—20.9 ± 1.2 days) and number of inflorescences (*K. blossfeldiana* ‘0089A’—7.6 ± 0.4 vs. *K. marnieriana*—6.4 ± 0.5

Fig. 4 Principal component analysis plot of interspecific hybrids ▶ of *Kalanchoë* and parental species based on the correlation of 10 characters; *red* (*1*)—maternal species, *blue* (*2–7*) – hybrids and *green* (*8*)—paternal species; *T1–10*: traits used for the characterization (*T1*: number of days until first open flower, *T2*: number of days until first wilted flower, *T3*: flower longevity, *T4*: plant height, *T5*: broadest plant diameter, *T6*: number of inflorescences, *T7*: diameter of the flower, *T8*: length of the flower, *T9*: length of the style, *T10*: number of flowers). (Color figure online)

Table 3 Morphological characteristics of parental plants and interspecific hybrids—generative traits

Genotype	Flower direction	No. of petals	Corolla tube	Corolla limb	Corolla color[a]	Filament attachment
K. blossfeldiana ‘0089A’	Erect	4	Narrow	Lobes spreading	Red 41A	Above the middle of the corolla tube
K. blossfeldiana ‘Jackie’	Erect	4	Narrow	Lobes spreading	Red 44A	Above the middle of the corolla tube
K. nyikae	Erect	4	Narrow	Lobes spreading	Light yellow 8C	Above the middle of the corolla tube
K. pubescens	Pendant	4	Wide	Lobes spreading	Orange 28A—orange yellow 14A	Below the middle of the corolla tube
K. marnieriana	Pendant	4	Wide	Lobes slightly spreading	Orange red 41B	Below the middle of the corolla tube
K. bloss. ‘0089A’ × *K. nyikae* hybrids	Erect	4	Narrow	Lobes spreading	Orange red 39B	Above the middle of the corolla tube
K. nyikae × *K. bloss.* ‘0089A’ hybrids	Erect	4	Narrow	Lobes spreading	Orange red 39B	Above the middle of the corolla tube
K. bloss. ‘0089A’ × *K. pubescens* hybrids	Erect	4 and 5, rarely less, frequently more	Intermediate	Lobes spreading	Red 41A, 44A	In the middle of the corolla tube
K. bloss. ‘0089A’ × *K. marnieriana* hybrids	Pendant to horizontal in bud, erect in flowering	4	Narrow	Lobes spreading	Red 41A, orange red 41B, orange 32A	In the middle of the corolla tube
K. bloss. ‘Jackie’ × *K. nyikae* hybrids	Erect	4	Narrow	Lobes spreading	1: orange red 39B, 2–4: dark pink red 52A	Above the middle of the corolla tube
K. bloss. ‘Jackie’ × *K. pubescens* hybrids	Erect	4 and 5, rarely less, frequently more	Intermediate	Lobes spreading	Red 41A, 44A, 47A	In the middle of the corolla tube

[a] Color was determined by using the Royal Horticultural Society Colour Chart, London, 2005


A
K. blossfeldiana '0089A' x K. nyikae
PC 1 (65.8 %)
PC 2 (12.1 %)
B
K. nyikae x K. blossfeldiana '0089A'
PC 1 (60.5 %)
PC 2 (16.1 %)
C
K. blossfeldiana '0089A' x K. pubescens
PC 1 (44.5 %)
PC 2 (16.1 %)
D
K. blossfeldiana '0089A' x K. marnieriana
PC 1 (47.5 %)
PC 2 (21.1 %)
E
K. blossfeldiana 'Jackie' x K. nyikae
PC 1 (67.5 %)
PC 2 (10.8 %)
F
K. blossfeldiana 'Jackie' x K. pubescens
PC 1 (53.2 %)
PC 2 (20.6 %)
T1
T2
T3
T4
T5
T6
T7
T8
T9
T10

and *K. blossfeldiana* 'Jackie'—6.6 ± 0.3 vs. *K. pubescens*—5.1 ± 0.4) (Online Resource 1).

Mean values for traits in the hybrids were equal to, or intermediate between parental species in all cases except for days until first open flower, plant height, number of inflorescences, flower diameter and number of flowers where specific hybrids significantly exceeded ($P \leq 0.05$) the mean values of parental species (Online Resource 1). All hybrids of *K. blossfeldiana* '0089A' × *K. pubescens* (Fig. 5a), hybrid 1 and 2 of *K. blossfeldiana* 'Jackie' × *K. nyikae* (Fig. 5b) and hybrid 1–3 of *K. blossfeldiana* 'Jackie' × *K. pubescens* flowered earlier than any of the parental species (i.e. *K. blossfeldiana* '0089A' ♀—82.1 ± 1.0 days, hybrid 1–72.0 ± 0.7 days, hybrid 2—74.4 ± 0.8 days, hybrid 3—77.3 ± 0.9 days, hybrid 4—73.8 ± 0.7 days, hybrid 5—76.6 ± 0.7 days, hybrid 6—74.4 ± 1.0 days, *K. pubescens* ♂—83.2 ± 0.7 days; *K. blossfeldiana* 'Jackie' ♀ – 81.9 ± 0.7 days, hybrid 1—68.1 ± 0.6 days, hybrid 2—70.5 ± 0.7 days, *K. nyikae* ♂—73.8 ± 0.6 days; *K. blossfeldiana* 'Jackie' ♀—77.5 ± 1.2 days, hybrid 1—70.9 ± 0.6 days, hybrid 2—70.5 ± 0.6 days, hybrid 3—72.0 ± 1.1 days, *K. pubescens* ♂—83.2 ± 0.7 days, respectively). The hybrid 5 and 6 of *K. blossfeldiana* '0089A' × *K. marnieriana* (Fig. 5c) were significantly taller when compared to parental species (*K. blossfeldiana* '0089A' ♀—46.4 ± 1.1 cm, hybrid 5—56.2 ± 1.9 cm, hybrid 6—53.9 ± 1.6 cm, *K. marnieriana* ♂—34.9 ± 1.1 cm). The number of inflorescences in hybrid 2 of *K. blossfeldiana* '0089A' × *K. pubescens,* hybrid 5 and 6 of *K. blossfeldiana* '0089A' × *K. marnieriana,* and hybrid 1, 3 and 5 of *K. blossfeldiana* 'Jackie' × *K. pubescens* (Fig. 5d) exceeded values observed in parental species (i.e. *K. blossfeldiana* '0089A' ♀—7.6 ± 0.4, hybrid 2—9.8 ± 0.4, *K. pubescens* ♂—5.1 ± 0.4; *K. blossfeldiana* '0089A' ♀—7.6 ± 0.4, hybrid 5—10.8 ± 0.7, hybrid 6—11.5 ± 0.5, *K. marnieriana* ♂—6.4 ± 0.5; *K. blossfeldiana* 'Jackie'♀—6.6 ± 0.3, hybrid 1—8.8 ± 0.4, hybrid 3—9.0 ± 0.3, hybrid 5—8.6 ± 0.4, *K. pubescens* ♂—5.1 ± 0.4, respectively). Moreover, the average flower diameter of hybrid 2 of *K. blossfeldiana* 'Jackie' × *K. nyikae* (Fig. Fig. 5e) was greater than any of the parental species (*K. blossfeldiana* 'Jackie' ♀—18.4 ± 0.1 mm, hybrid 2—24.8 ± 0.1, *K. nyikae* ♂—23.6 ± 0.2 mm). The hybrid 5 and 6 of *K. blossfeldiana* '0089A' × *K. marnieriana* (Fig. 5f) had a number of flowers that exceeded parental values (*K. blossfeldiana* '0089A'♀—334.3 ± 25.0, hybrid 6—404.5 ± 38.6, *K. marnieriana* ♂—24.7 ± 2.5). Additionally, the hybrid 1 of *K. blossfeldiana* '0089A' × *K. pubescens* had flowers significantly smaller ($P \leq 0.05$) than any of the parental species (*K. blossfeldiana* '0089A'♀—18.2 ± 0.11 mm, hybrid 1—17.5 ± 0.1 mm, *K. pubescens* ♂—25.0 ± 0.3 mm) (Online Resource 1).

Pollen analysis

The percentage of viable pollen was generally high for the *Kalanchoë* species ranging from 51.0 ± 1.8 % for *K. blossfeldiana* 'Jackie' to 91.8 ± 0.7 % for *K. blossfeldiana* '0089A' (Fig. 6a). Several hybrids resulted from crosses between *K. blossfeldiana* cultivars and *K. pubescens* i.e. hybrids 6 where '0089A' was the maternal plant, and hybrids 1, 4, 5 and 6 where 'Jackie' was the maternal plant, were sterile. For several hybrids it was not possible to determine the percentage of viable pollen due to aggregation of pollen grains. The single stained pollen grains were, however, visible in the clumps of pollen grains. The other hybrids exhibited low viability of pollen between 3.1 ± 0.3 recorded for the hybrid 3 of *K. blossfeldiana* 'Jackie' × *K. pubescens*, and 19.0 ± 1.5 % for the hybrid 3 of *K. blossfeldiana* '0089A' × *K. marnieriana* (Fig. 6a, b).

Discussion

Interspecific hybridization represents a leading strategy in the improvement of ornamental plants. Since the beginning of the breeding of *Kalanchoë* cultivars, the use of wild species was an important approach in the development of new ornamental plants. Interspecific crosses, however, were mainly limited to intra-sectional combinations (Izumikawa et al. 2007). Wild species of the *Kalanchoë* genus may due to their genetic variability significantly improve the existing cultivars (Kuligowska et al. 2015).

In the present study, the interspecific *Kalanchoë* hybrids were characterized regarding their qualitative and quantitative traits. The comparison of hybrids and parental species revealed that characters of hybrids were mostly intermediate. A similar situation was observed following interspecific hybridization between

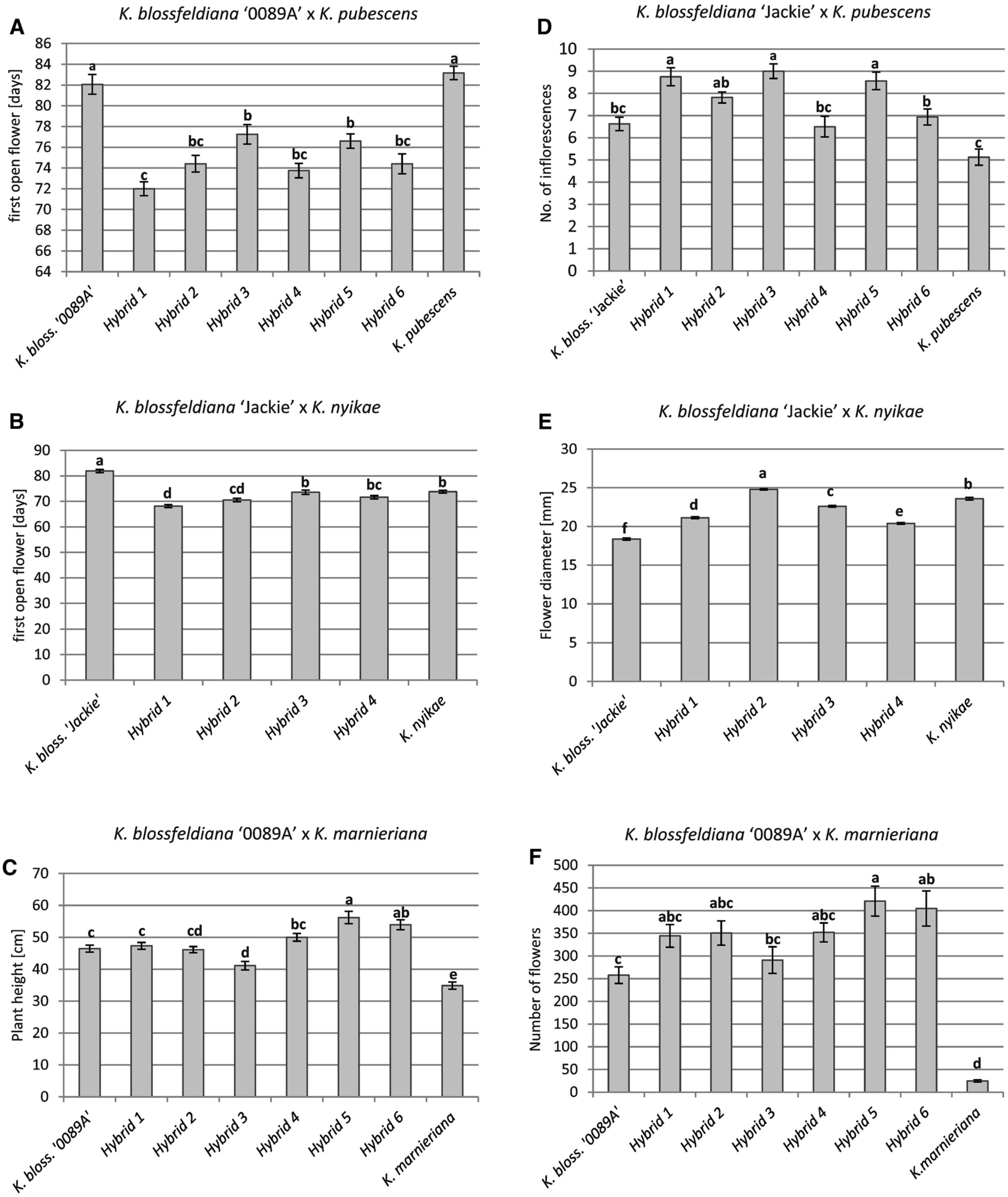


Fig. 5 Selected characteristics of interspecific *Kalanchoë* hybrids and parental species; Values presented are means (± S.E.), values followed by different letters are significantly different ($P \leq 0.05$) according to Tukey's honestly significant difference test

two *Kalanchoë* species, where the progeny generally exhibited intermediate phenotypes (Izumikawa et al. 2007). Principal component analysis was used to summarize patterns of correlations among variables. The overall characterization of interspecific hybrids and parental species based on quantitative features clearly

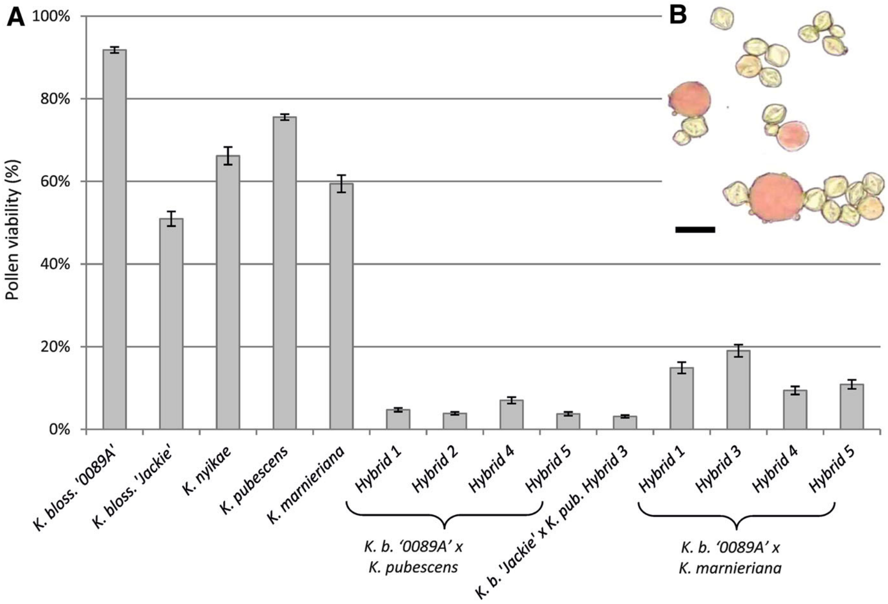


Fig. 6 Pollen viability of parental species and interspecific hybrids; **a** Values presented are means (± S.E.); **b** Acetocarmine staining of pollen of hybrid 2 of *K. blossfeldiana* '0089A' × *K. pubescens, Scale bar:* 50 µm

showed separation of plants into three clusters (Fig. 4). Moreover, the study presented clear differences existing between the parental species used in the hybridization process. Thus, PCA highlighted the intermediate state of hybrids. The intermediacy of hybrid features can be explained by inheritance pattern based on polygenic control with additive effects (Schwarzbach et al. 2001).

The flower color of the majority of interspecific hybrids had an intermediate character (Fig. 2; Table 3), thus the color was a mixture of those of parental species. The heredity of flower color may be controlled by genes with partial dominance as demonstrated in *Pharbitis purpurea* and *Mirabilis jalapa* (Engels et al. 1975; Habu et al. 1998). Partial dominance can also be assumed for the traits such as lobed leaf margin in crosses where *K. blossfeldiana* '0089A' was one of the parental plants.

Some of the morphological features of the hybrids observed in our study had a uniparental character. The formation of violet spots at the base of crenations of leaf margin was successfully transmitted into hybrid progeny of *K. blossfeldiana* '0089A' and *K. marnieriana* from paternal parent. The presence of this characteristic suggests simple dominant inheritance pattern (Schwarzbach et al. 2001). Morphological characterization of intersectional hybrids did interestingly not show formation of viviparous plants in the hybrids. Thus, this trait has most likely a quantitative inheritance background (Izumikawa et al. 2007).

The novel features of hybrids were observed in relation to morphological traits (Tables 2, 3). Several hybrids displayed a significant transgressive segregation and heterosis for a number of traits (Fig. 5). The hybrid 6 of *K. blossfeldiana* '0089A' × *K. marnieriana* had a significantly higher number of flowers at the time point of the first wilted flower, compared with the parental species. This situation can be explained by its high number of inflorescences that combined with relatively long flower longevity may result in exceed of parental values. The interspecific hybrids were generally characterized by vigorous growth. Several hybrids exhibited earlier flowering compared to the parental species. This remarkable feature may be attributed to faster developmental rate of hybrids as demonstrated in *Petunia* (Warner and Walworth 2010).

The explanation of the expression of transgressive traits in interspecific hybrids includes the complementary action of new combinations of existing alleles, epistasis and elevated mutation rate (Rieseberg and Carney 1998; Rieseberg et al. 1999). More recent research suggests the role of genomic shock that induces qualitative and quantitative changes in epigenetic regulation and can lead to morphological changes and transgressive segregation in hybrid progeny. The nature of genomic shock includes chromosomal rearrangement, gain and loss of chromosome segments, gene repression and activation, transposon activation and changes in the patterns of cytosine methylation (Ng et al. 2012; Wang et al. 2014).

The obtained hybrids exhibited low or no fertility of the examined pollen samples (Fig. 6). Thus, this fact can limit their usefulness in further breeding programs. Surprisingly, the hybrids resulted from hybridization of species of the same ploidy level were sterile in our studies (*K. blossfeldiana*—$2n = 4x = 68$ and *K. nyikae* $2n = 4x = 68$), whereas the interploidy crosses (*K. blossfeldiana*—$2n = 4x = 68$ and *K. pubescens* $2n = 2x = 34$) were both fertile and sterile or only fertile (*K. blossfeldiana*—$2n = 4x = 68$ and *K. marnieriana* $2n = 2x = 34$) (Kuligowska et al. 2015). These results were opposite to what can be expected, they however agreed with previous studies on interploidy crosses between *K. spathulata* and *K. laxiflora* (Izumikawa et al. 2007). The possible reason for hybrid sterility is the effect of chromosomal rearrangements in meiotic pairing that result in the production of unviable gametes (Van Tuyl and Lim 2003). There are also studies that demonstrate that improper interactions between single genes or alleles may cause sterility of hybrids (Rieseberg and Carney 1998; Bomblies 2010). The formation of viable pollen in *Kalanchoë* interspecific hybrids can be attributed to the formation of unreduced gametes, a phenomenon frequently described in hybrids following distant hybridization (Van Tuyl and Lim 2003). Alternatively, the alloploid nature of the obtained hybrids cannot be excluded. A previous report demonstrated spontaneous chromosome doubling of hybrids obtained from the cross between *K. blossfeldiana* and *K. pubescens* (Izumikawa et al. 2008). Our results suggest that the differences in *Kalanchoë* chromosome numbers are not a strong barrier to hybrid formation, as it similarly was demonstrated for *Salvia* (Tychonievich and Warner 2011). Nevertheless, more research is needed to determine the possibility of self-pollination and back crossing of the obtained hybrids.

The present study presents a thorough qualitative and quantitative analysis of novel interspecific *Kalanchoë* hybrids and comparison with their parental species. The hybrids showed intermediate phenotypes between the parents. As a result, the favorable features of *K. blossfeldiana* cultivars such as the long flower longevity and high number of flowers have been transmitted from the parental genotypes. These characters are unquestionably important in ornamental breeding and production. Moreover, the hybrids were characterized by vigorous growth and were easily propagated by cuttings. Several lines of hybrids were characterized by increased plant height and moderate branching that were the characters transmitted from wild species. These features can be interesting for the development of new *Kalanchoë* cultivars suitable as cut flowers, a new direction in the breeding programs of *Kalanchoë*. The early flowering of hybrids can also be economically important due to the possibility of shortening of the production time.

Some undesirable characteristics of wild species such as leaf dropping of *K. marnieriana* and stem fragility of *K. nyikae* were also observed in several hybrids. Thus, it may be necessary to perform backcrossing to eliminate unwanted characteristics. This will also requires further investigation of the sterility of hybrids.

The present study clearly shows the possibilities of improvement of *Kalanchoë* plants by interspecific crosses with wild species that belong to the *Kalanchoë* genus. The obtained hybrids may contribute to the broadening of genetic variability of the cultivated material within this economically important genus.

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Conflict of interest The authors declare that they have no conflict of interest.

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