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Abstract: Floral scent plays an important role in attracting and guiding pollinators and is composed of a bouquet of volatile organic compounds (VOCs). Alstroemeria is a commercially important cut flower, however breeding efforts have focussed on flower color and size rather than scent. Recently analysis of two scented cultivars derived from the scented A. caryophyllaea revealed a surprising divergence in VOC profiles. Here 13 scented lines of A. caryophyllaea derived from selfing were characterised including morphology, evaluation of the floral scent through GC-MS and sensorial analysis. Leaf shape, stem length, flower size, shape, coloration and productivity all varied between lines. Sensorial analyses indicated that two lines (C013 and C017) were most highly rated for their appearance and C017 was also scored highest for its scent contrasting with C004 which scored lowest. Analyses of scent bouquets from six of the lines revealed 23 terpenoid compounds. All lines showed the same most abundant compound putatively identified as β-trans-ocimene, and three further compounds were discriminatory amongst the lines following PCA. Genomic organization of AlstroTPS, a previously identified myrcene synthase, showed substantial polymorphism between lines. The multifactorial characterization performed in this study showed differences among the lines confirming parental heterozygosity.

Floral scent evaluation of segregating lines of *A. caryophyllaea*.

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Floral scent plays an important role in attracting and guiding pollinators and is composed of a bouquet of volatile organic compounds (VOCs). *Alstroemeria* is a commercially important cut flower, however breeding efforts have focussed on flower color and size rather than scent. Recently analysis of two scented cultivars derived from the scented *A. caryophyllaea* revealed a surprising divergence in VOC profiles. Here 13 scented lines of *A. caryophyllaea* derived from selfing were characterised including morphology, evaluation of the floral scent through GC-MS and sensorial analysis. Leaf shape, stem length, flower size, shape, coloration and productivity all varied between lines. Sensorial analyses indicated that two lines (C013 and C017) were most highly rated for their appearance and C017 was also scored highest for its scent contrasting with C004 which scored lowest. Analyses of scent bouquets from six of the lines revealed 23 terpenoid compounds. All lines showed the same most abundant compound putatively identified as β-trans-ocimene, and three further compounds were discriminatory amongst the lines following PCA. Genomic organization of *AlstroTPS*, a previously identified myrcene synthase, showed substantial polymorphism between lines. The multifactorial characterization performed in this study showed differences among the lines confirming parental heterozygosity.

195 words

Key words: *Alstroemeria caryophyllaea*; breeding; Floral scent; genomic organization; sensorial attributes.

1. Introduction

The main function of floral scent is to attract and guide pollinators (Ando et al. 2001; Reinhard et al. 2004; Dudareva et al. 2004; Jürgens et al. 2003) although Dudareva and Pichersky (2000) suggest that flowers can also emit specific volatile organic compounds (VOCs) to repel non-beneficial insects, for example pollen or nectar 'thieves' or destructive insects. Furthermore some flowers are able to emit VOCs with anti-microbial or anti-herbivore activity to protect their reproductive organs (Friedman et al. 2002; Hammer et al. 2003; Farré-Armengola et al. 2013). Scent thus forms a key component of the 'pollination syndrome'. This includes other pollination rewards and attractants such as petal color and form, and nectar production, and has been an important driver in floral evolution (Fenster et al. 2004; Galliot et al. 2006).

Floral scent is composed of a bouquet of VOCs, including aromatic compounds, terpenoids and fatty acid derivatives (Knudsen et al. 2006). The composition and relative amounts of the VOCs in the final bouquet determines the overall scent perceived by the human olfactory system. Considering both the important role of floral scent in plant reproduction and its aesthetic value, composition of floral scent has been characterised in several ornamental species such as *Dianthus inoxianus* (Balaoa et al. 2011), snapdragon (*Antirrhinum majus*; Dudareva et al. 2005), carnation (*Dianthus* spp.; Jürgens et al. 2003) and petunia (*Petunia axillaris* ; Kondo et al. 2006).

Sensorial analyses (Morinaka et al. 2001) provide information on the aesthetic qualities of the bouquet, based on the specific recognition and discrimination between a vast variety and combinations of odour molecules, by a large gene family of odorant receptors connected to the human olfactory system (Zhao and Firestein, 1999). Humans have very varied thresholds for different VOCs, so concentration in the mixture does not directly relate to what is perceived. For some compounds our olfactory system can be more sensitive than analytical tools, such as

GC-MS, for evaluating floral scent (Hinterholzer and Schieberle, 1998). In addition, GC-MS alone cannot predict what the perceived odour type will be as combinations of compounds can have unique and specific aromas that are not the same as the individual compounds. On the other hand, GC-MS provides a very sensitive measure of bouquet composition including chemical identification of specific VOCs. (Yeon Oh et al. 2008; Kondo et al. 2006; Klahre et al. 2011). Furthermore it is highly reproducible and a total of 1719 chemical compounds have been identified from samples of floral scent in 991 species of flowering plants to date (Knudsen et al. 2006). In addition to identification and quantification by GC-MS and sensory evaluation of what is perceived by the human, it is also important to determine whether the aroma is well liked. Therefore, selection of new ornamental lines benefits from a combination of sensory and analytical approaches.

Alstroemeria is a commercially important cut flower originating in South America with main centres of diversity in Brazil and Chile (Bayer, 1987; Muñoz and Moreira, 2003). Breeding has mainly targeted flower size and color through interspecific hybridisation (Buitendijk et al. 1995; Burchi et al. 1997), mutant selection (Aros et al. 2012a), development of polyploids (Lu and Bridgen 1997; Takayuki, 1999) and more recently transformation (Akutsu et al. 2004). However little attention has been played to scent, as few native species are scented and breeding programmes have used a narrow genetic background (Aros et al. 2006). Recently there has been increased interest in scented flowers (Pichersky and Dudareva 2007) and two scented *Alstroemeria* cultivars: 'Sweet Laura' and 'Ajax' have been developed by crosses between the Brazilian scented *A. caryophyllea* and non-scented lines (Pounders et al. 2003; R. Meijles personal communication). VOC analyses of these genetically related cultivars (Aros et al. 2012b) revealed a surprising divergence in VOC profiles. Both cv. 'Ajax' and *A. caryophyllea* produce a single major terpenoid compound, though different to each other, while cv. 'Sweet Laura' produces at least three major and many more minor terpenoid compounds including (*E*)-ocimene, (*E*)-caryophyllene, humulene and myrcene. Bouquet composition has been previously reported to vary even between cultivars of the same species (e.g. in Petunia,

106 Klahre et al. 2011), although the precise composition of VOC bouquets as a result of
107 segregation has not been extensively studied (Andargie et al. 2014).
108
109 Terpenes are one of the most common VOCs in floral scent, particularly mono- and
110 sesquiterpenes (Knudsen et al. 2006). Terpenoids comprise a large number of primary and
111 mostly secondary metabolites with a wide variety of structural types and their biosynthesis is
112 controlled by the action of terpene synthases (TPS). TPS genes have been identified from a
113 wide range of species (Bohlmann et al. 2000; Dudareva et al. 2003; Yang et al. 2013) to
114 examine evolutionary relationships within the gene family (Trapp and Croteau, 2001; Lee and
115 Chappell, 2008). In *Alstroemeria* Aros et al. (2012b) identified a terpene synthase (*AlstroTPS*)
116 with myrcene synthase activity, which is highly expressed in cv. 'Sweet Laura' tepals during
117 peak VOC production. Gene structure of *AlstroTPS* placed it amongst class III terpene
118 synthases according to the classification of Trapp and Croteau (2001), but with 5 introns instead
119 of the 6 introns common for this class. Expression of *AlstroTPS* has also been confirmed in *A.*
120 *caryophyllaea* tepals (Aros et al. 2012b), but its genomic structure has not been characterized in
121 this species.
122
123 An analysis is presented here of a population of F1 lines produced through self
124 pollination of *A. caryophyllaea* revealing morphological polymorphism followed by a
125 multifactorial characterization of their floral scent, using both GC-MS and sensorial analysis to
126 identify levels of variation in bouquet composition as well as aesthetic qualities. An analysis of
127 genomic organization of *AlstroTPS* in these lines is also presented showing substantial
128 polymorphism at the level of scent-related genes.
129
130 **2. Materials and Methods**
131
132 2.1. Plant material
133

Self-pollination was performed on an *A. caryophyllaea* individual inflorescence. Seeds were collected from dried fruits 60 – 75 DAP and were soaked in warm water (30-40 ºC initial temperature) for 48 h and then stratified at 4 ºC for 2 weeks. Plants were grown at the University Botanical and Research Garden, Cardiff University (Cardiff, UK) in a greenhouse at a minimum of 14 ºC. Humidity and light were not controlled. After the stratification, seeds were sown in pots containing a mixture of sand (25 %), coarse grit (25 %) and compost (50 %). A total of 17 new lines were obtained of which 13 were morphologically characterized and the 5 most promising, from an ornamental point of view, were selected for further evaluation. Each line was vegetatively propagated in pots containing the same soil mixture as above.

2.2. Morphological characterization

Morphological characterizations were performed on 13 lines (C001, C002, C003, C004, C005, C006, C008, C009, C010, C013, C014, C016, C017) when plants were in full bloom during two flowering seasons (2008/2009 and 2009/2010). Leaf shape was assessed according to Hickey and King (1997). Stem length was considered as the distance between the soil level and the highest flower. Flower size was measured as height x width (cm). Width was considered as the maximum distance between the external margins of the two highest inner tepals or between the external margins of the two basal outer tepals. Height was considered as the distance between the margins of the highest outer tepal and the basal inner tepal. Flower shape was assessed as the ratio of height: width. Flower color was assessed as the main two or three colors covering the six tepals. Flower markings were assessed by the identification of dots/stripes covering the tepals in terms of location (inner or outer tepals), abundance (rare, medium or abundant) and shape (dots or stripes). Pollen abundance was assessed as abundant, medium or poor.

2.3. Sensorial Evaluation of Scent

Seventy untrained participants were asked to evaluate five different scented lines of selfed *A. caryophyllaea* (C001, C004, C010, C013 and C017). The demographic composition represented both genders and five age ranges (Table 1). Participants were asked to score their flower purchasing frequency (Table 1) and their purchasing priorities in terms of the relative importance given to stem length, flower size, flower color, floral scent and vase life by answering the question "when you buy/look at flowers the character you appreciate most is". A 5-point category scale was used of "strongly disagree"; "disagree"; "neutral"; "agree" and "strongly agree". Samples consisted of three floral stems cut in proportion to their natural length and placed in a 500 ml measuring cylinder in water. Lines were evaluated individually by each participant in terms of liking of appearance and scent. Both were rated using a standard 9-point hedonic category scale: "Dislike extremely"; "dislike very much"; "dislike moderately"; "dislike slightly"; "neither like nor dislike"; "like slightly"; "like moderately"; "like very much" and "like extremely". Intensity of the scent was rated using a 9-point intensity category scale: "Extremely low"; "very low"; "moderately low"; "slightly low"; "neither high nor low"; "slightly high"; "moderately high"; "very high" and "extremely high". Appearance was rated after scent characters and participants were asked to wait for about one minute between evaluating each sample.

2.4. GC-MS Evaluation

VOC sampling was performed on six selfed *A. caryophyllaea* scented lines (C001, C003, C004, C008, C013 and C017) as described by Aros et al. (2012b). Briefly, three open flowers stage 4-5 (anthesis) were enclosed in 250 ml polystyrene bottle with 30 ml of distilled water and VOCs collected by solid phase microextraction (SPME). A 50/30 µm divinylbenzene/carboxene/PDMS composite SPME silica fibre on 2 cm fused silica (grey fibre, Sigma Aldrich, Gillingham, UK) was exposed to the head space for 4h. VOCs were desorbed from fibres for 2 min at 240°C in the injection port of the gas chromatograph (GC 6890, Agilent, Wokingham, UK) and separated on a 30 m, 0.25 mm ID capillary column over 0.25 µm

190 HP-5MS (Agilent) using the following temperature programme: initial temperature 35°C for 2.5
191 min, first step increase 3°C/min to 140°C, second step increase 16°C/min to 300, followed by
192 1.5 min at 300°C constant. Electron Impact mass spectra were recorded in full scan mode from
193 35 – 500 m/z (70 eV, MSD 5873, Agilent) coupled to a GC (GC 6890, Agilent). To monitor
194 system performance and provide retention time references for calculation of retention (Kovats)
195 indices (RIs). A C_8–C_{20} alkane standard was analysed regularly (0.1 µL direct injection,
196 Supelco, Gillingham, UK). Data were analysed using AMDIS 2.71. Signals were integrated
197 using total ion count and areas normalised. Putative identification was achieved by comparison
198 of mass spectra with the NIST mass spectra library (v. 2.0, 2011) taking into account available
199 information on Kovats indices.
200
201 2.5. Genomic Organization
202
203 A DNeasy Plant mini kit (QIAGEN, Venlo, The Netherlands) was used to extract
204 genomic DNA from young leaves of selfed *A. caryophyllaea*, scented lines C001, C003, C004,
205 C006, C010, C013, C017 and the non-scented *A. psittacina* which was included as a
206 comparison. Three primer sets were used to amplify the full length sequence of the *AlstroTPS*
207 gene: TPS-3` (Fw 5`-ATGGCTTCCCATCTTCCTCTTC-3` and Rev 5`-
208 CCCGTTGTTTCGTCATAGAACC-3`), TPS-I (Fw 5`-
209 CGCCGAATGTTATGTTACGCATCA-3` and Rev 5`-
210 CCCCTATCGAAATCCCTGCATTCT-3`) and TPS-5` (Fw 5`-
211 GCTTTGCACGATTCAGTGGT-3 and Rev 5`-AGGTTCCACCAACATTGCCA-3)
212 Amplification was carried out in a PTC-100 machine (MJ Research, St Bruno, Canada) using
213 GoTaq Master Mix (Promega, Madison, USA) following the thermal profile: 4 min at 95°C; 35
214 cycles of 95°C for 30 s, 57°C for 20 s, and 72°C for 40 s; and a final extension at 72°C for 5
215 min. PCR products were purified from agarose gels using a GeneJET Gel Extraction Kit
216 (Thermo scientific, Loughborough, UK), sequenced and sequences aligned using Geneious
217 3.6.2. Variations on the conserved domains of the gene were detected using InterProScan

218 software (The European Bioinformatics Institute, Cambridge, UK) available on line
219 (http://www.ebi.ac.uk/Tools/pfa/iprscan/).
220
221 2.6. Statistical Analysis
222
223 The scales described for hedonic and intensity evaluation were translated into scores.
224 For the hedonic scale the scores were: Dislike extremely = 1 and like extremely = 9; for
225 intensity scale the scores were: Extremely low = 1 and extremely high = 9. Standard deviation
226 (STEDV) and standard error (SE) were calculated. Analysis of variance (ANOVA) was
227 performed through SPSS 17.0 for Windows, using Tukey's HSD (Honestly Significant
228 Difference) test for multiple pairwise comparisons with a significance level of 0.05. Principal
229 Component Analysis (PCA) (Jolliffe 1986) was applied to the VOC dataset to summarize
230 graphically differences in VOC abundance between the *A. caryophyllaea* lines and also to assess
231 biological reproducibility. To prevent the VOC peaks of greatest intensity from dominating the
232 PCA model, data were first standardized (value-average/STDEV) thus giving all VOCs a
233 normal distribution. Univariate analysis (Kruskal-Wallis non parametric N-Way ANOVA) was
234 also performed to test for significant VOC differences between the lines.
235
236 **3. Results**
237
238 3.1. Selfed progeny of *A. caryophyllaea* show morphological differences in both leaves and
239 flowers
240
241 The most common leaf shape was lanceolate, observed in eight out of the 13 lines
242 evaluated (C001, C002, C006, C009, C010, C013, C014 and C016). Elliptic leaves were
243 observed in three of the lines (C004, C005 and C008), while linear leaves were observed only in
244 C003 and C017 (Table 2). C004 showed the longest stem with an average of 70.6 cm (n=49),
245 that was statistically different ($P<0.05$) to six of the other lines, among them C001 and C003,

246 which showed the shortest stems with averages of 35.5 (n=34) and 39.1 cm (n=37) respectively
247 (Figure 1). C004 had flowers of greatest height (7.48 cm, n=49) and shared greatest width (5.45
248 cm, n=49), with C001 (5.51 cm, n=38) (Figure 2). The smallest flowers were observed in C005,
249 C006 and C014. Both mean height and width were lowest in C005 with averages of 5.0 and 4.1
250 cm respectively although there was a lot of variability especially in height and thus the flower
251 size of C005 was not statistically distinguishable from the other two lines. The ratio between
252 height and width showed average values of > 1 in all cases indicating a vertical elliptical shape.
253 C001 presented the most rounded flowers (ratio 1.13), although not statistically different from
254 six other lines, while C016 the most elliptical flowers (ratio 1.44) with no statistical differences
255 with five other lines (Table 3). C006 produced the highest number of single flowers per floral
256 stem (average value=6.5), significantly higher than all the other lines, excepting C009 and
257 C014, both with an average of five flowers per stem. The poorest line was C017 producing only
258 3.6 flowers per stem, although only statistically different to the highest producer, C006 (Figure
259 3).
260
261 All the lines were white in background color, while different intensities of pink, red and
262 green were seen as cover color on the margins of the tepals (Figure 3). Markings over the tepals
263 were abundant in the majority of the lines, excepting on lines C001, C006 and C009 where they
264 were scored as 'rare' (Figure 3). Characterization of the anthers showed one sterile line with no
265 pollen (C006), while anthers of the rest of the lines produce morphologically normal pollen
266 (data not shown).
267
268 3.2. The selfed *A. caryophyllaea* lines differ in their sensorial attributes
269
270 Sensorial analysis was performed to determine whether there were any perceived
271 significant differences in appearance or scent bouquet between five of the selfed *A.*
272 *caryophyllaea* lines. The 70 participants in the analysis were first asked to rate their

purchasing/rating priorities revealing that both flower color and scent were highly rated and significantly different from stem length, flower size and vase life (Table 1).

C013 and C17 were most highly rated in liking for their appearance (significantly compared to the other lines), and C017 also scored most highly for liking and intensity of its floral scent bouquet although it was only significantly different from the lowest rated line, C004 (Figure 4). C004 was rated lowest for both scent intensity and liking of scent as well as liking of appearance, although liking of scent was only significantly different to C017 and appearance was only significantly lower than C017 and C013.

3.3. Scent profiles differ amongst the selfed *A. caryophyllaea* lines

VOCs from four of the lines that had been evaluated sensorially (C001, C004, C013 and C017) were analysed by GC-MS as well as two further lines (C003 and C008). In total 23 compounds, all terpenoids, were detected and putatively identified with high confidence (Table 4). All lines showed the same major peak as well as several minor peaks. The most abundant peak was identified as the monoterpene β-trans-Ocimene (C10) (RI: 1038), based on a NIST library search and on the Kovat index (1040) reported by Porta et al, 1999 (Figure 5A). Three compounds: 3-Thujene (C3), o/m Ethilanisol (C15) and an unidentified sesquiterpene (sesquiterpene 7, (C28) were only detected in line 008 ($P < 0.05$).

Sabinene (C5) was detected differentially in three lines, being most abundant in C003 followed by C008 and C017, but was below detection in lines C001, C004, and C013 ($P<0.0005$) Another unidentified sesquiterpene, (sesquiterpene 2, C19), was detected in lines C001 and C004 but was below detection in the other lines. Eudesma-4(14),11-diene (C25, circled black in Figure 5C, Figure 6C) and sesquiterpene 6 (C26) were undetectable in C003 and higher in lines C001 and C004, though both were only significantly higher in C004

300 compared to C008, C013 and C017) ($P<0.05$) No significant differences in levels of Myrcene
301 were found between lines (Figure 6E).
302
303 PCA analysis of VOC levels showed that 48% of variance of lines were explained by
304 PC1 and PC2. The biplot of the components showed clear separation of lines C003, C004 and
305 C017 along PC2, strong clustering of line C003 and separation of line C008 on PC1 (Figure
306 5B). The same biplot showing the chemical components (Figure 5C) reflected the role of
307 compounds mentioned above; position of C3, C28 and C15 clustered strongly at the right end of
308 PC1 (circled red in Fig 5C), while sabinine (circled blue in Fig 5C) was discriminatory for lines
309 C003, C008 and C017. C19 (circled green) was strongly discriminatory for lines C001 and
310 C004 while C26 (circled black) was a strong indicator for line C004.
311
312 3.4. Genomic organization
313
314 Although myrcene levels did not appear to differ between the lines, as this is the only
315 terpene synthase gene available in *Alstroemeria*, it was used to investigate whether the
316 differences in VOC profiles were reflected in polymorphism amongst the lines in the structure
317 of scent-related genes. The genomic organization of the myrcene synthase is constituted by two
318 domains, near the N terminus is the terpene synthase domain, and at the C terminal end of the
319 protein is the terpene synthase metal binding domain (Figure 7). This gene has six exons and
320 five introns in *Alstroemeria* and for all the *A. caryophyllaea* lines and *A. psittacina* evaluated
321 the presence of an insertion of 105 bp was observed in the fourth exon of the myrcene synthase
322 genetic sequence (Figure 7). The insertion was located at the same position in all seven of the
323 selfed *A. caryophyllaea* evaluated showing the same sequence. In the fifth exon a deletion of 61
324 bp was identified only in lines C001, C004, C006, C010 and C013 (Figure 7), generating a stop
325 codon in the middle of the terpene synthase metal binding domain of the gene and producing a
326 shorter protein. Finally an insertion of 4 bp in *A. psittacina* was observed, changing the reading
327 frame of the gene and generating a stop codon in the first part of the fourth exon of the gene.

328 This insertion would produce a truncated protein lacking most of the terpene synthase metal
329 binding domain of the myrcene synthase, and the insertion was not present in any of the *A.*
330 *caryophyllaea* selfed lines.
331
332 **4. Discussion**
333
334 Although most of the lines evaluated were similar to the parental plant and to the
335 botanical descriptions available for *A. caryophyllaea* (Assis 2004 and Foster 1945), some
336 characters showed segregation suggesting a possible underlying heterozygosity within the
337 parent plant. This suggestion is supported by the natural outcrossing habit described for species
338 of this genus (Aizen and Basilio 1998; Cavieres et al. 1998; Valdivia and Niemeyer 2005).
339 Some of this segregation was observed in the differences in leaf shape, stem length, flower size,
340 shape and color. Line C004 appeared distinctive in several morphological characters including
341 leaf size, stem length and flower size, however lines C006, C009 and C014 were more
342 productive in terms of flower number compared to the other lines, and C017 flowers were of the
343 most intense color (Figure 3). QTLs have been identified in *A. aurea* for leaf length and width,
344 flower color and size (Han et al. 2002), and may be relevant to the characters seen in this study.
345 The main pigments found in *Alstroemeria* flowers are anthocyanins, flavonoids and carotenoids,
346 with the orange/red colors as seen in *A. caryophyllaea* due mainly to anthocyanins, specifically
347 6-hydroxypelargonidin glycosides (Tatsuzawa et al. 2003). An analysis of the inheritance of
348 flower color in *Alstroemeria* species and cultivars (Nørbæk et al. 1998) showed a complex
349 composition and inheritance of various anthocyanin pigments. However Han et al. (2002)
350 inferred from a QTL analysis of *A. aurea* that flower color inheritance was relatively simple.
351 The differences in the color intensity and patterning seen here would support a more complex
352 control given the variability revealed from selfing.
353
354 Although flower color was the most important character influencing purchasing of
355 flowers by the participants of the sensorial study here, floral scent (in terms of liking and

356 intensity) also scored highly. Floral appearance, floral scent liking and scent intensity were
357 therefore scored. Both genders were well-represented in the sample, which is important as
358 olfaction is generally superior in females (Brand and Millot 2001). The design of the sensorial
359 tests asked participants to score the three criteria separately, however an interaction between
360 floral scent and appearance cannot be excluded as the scent evaluation was performed in the
361 presence of the visual stimulus and vice versa and the two stimuli are known to interact
362 (Gottfried and Dolan 2003). Line C017 was more valued in the sensorial analysis both for
363 appearance and floral scent bouquet, and was one of the three lines rated highest for floral
364 intensity suggesting a link between these three criteria. Furthermore, line C004 was distinct
365 from C017 in all three criteria and was least valued in floral scent. VOC analyses were
366 performed to explore further the differences in sensorial value. All five lines analysed showed
367 the same major peak that was identified as the monoterpene β –Ocimene. This was distinct from
368 the single peak perviously identified from the parental *A. caryophyllaea* (Aros et al. 2012b)
369 which could not be unequivocally identified, but which had a different RI value to β –Ocimene.
370 Based on PCA and ANOVA analysis distinctness of the VOC profiles of some of the lines
371 tested could be ascribed to a small number of compounds. Of particular interest was the higher
372 level of sabinine in line C0017 compared to line C004 and the higher levels of two
373 sesquiterpenes putatively identified as eudesma-4(14),11-diene (also known as β-selinene) and
374 selina-3,5-diene in line C004 compared to line C017. These three compounds are thus good
375 candidates for a differential sensorial evaluation of these two lines. Sabinene is emitted by a
376 number of scented species contributing to the spicy fragrance of nutmeg and some varieties of
377 black pepper (Shulgin et al. 1967; Schenk and Lamparsky 1981; Richard et al. 1971). β-selinene
378 is a minor component of celery seed oil VOCs (Mcleod et al. 1988; Mcleaod and Ames 1989)
379 and is described as having a “herbal” or “green, fragrant” odour. Selina-3,5-diene is a minor
380 component of cashew nut (*Anacardia occidentale*) leaf essential oils (Dzamic et al. 2009).
381 Further sensorial analyses linked to GC separation would be needed to determine whether these
382 compounds do in fact make an important contribution to the sensorial value of the lines.
383 Myrcene was detected as a minor component of the VOC profile in all the lines analyzed, and

384 although there were apparent differences between lines, these were not statistically significant
385 and are thus unlikely to contribute to the sensorial value differences.
386
387 All the lines evaluated, including *A. psittacina*, showed the same genomic organization
388 composed by 6 exons and 5 introns for the *Alstroemeria* myrcene synthase gene (*AlstroTPS*).
389 This organization is the same shown by Aros et al. (2012b) for *A*. cv. 'Sweet Laura' which
390 clustered as an anomalous member of class III according to the classification of Trapp and
391 Croteau (2001). However differences in the size of exons and introns were observed among the
392 lines: an insertion of 105 bp in the fourth exon was observed in both the scented *A.*
393 *caryophyllaea* lines and the non-scented *A. psittacina*, but not in the scented *A*. cv. 'Sweet
394 Laura'. Therefore this insertion seems to be a common pattern for the Brazilian native species
395 and not related to the scent character. Furthermore a deletion of 61 bp was identified in the fifth
396 exon of the myrcene synthase only in five of the seven lines analysed (Figure 7). This indicates
397 polymorphism at this gene locus, suggesting that this gene is heterozygous in the parent plant
398 and the two alleles are segregating amongst the selfed F1 lines. The fact that the insertion noted
399 in all the F1 lines introduces a stop codon, thus truncating the open reading frame suggests that
400 this gene may not be functional. This is in contrast to *Alstroemeria* cv. Sweet Laura which was
401 derived from a cross between Chilean non-scented *A. aurea* and scented *A. caryophyllaea*
402 (Pounders et al. 2003; M. Bridgen, personal communication). This indicates that the functional
403 *AlstroTPS* in cv Sweet Laura may in fact derive from the unscented *A. aurea* parent, and
404 perhaps have been activated in the new genetic background. It also indicates that other myrcene
405 synthases are active in *A. caryophyllaea* since myrcene was detected in the scent bouquet by
406 GC-MS.
407
408 **5. Conclusions**
409
410 In conclusion this work has shown that selfed progeny of the scented Brazilian *A.*
411 *caryophyllaea* all resemble the parent plant but differ in a number of important morphological

features including flower color, size, productivity and markings, reflecting parental heterozygosity. All the F1 selfed lines are scented, but differ in their VOC profiles from each other and from their scented parent. Furthermore, the differences in flower morphology and scent result in differing sensorial value. Thus, even without performing crosses, some of the underlying diversity in *Alstroemeria* can be released for the development of new scented lines.

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TABLES

Table 1: Demographic distribution and consuming habits of volunteers in sensorial analyses

Age (years)	**%**
18-22	52
23-28	17
29-35	20
36-42	1
43+	10
Gender	**%**
Male	34
Female	66
Flower purchasing frequency	**%**
Weekly	1
Monthly	11
Special occasions	74
Never	14
Character most appreciated	Sensorial scale (1 to 5)
Stem length	2.75 a
Flower size	3.28 ab
Vase life	3.73 b
Floral scent	4.35 c
Flower colour	4.73 c

Table 2. Leaf shape observed in 13 selfed lines of *A. caryophyllaea*.

Line	Leaf shape
DANCAR 001	Lanceolate
DANCAR 002	Lanceolate
DANCAR 003	Linear
DANCAR 004	Elliptic
DANCAR 005	Elliptic
DANCAR 006	Lanceolate
DANCAR 008	Elliptic
DANCAR 009	Lanceolate
DANCAR 010	Lanceolate
DANCAR 013	Lanceolate
DANCAR 014	Lanceolate
DANCAR 016	Lanceolate
DANCAR 017	Linear
Parent plant	Linear to lanceolate

Table 3. Average ratios calculated from the relationship between flower height and width (±SE, n=4 to 54)* evaluated on 13 selfed lines of *A. caryophyllaea,* during two periods of flowering (’08/’09 and ’09/’10).
* The number of flowers evaluated (n) depends on the productivity of each line.
Different letters indicate statistically significant differences $P<0.05$.

Line	Ratio (height:width)	SE
C001	1.13 a	0.01
C002	1.41 d	0.01
C003	1.35 cd	0.01
C004	1.38 cd	0.02
C005	1.22 abc	0.14
C006	1.15 ab	0.03
C008	1.21 abc	0.00
C009	1.17 ab	0.00
C010	1.22 abc	0.04
C013	1.23 abc	0.01
C014	1.31 bcd	0.02
C016	1.44 d	0.05
C017	1.32 bcd	0.03

Table 4. Volatile compounds detected in Alstroemeria dancar flowers via Solid Phase Microextraction, Gas Chromatography – Mass Spectrommetry (SPME GC-MS).

Compound	VOC#	Common name	RI (DB-5)	RT	RI present in Terpenedata
Hexanal	C2	Hexanal	799.9	4.2327	771
2-Methyl-5-(1-methylethyl)-bicyclo[3.1.0]hex-2-ene	C3	3-Thujene	922.3	8.6598	932
α.-Pinene	C4	α.-Pinene	926.8	8.8608	936
α -Phellandrene	C5	Sabinene	967.5	10.705	973
α -Pinene	C6	Myrcene	990.2	11.7346	987
3-Isopropenyl-5,5-dimethyl-cyclopentene	C7	3-Carene	1006.1	12.4776	1000
Unidentified Monoterpene	C8	Monoterpene	1028.2	13.4796	
D-Limonene	C9	D-Limonene	1024.2	13.2675	1025
3,7-Dimethyl-, (E)-1,3,6-octatriene	C10	β-trans-Ocimene	1038.8	14.0722	1029
11-Methyl-4-(1-methylethyl)-,4-cyclohexadiene	C12	Moslene	1057.4	14.957	
(+)-4-Carene	C13	Terpinolene	1081.1	16.3223	1082
1-Ethyl-6-ethylidene-cyclohexene,	C1	p-Ethylanisol	1091.5	16.7012	
1-Ethyl-6-ethylidene-cyclohexene (20)	C15	o/m-Ethylanisol	1070	15.5046	
1,3,5,5-Tetramethyl-1,3-cyclohexadiene	C16	Allo-ocimene	1128.5	18.5267	
2,6-Dimethyl-, (E,Z)- 2,4,6-octatriene (terpene)	C17	Allocymene	1140	19.0927	
Unidentified sesquiterpene	C19	Sesquiterpene 2	1387.6	30.6225	
Unidentified esquiterpene	C20	Sesquiterpene 3	1410.9	31.1126	
Caryophyllene	C21	Caryophyllene	1411.5	31.9224	
Unidentified esquiterpene	C23	Sesquiterpene 5	1387.6		
α -Caryophyllene	C24	à-Caryophyllene	1446.3	33.2645	
Eudesma-4(14),11-diene	C25	Eudesma-4(14),11-diene	1478.8	34.6524	
Unidentified sesquiterpene	C26	Sesquiterpene 6	1488.4	35.0607	
Unidentified sesquiterpene	C28	Sesquiterpene 7	1445.7	33.1326	

FIGURES

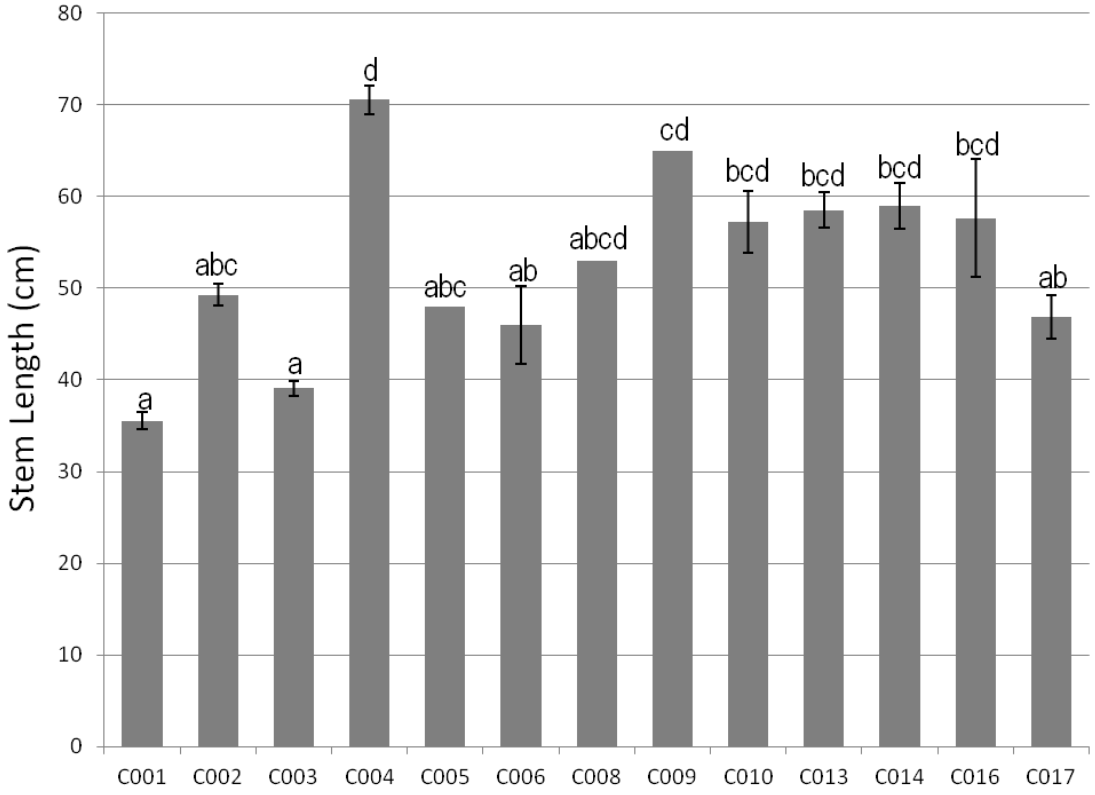

Figure 1. Average stem length (±SE, n=3 to 49)* observed in selfed *A. caryophyllaea* lines, evaluated during two periods of flowering (2008/2009 and 2009/2010).
* The number of stems evaluated (n) depends on the productivity of each line.
Different letters indicate statistically significant differences $P<0.05$.

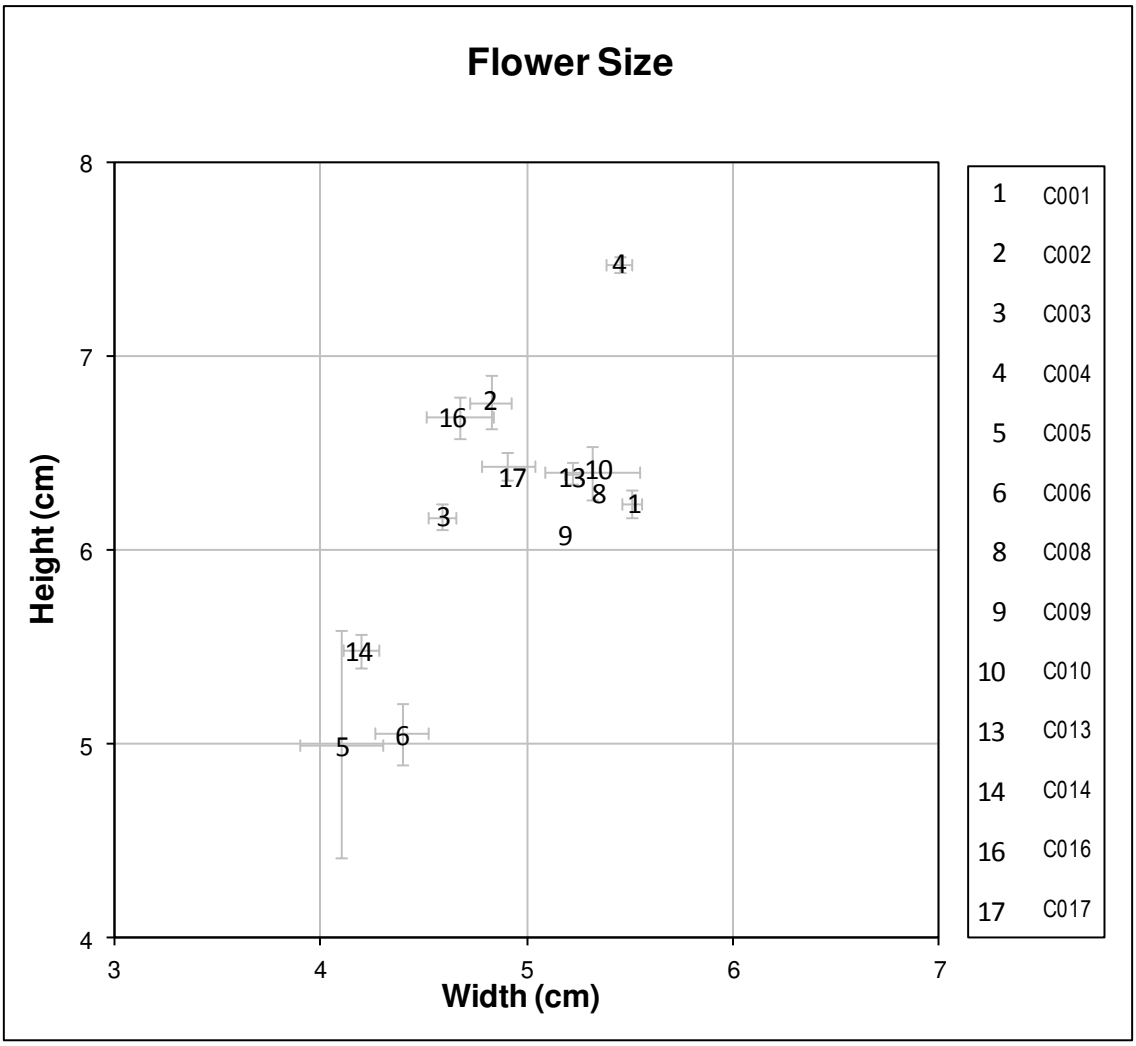

Figure 2. Average flower heights and widths (±SE, n=4 to 54)* evaluated on 13 selfed lines of *A. caryophyllaea,* during two periods of flowering (2008/2009 and 2009/2010).
* The number of flowers evaluated (n) depends on the productivity of each line.

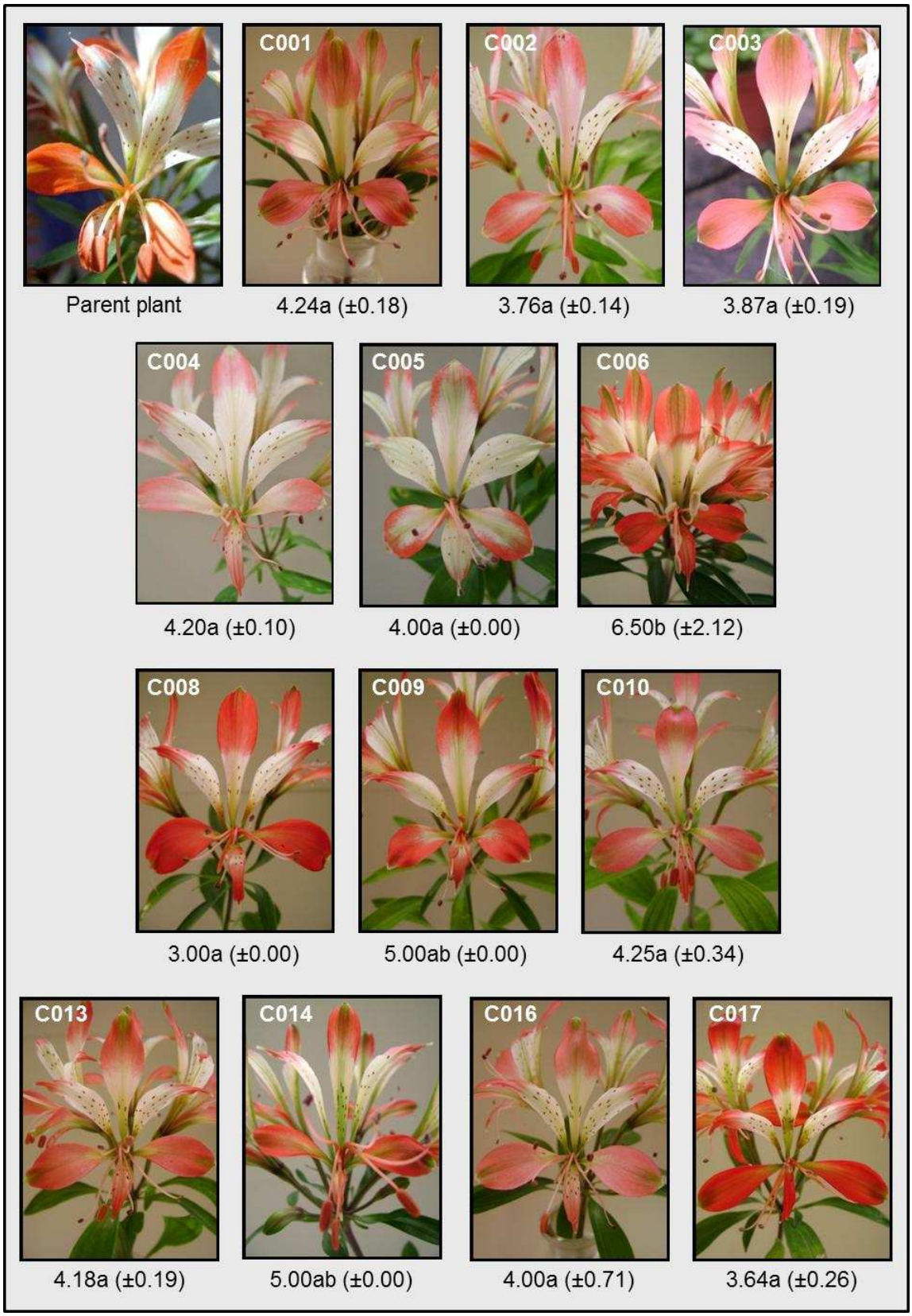

Figure 3. Flowers observed in 13 selfed lines of *A. caryophyllaea* and the parental line. Below each picture the average number of flowers per floral stems produced (±SE, n=4 to 54)* is shown. Evaluations were performed during two periods of flowering (2008/2009 and 2009/2010).

* The number of floral stem evaluated (n) depends on the productivity of each line.

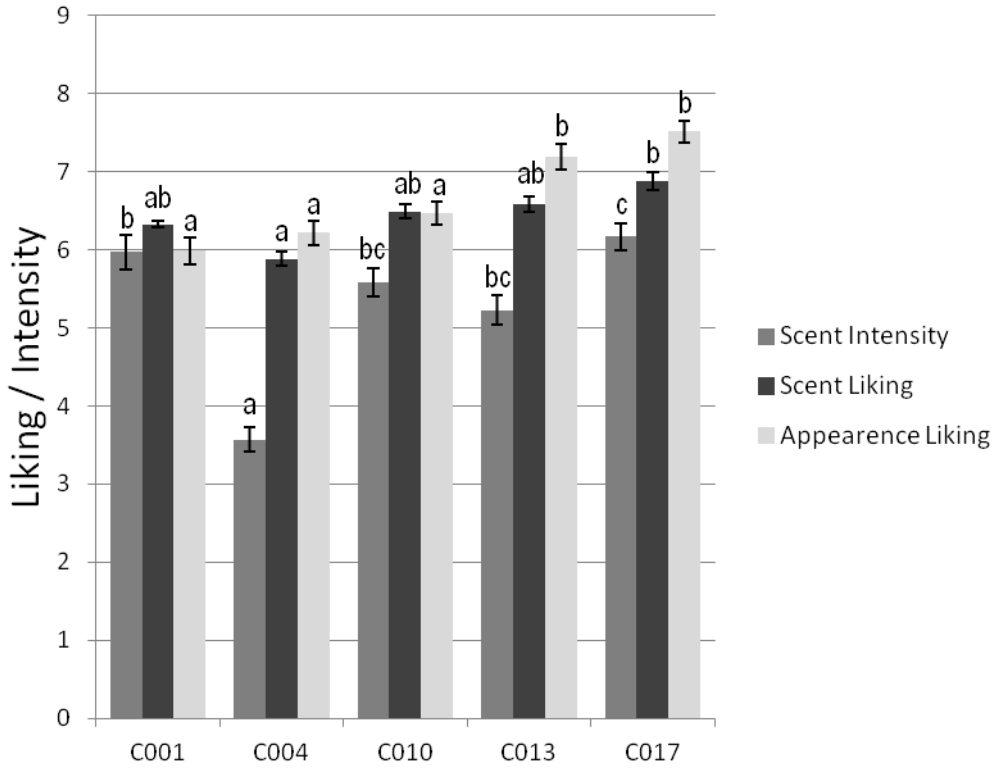

Figure 4. Means of floral scent liking and intensity and appearance liking (± SE, n=70) evaluated on 5 selfed lines of *A. caryophyllaea.* The scale ranges from 'dislike extremely' (= 1) to 'like extremely' (= 9) for liking; and from 'extremely low' (=1) to 'extremely high' (=9) for intensity. Letters above same-shaded bars indicate statistically significant differences (n=70; $P<0.05$).

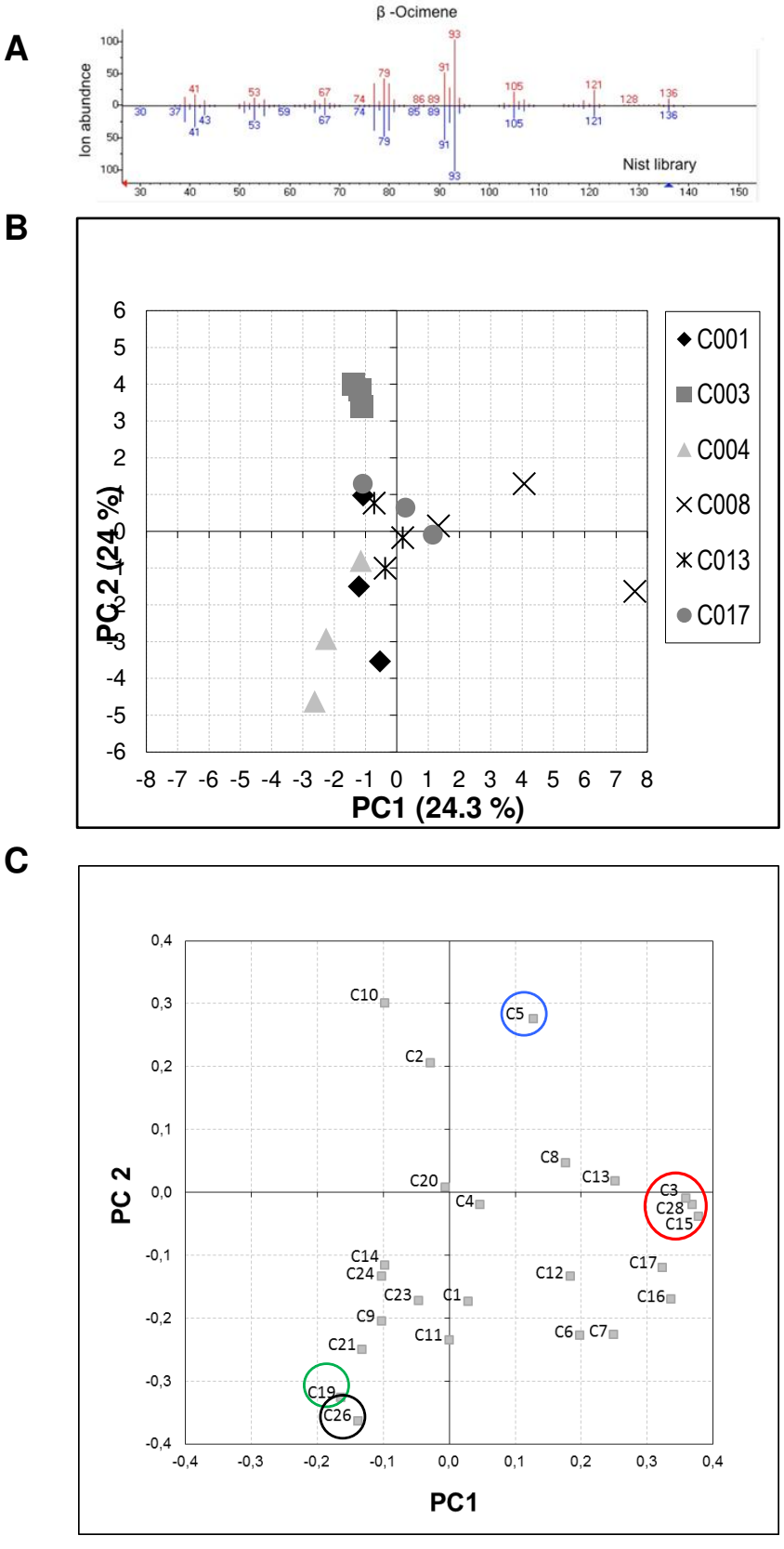

Figure 5. VOC data from six selfed *A. caryophyllaea* lines analysed by GC-MS. (A) Mass spectrum of the peak for β –Ocimene from line C001 and the match from NIST library. (B) PC biplot based on three biological replicates. Identification names of the PC applied (C1 to C28) are listed in Table 3.

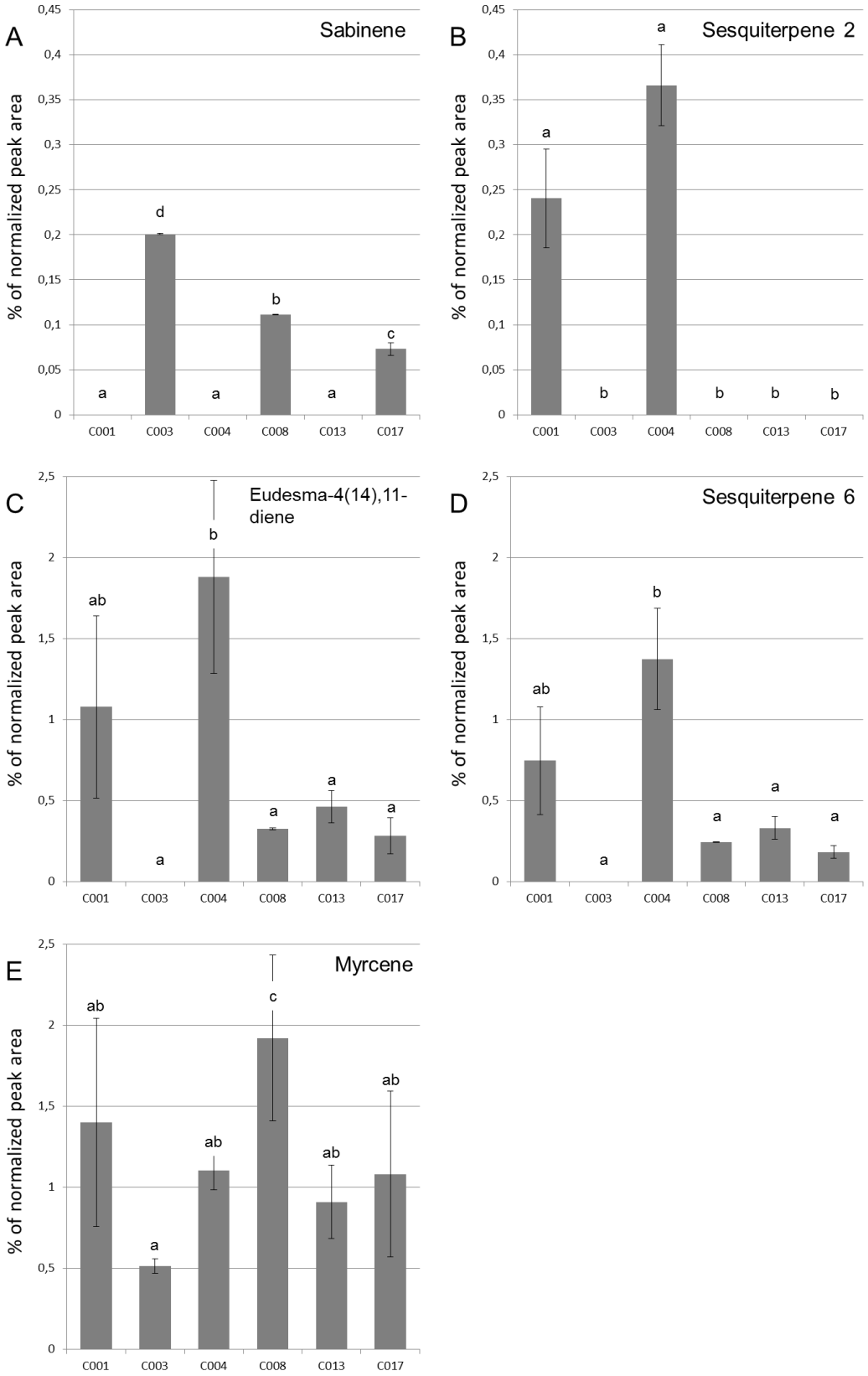

Figure 6. Average relative abundance of selected compounds in selfed *A. cayophyllaea* lines (A-D) as identified by PCA and Myrcene (E). Letters above bars indicate statistically significant differences (n=3; $P<0.05$).

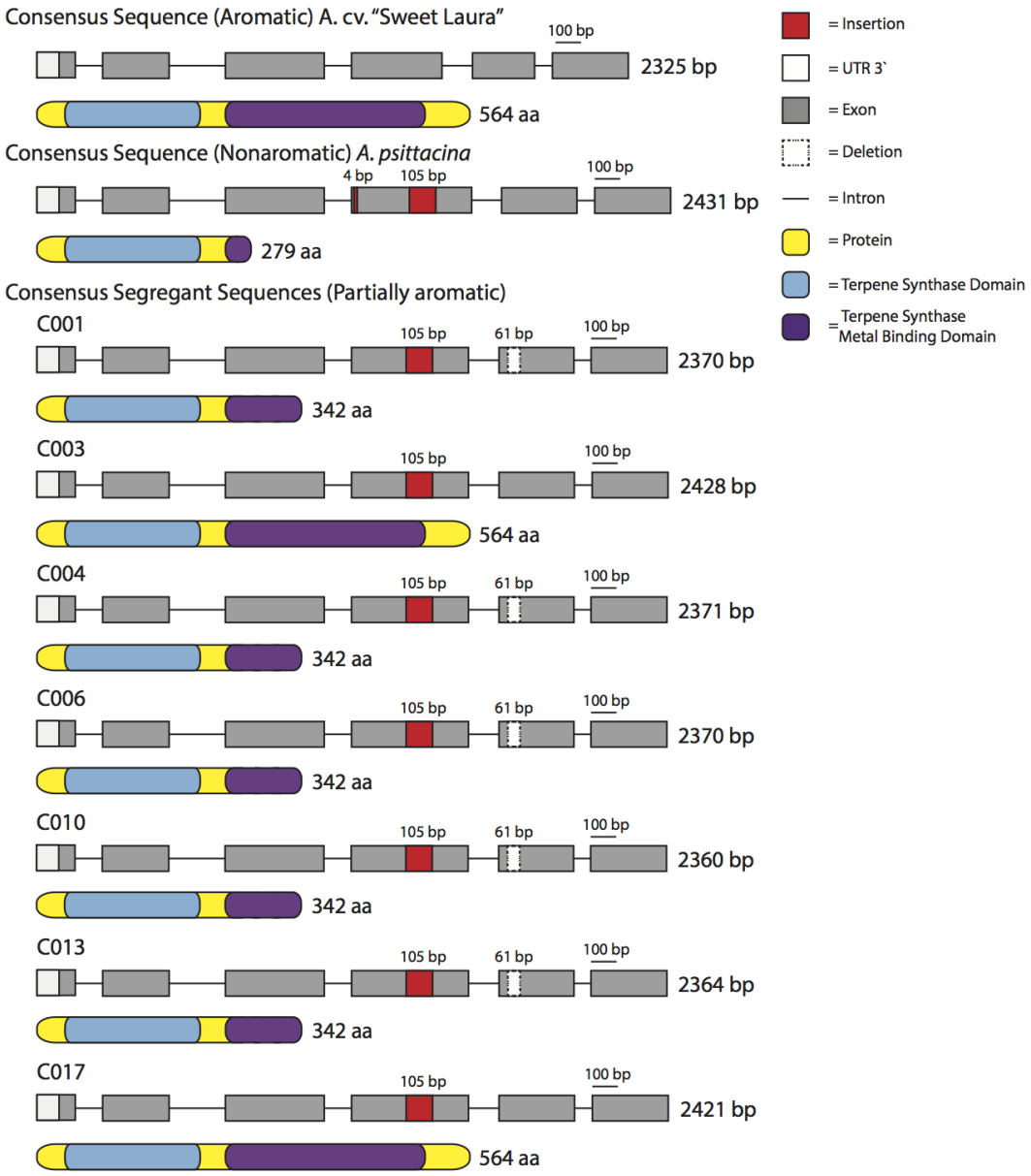

Figure 7. Genomic organization of *AlstroTPS* observed in 7 selfed lines of *A. caryophyllaea*, compared to the scented *A.* cv. Sweet Laura and the non-scented *A. psittacina*.