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Genetic control of flower petal number in *Rosa x Damascena* Mill f. trigintipetala

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ABSTRACT

We studied the petal number trait in a population obtained after self-pollination of *R. x damascena* f. trigintipetala following analysis of molecular markers which have previously been mapped near the major dominant locus *Blfo/d6* determining this trait in other rose species including *R. multiflora* and *R. hybrida*. The results showed that the same genetic mechanism, which determines the petal number trait in *R. multiflora* and *R. hybrida* also controls the trait in *R. x damascena* f. trigintipetala and is related to the dominant effect of a single copy allele in the tetraploid genome of this species. We also analyzed the expression of several flower homeotic genes including *R. x damascena APETALA1/FUL-like* (paleo AP1 type), *R. x damascena euAPETALA 3* (euAP3 line) and *R. x damascena AGAMOUS* in early stage flower buds corresponding to plants with double and simple flowers. The obtained results showed that only *R. x damascena AGAMOUS* was differentially expressed between the samples of double and simple flowers, its relative expression being upregulated 3.5-fold in simple flowers. We further cloned and sequenced the four genomic clones of *R. x damascena AGAMOUS* and studied the potential additive effect of this gene by analysing the segregation of its four alleles in the population of self-pollinated *R. x damascena*. Analysis of variance of the data for petal number and allele segregation did not show a statistically significant effect of any allele configuration of the *AGAMOUS* gene on the petal number trait in *R. x damascena* f. trigintipetala.

Introduction

Although more than 200 species have been identified in the genus *Rosa*, only a few of them have been industrially grown for production of rose essential oil products, which are widely applied as base components in perfumery and cosmetics products by the biggest producers in the market [1]. *Rosa x damascena* Mill, also known as the oil-bearing rose, has been grown for centuries for its highly scented flowers containing high amounts of essential oil components. The production of rose oil as well as other aromatic products including rose water, rose concrete and rose absolute from the flowers of *R. x damascena* is an important traditional industry in a number of countries in Europe, the Middle East and Northern Africa including Bulgaria, Turkey, Iran, India, Pakistan, Morocco and several others [2]. Although other rose species like *R. gallica* and *R. centifolia* have also been grown for rose oil production during the years, currently the

predominant rose species grown worldwide for industrial production of rose essential oil products is *R. x damascena* Mill.

The main product obtained from the flowers of *R. x damascena* is the essential oil produced by water–steam distillation of its flowers. The two biggest producers of rose oil supplying more than 90% of the rose oil to the world market are Bulgaria and Turkey [3]. Several studies have shown that the entire industrial cultivation of *R. x damascena* in these two countries is based on a single genotype of this rose species, namely the f. trigintipetala form, which has 30–35 petals in its flowers and has been vegetatively propagated for centuries from a common ancestor [4–6]. In contrast, a number of other genotypes of *R. x damascena* exist in Iran and other neighbouring countries, as today Iran and its region is considered to be the geographic origin of this species [7,8]. These studies, as well as the general trend towards diversification

of natural products nowadays, clearly demonstrate that it is necessary to expand the local genetic resources of *R. x damascena* in the two countries that are the main producers of rose oil in the world. Several reviews have discussed the prospects of improving the oil rose and expanding its genetic resources in Bulgaria and Turkey through breeding based on cross-pollination involving intra- or inter-specific hybridization strategies [9,10]. A key element when implementing such strategies would be to maintain or increase the number of flower petals, whose epidermal layer is the main source of essential oil compounds [11]. The number of petals in the diploid *R. multiflora* has been demonstrated to be a monogenic trait controlled by a major dominant allele of the *Blfo/d6* locus mapped on LG3 in the unified genetic map of diploid roses [12,13]. Later studies also mapped the same locus in tetraploid roses based on QTL analysis [14]. Additive effects of other loci in the rose genome are expected to further contribute to the petal number trait [15].

Rosa x damascena is a tetraploid rose species with a possible triparental origin [16]. Due to its complex genome and the hybrid nature of this species, genetic studies related to segregation of traits in populations developed with *R. x damascena* as a parent have not been implemented so far. In a recent study, Baydar et al. [17] analysed a population obtained after self-pollination of *R. x damascena* f. trigintipetala in Turkey and observed large segregation of the number of petals in the population ranging from 5 to 114. In the present study, we analysed the segregation of the petal number trait and molecular markers located in the vicinity of the *Blfo/d6* locus in a population of roses obtained after self-pollination of *R. x damascena* f. trigintipetala. We further analysed the expression of several flower homeotic genes in simple and double flower plants during the early stages of flower bud development and analysed the potential additive effect of the class C flower homeotic gene *R. x damascena AGAMOUS*.

Material and methods

Plant material and genomic DNA purification

Fully developed flowers of *R. x damascena* Mill f. trigintipetala and 143 plants derived from self-pollination of *R. x damascena* f. trigintipetala corresponding to phase 6 according to Rusanov et al. [18] were picked in the early morning hours in June 2017, and were immediately frozen in liquid nitrogen for subsequent purification of genomic DNA. The frozen flowers were stored at −80 °C until they were milled into fine powder using a TissueLyser II (Qiagen) Laboratory Mill. Genomic DNA was purified using a GeneJET Plant Genomic DNA purification kit (Thermo Scientific, Waltham, MA) according to the manufacturer's instructions. The concentration of the resulting genomic DNA was determined using Nanodrop 2000 (Thermo Scientific).

Morphological characterization of rose flowers

At least three fully developed flowers corresponding to phase 6 according to Rusanov et al. [18] were collected from each of the analysed rose plants in the early morning hours in June 2017 and used for analysis of flower morphology. The parameters measured included flower diameter, number of petals and number of stamens. Flowers with 6 ± 1 petals were scored as simple flowers, flowers with petals >7 ≤ 20 were scored as semi-double flowers and flowers with petals >20 were scored as double flowers.

qPCR analysis of flower homeotic genes

Flower buds at the early stage of flower development corresponding to stage 1–4 according to Dubois et al. [19] were used for total RNA purification. The buds were sampled from a plant possessing double flowers (30–35 petals) corresponding to the original genotype of *R. x damascena* f. trigintipetala and from a plant with simple flowers (six petals) obtained after self-pollination of *R. x damascena* f. trigintipetala. The flower buds were collected on 10 May 2017, prior to the start of the flowering season, and were immediately frozen in liquid nitrogen prior to purification of total RNA. Total RNA was purified using a CloneJET Plant RNA Purification Mini Kit (Thermo Scientific) according to the manufacturer's instructions. Genomic DNA residues were removed using DNase I (Thermo Scientific) according to the manufacturer's instructions. The concentration of the resulting total RNA was determined using Nanodrop 2000 (Thermo Scientific). SensiFAST™ SYBR Hi-ROX One-Step Kit (Thermo Scientific) was used to perform qPCR analysis using 40 ng of total RNA. Two genes with proven stable expression in *R. hybrida* encoding Translational Controlled Tumor Protein (TCTP) and Elongation Factor 1-alpha (EF1-alpha) (Table 1) were used for signal normalization. The primer pairs used and the homoeotic genes analyzed are presented in Table 1. The online version of Primer 3 (http://www.bioinformatics.nl/cgi-bin/primer3-plus/primer3plus.cgi) was used for design of primers for *R. x damascena euAPETALA3* (euAP3 line) and *R. x damascena APETALA1/FUL*. The assay was performed

Table 1. Primers used for analysis of expression of flower homeotic genes in early stages of flower development in *Rosa x damascena* by qPCR.

Gene	Primer designation	Primer sequence
R. hybrida AGAMOUS [19]	RhAG-s1	5′-AAAAGGGAACTTGACTTGCACAAC-3′
	RhAG-as1	5′-TCAAACTAATTGAAGGGAAATCTGG-3′
R. x damascena euAPETALA3 (euAP3 line)	AP3QPCRse	5′-TGGCGAGAGGTAAGATCCAG-3′
(Rusanov et al., unpublished data)	AP3QPCRas	5′-TGCTTTGTCGTGGTGGAG-3′
R. x damascena APETALA1/FUL (Rusanov et al., unpublished data)	AP1QPCRas	5′-TCGACACTACTAATGTGAGAAGACG-3′
	AP1QPCRse	5′-GAAAATAAGGAATTACACGGGAGA-3′
R. hybrida TCTP [19]	RhTCTPs	5′-GATGCTGATGAGGGTGTTGA-3′
	RhTCTPas	5′-CTTGGTTGCTCCCTCAATGT-3′
R. hybrida EF1-alpha [19]	RhEF1-QS1	5′-GGGTAAGGAGAAGGTTCACATC-3′
	RhEF1-QAS1	5′-CAGCCTCCTTCTCAAACCTCT-3′

on an ABI 7300 Real-time PCR system (Applied Biosystems) using the following temperature programme: reverse transcription at 45 °C for 10 min, activation of the polymerase at 95 °C for 2 min; 40 cycles of denaturation at 95 °C for 5 s, primer hybridization/elongation at 60 °C for 27 s (fluorescence reading); dissociation step for analysis of melting curve including 95 °C for 15 s, 60 °C for 1 min, 95 °C for 15 s. Biorad Gene Expression Macro™ Version 1.1 software was used to determine the relative expression of the analysed genes.

Cloning of the allelic forms of *R. x damascena* AGAMOUS

Cloning of the allelic forms of *R. x damascena* AGAMOUS, an ortholog of *R. rugosa* MASAKO C1, was done using a forward (5′-TGGACTCTGATGCCCAAAGA-3′) and reverse primer (5′-GAAGGGAAATCTGGTCATGGC-3′) constructed using the online version of the Primer 3 software and GenBank deposited sequences of cDNAs from *R. rugosa* and *R. hybrida*. The constructed primer pair was used for PCR amplification of genomic DNA from R. x damascena f. trigintipetala. PCR reactions were performed on an Applied Biosystems 9700 PCR apparatus in a volume of 30 μL using 40 ng genomic DNA, 20 pmol right and reverse primer, and Phusion High-Fidelity PCR Kit (Thermo Scientific) according to the manufacturer's conditions using the following temperature programme: denaturation at 98 °C for 30 s; 35 cycles of denaturation at 98 °C for 10 s, hybridization at 62 °C for 30 s, elongation at 72 °C for 4 min and a final elongation step at 72 °C for 10 min. The resulting PCR products were separated in an 0.8% agarose gel prepared with Top Vision Agarose (Thermo Scientific) visualized using Gelred (Biotium) and the approximate size determined using O'GeneRuler 1 kb DNA ladder (Thermo Scientific). The PCR products were purified from the gel using GeneJET Gel Extraction Kit (Thermo Scientific). The resulting purified PCR fragments were used for cloning in pJET1.2/blunt vector using a CloneJET PCR cloning

kit (Thermo Scientific) and the obtained recombinant plasmids were transformed into competent *Escherichia coli* Dh5α cells by heat shock [20]. Plasmid vectors from individual colonies were used for sequencing using pJET Fw (5′-CGACTCACTATAGGGAGAGCGGC-3′), pJET Rev (5′-AAGAACATCGATTTTCCATGGCAG-3′) as well as internal primers developed for primer walking. Sequencing was performed by Macrogen Inc. (Seoul, Republic of Korea) The four identified allelic forms were deposited in Genebank under the following accession numbers: MH593872, MH593873, MH593874 and MH593875.

Analysis of allelic configurations of *R. x damascena* mill AGAMOUS

The fluorescently labelled primers for amplification of allele-specific PCR products were designed using the online version of the Primer 3 software based on the sequences of the four identified alleles of *R. x damascena* AGAMOUS. The constructed primer pair consisted of the forward primer AGM2F (FAM-5′-TCAATAAACTGTGGCAGTGAAC-3′), fluorescently labelled at the 5′-end with FAM and the reverse primer AGM2R (5′-TTTGCTTGGTCATTGATCCT-3′). The temperature programme used for PCR amplification was as follows: denaturation at 95 °C for 3 min; 35 cycles of denaturation at 95 °C for 15 s, hybridization of the primers at 53 °C for 15 s, elongation at 72 °C for 30 s and a final elongation step at 72 °C for 5 min. Analysis of the amplified fragments was performed on an ABI 3130 (Thermo) automated sequencer using capillaries with a length of 36 cm, POP7 polymer and GeneScan 600 LIZ dye Size Standard (Thermo Scientific). Sizing of the PCR fragments was performed using the GeneMapper 4.0 software (Applied Biosystems, Foster City, CA).

Analysis of microsatellite loci

The PCR reactions were performed in a volume of 20 μL, comprising 40 ng genomic DNA, 5 pmol locus specific forward primer containing a 14-bp M13 tail at

Table 2. Primers used for analysis of microsatellite loci.

Locus	Primer pair sequence*	τ annealing (°C)
Rh58	Fw 5′-**AAACGACGGCCAGT**ACAATTTAGTGCGGATAGAACAAC-3′	59
	Rev 5′-GGAAAGCCCGAAAGCGTAAGC-3′	
Rh50	Fw 5′-**AAACGACGGCCAGT**TGATGAAATCATCCGAGTGTCAG-3′	59
	Rev 5′-TCACTTTCATTGGAATGCCAGAAT-3′	
Rh65	Fw 5′-**AAACGACGGCCAGT**AGTACGCCGACGCAGATCCAGTGA-3′	67
	Rev 5′-ACGGCGTTGTAGGTCGTCATTCTC-3′	
Rhl402	Fw 5′-**AAACGACGGCCAGT**TCCCATCTTGCTAAGTGCCTT-3′	62
	Rev 5′-GTTTCTTCAGGGTAACTGAGCCGATT-3′	
Rw11E5	Fw 5′-**AAACGACGGCCAGT**GATACCGCGAAGGTGTAGT-3′	55
	Rev 5′-GAGTGAAAACTCTGCAATCA-3′	

*Note: The M13 tail in each forward primer is shown in bold.

Table 3. Flower biometric analysis* of plants obtained after self-pollination of *Rosa x damascena* f. trigintipetala.

Flower type	Value	Flower diameter, mm	Number of petals	Number of stamens
Simple flower	Aver.	62 ± 6	6 ± 1	110 ± 12
	Min.	50	5	82
	Max.	75	7	132
Semi-double flower	Aver.	61 ± 8	16 ± 5	76 ± 24
	Min.	46	8	36
	Max.	74	20	107
Double flower	Aver.	60 ± 10	43 ± 20	70 ± 28
	Min.	41	21	0
	Max.	112	103	134

*Note: Mean values ± standard deviation. Simple flower defined as 6 ± 1 petals, semi-double flower as $>7 \leq 20$ petals, double flower as >20 petals.

the 5′-end (Table 2), 20 pmol locus specific reverse primer (Table 2), 20 pmol forward M13 primer labelled at the 5′-end with FAM (FAM-5′-GTAAAACGACGGCCAGT-3′) used for fluorescent labelling of the products and 2× PCR mix MyTaq HS Mix, 2× (Bioline USA Inc.). The temperature programme included: denaturation at 95 °C for 3 min, 30 cycles of denaturation at 95 °C for 15 s, primer annealing at the temperatures shown in Table 3 for 15 s, elongation at 72 °C for 30 s followed by 10 cycles of denaturation at 95 °C for 15 s, hybridization at 53 °C for 15 s, elongation at 72 °C for 30 s and a final elongation step at 72 °C for 10 min. Fragment analysis was performed on an ABI 3130 (Thermo) automated sequencer using capillaries with a length of 36 cm, POP7 polymer (Thermo) and GeneScan 600 LIZ dye Size Standard (Thermo). Sizing of the PCR fragments was performed using the GeneMapper 4.0 software (Applied Biosystems) and the allele lengths of the analysed loci were calculated after subtracting 17 bp corresponding to the length of the M13 primer used for fluorescent labelling.

Statistical analysis and graphical presentation of data

Correlation analysis as well as analysis of variance (ANOVA) was performed using SPSS v. 21 (IBM SPSS Statistics, Armonk, NY). Microsoft Office 2016 was used for building box and whiskers plots.

Results and discussion

Flower morphology

A total of 143 plants from a population obtained after self-pollination of *R. x damascena* f. trigintipetala, were used for characterization of flower morphology parameters. Significant variation in the population was observed for all three measured parameters including flower diameter, number of petals and number of stamens (Figure 1, Table 3). Forty plants (28%) were identified as possessing simple flowers (6 ± 1 petals), 9 plants (6%) with semi-double flowers ($>7 \leq 20$ petals) and 95 plants (66%) with double flowers (>20 petals). Correlation analysis showed statistically significant ($p<.01$) strong negative correlation ($r= -0.785$) between the number of petals and the number of stamens in the flowers. There was weak negative correlation between the number of petals and flower diameter ($r= -0.242$, $p<.01$) and weak positive correlation between the number of stamens and flower diameter ($r= 0.269$, $p<.01$). Roses with simple flowers were characterized by a large number of stamens, 110 ± 12, with a maximum number of 132. At the same time, significant variation in the number of both petals and stamens with mean values of 43 ± 20 and 70 ± 28, respectively was observed in plants with double flowers. The variation of petal number ranged from 21 to 103, while the number of stamens ranged from 0 to a maximum of 134, which was even higher compared to simple flowers.

Figure 1. Variation in flower morphology in plants obtained after self-pollination of *Rosa x damascena* Mill. f. trigintipetala. Simple flower (a), semi-double flower (b), double flower (c and d).
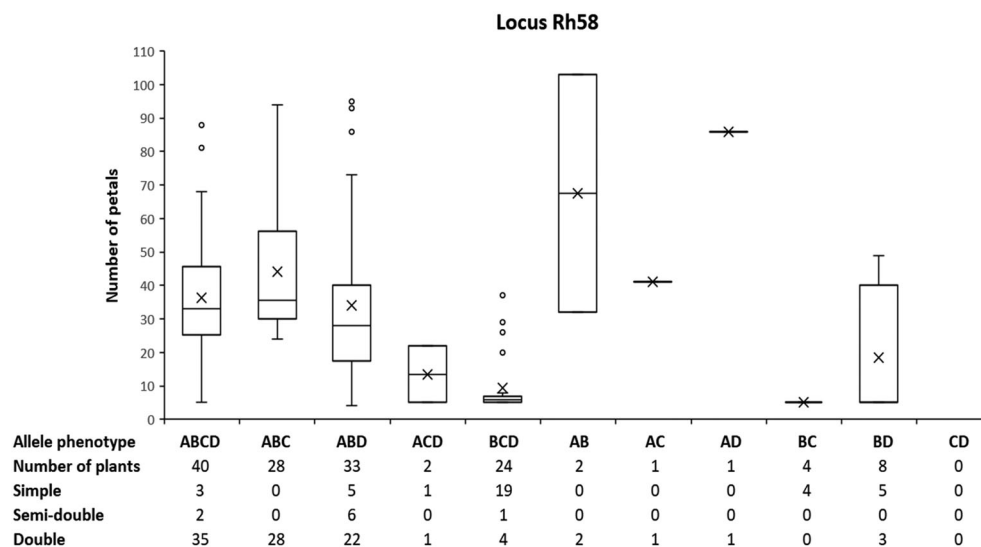

Allele phenotype	ABCD	ABC	ABD	ACD	BCD	AB	AC	AD	BC	BD	CD
Number of plants	40	28	33	2	24	2	1	1	4	8	0
Simple	3	0	5	1	19	0	0	0	4	5	0
Semi-double	2	0	6	0	1	0	0	0	0	0	0
Double	35	28	22	1	4	2	1	1	0	3	0

Figure 2. Box-and-whiskers plot showing the distribution of data for number of petals among plants with different allele phenotype of the microsatellite marker Rh58 in plants from a segregating population obtained after self-pollination of *Rosa x damascena* f. trigintipetala. A, B, C and D designate the scored allele sizes (A = 222 bp, B = 228 bp, C = 248 bp and D = 260 bp).

Segregation of microsatellite markers located near the *Blfo/d6* locus

Five microsatellite loci (Rh58, RhI402, Rh50, Rh65 and Rw11E5) derived from *R. hybrida* and *R. wichuraiana* and located in the region near the *Blfo/d6* locus on LG3 in the unified genetic map of diploid roses were analysed in the original genotype of *R. x damascena* f. trigintipetala. Of the five tested markers, only Rh58, RhI402 and Rh65 produced clear and reproducible PCR products with allele sizes of 222, 228, 248 and 260 bp for Rh58, 194, 200 and 206 bp for RhI402 and 128 bp for Rh65. Since only locus Rh58 had four different alleles in the original genotype of the tetraploid *R. x damascena*, this made it the most suitable candidate for analysis of allele segregation in the population. Figure 2 shows the data distribution for number of petals among the plants in the population for each observed allele phenotype of Rh58 in a population of 143 plants obtained after self-pollination of *R. x damascena* f. trigintipetala. ANOVA analysis showed that the observed allele phenotype of the analysed locus had a statistically significant effect on the number of flower petals ($p < .01$). The analysis of the results presented in Figure 2 showed a well-expressed relation between certain allele phenotypes of the Rh58 locus and the formation of double (>20 petals) or simple flowers (6 ± 1 petals). In 92% of the cases, the presence of the A allele (222 bp) was associated with the development of double or semi-double flowers, while in 81% of the cases its absence was associated with the development of simple flowers.

The obtained results show that the petal number trait in the tetraploid *R. x damascena* is controlled by the presence of a single copy of a dominant allele of the major locus designated as *Blfo/d6* on LG3 in the genetic linkage map of roses [12,13]. Until recently, specific genes associated with the major *Blfo/d6* locus had not been identified and isolated and its mapping in the *R. multiflora* genetic map was done following the segregation of the morphological trait as a monogenic trait and its linkage with certain molecular

markers. Recently, the complete genome sequence of *R. chinensis* was published, the authors also pointing out *APETALA2/TOE* as a candidate gene located in the region of the major locus controlling the petal number trait [21]. According to the ABC flower model, AP2 negatively regulates the expression of AGAMOUS [22]. However, further confirmation and verification of the role of APETALA2/TOE will be needed, which would then allow the use of gene-specific markers in breeding programmes without a compromise of potential recombination between the used marker and the locus determining the trait of interest.

qPCR and segregation analysis of R. x damascena *AGAMOUS*

The observed large variation in the group of double flower roses in the segregating population suggests the influence of other loci in addition to the major locus controlling the trait. The expression of *R. x damascena AGAMOUS* and two other flower homeotic genes including *R. x damascena APETALA1/FUL-like* (paleo AP1 type) and R. x damascena *euAPETALA 3* (euAP3 line) were studied during the early stages of flower development (stages 1–4 according to Dubois et al.[19]) in flower buds of the original genotype of *R. x damascena* f. trigintipetala possessing double flowers (30–35 petals) and in a plant obtained after self-pollination of *R. x damascena* f. trigintipetala characterized by simple flowers (6 ± 1 petals). Of the three genes tested, only *R. x damascena AGAMOUS* showed statistically significant difference in expression between the analysed samples (Figure 3): it was 3.4-fold upregulated in buds corresponding to simple flowers compared to double flower buds. Although the ortholog of *A. thaliana* AGAMOUS in roses has not been shown to be located in QTL loci controlling the petal number trait in roses and its location in the rose genetic map is on a different linkage group compared to the major *Blfo/d6* locus, its expression has a direct influence on the petal number trait and the transition of stamens to petals in double flowers [19]. We cloned and sequenced the four alleles of *R. x damascena AGAMOUS* and analysed their segregation in a population of 143 plants obtained after self-pollination of *R. x damascena* f. trigintipetala. Figure 4 shows the distribution of the data for petal number compared to the observed allele phenotype of *R. x damascena AGAMOUS* in the population. As seen from Figure 4, there was no direct relation between the number of petals and allele phenotype for the majority of allele phenotypes. Although some allele phenotypes like the
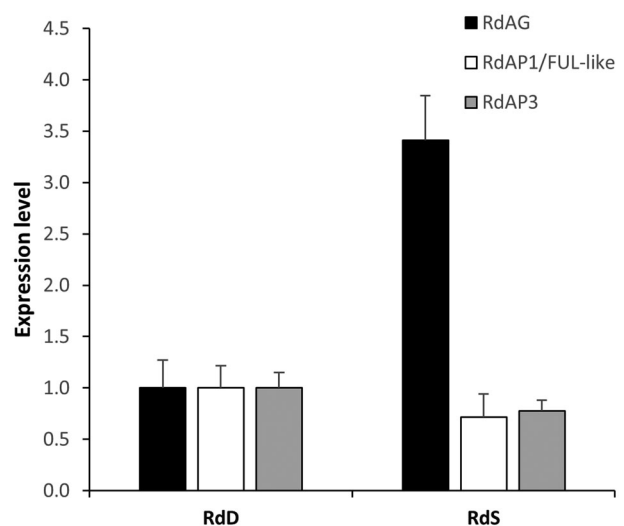

Figure 3. Expression level of *Rosa x damascena* AGAMOUS (RdAG), *R. x damascena* APETALA1/FUL-like (RdAP1/FUL-like) and *R. x damascena* euAPETALA3 (RdAP3). RdD, early stage flower buds of *R. x damascena* f. trigintipetala with double flowers. RdS, early stage flower buds of a simple flower plant (6 ± 1 petals) obtained by self-pollination of *R. x damascena* f. trigintipetala.

one designated as ABD in Figure 4 showed increased number of plants with double flowers, ANOVA analysis showed that the allelic configurations of *R. x damascena AGAMOUS* had no statistically significant influence on the number of petals (at $p<.05$). Additional ANOVA analysis after filtering out plants with simple flowers also showed no significant influence of the allelic configurations of *R. x damascena AGAMOUS* on the number of petals, thus suggesting that the gene is not likely to have an additive effect on the petal number trait in *R. x damascena*.

Our results related to the expression of the class C flower homeotic gene *AGAMOUS* are also consistent with the *A. thaliana* ABC model of flower development [22,23] and with the results of studies in *R. hybrida*, *R. gallica*, *R. chinensis* and *R. rugosa* [19], according to which the expression of the homoeotic gene from Class C *AGAMOUS* is directly related to the change in the number of petals, and its increased expression in the early stages of flower bud development is associated with a decrease in their number at the expense of the number of stamens. The analysis of the segregation of its four different alleles in the population of self-pollinated *R. x damascena* did not appear to statistically influence the trait or to infer an additive effect. This supports the hypothesis that the factor which determines the double flower phenotype is either the APETALA2/TOE gene itself or one located very close to it [21]. Analysis of other genes linked to QTL loci
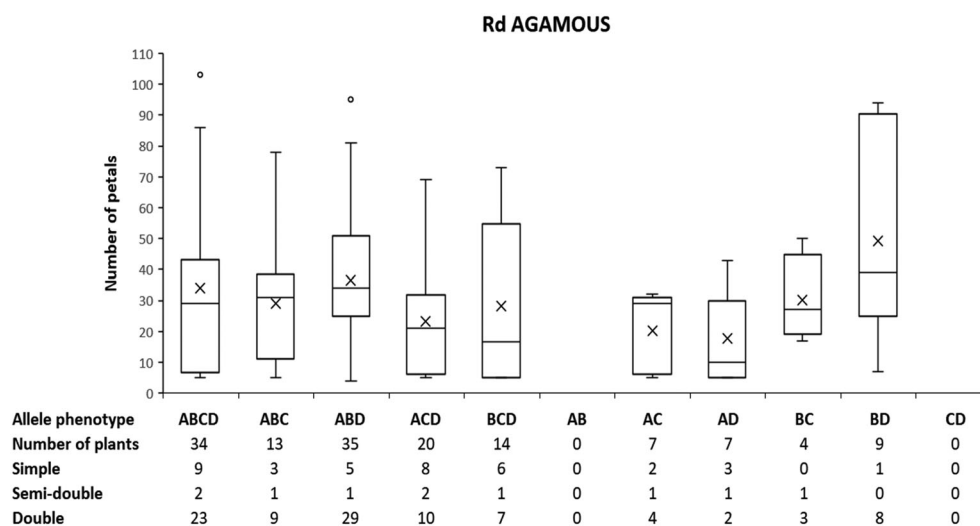
Rd AGAMOUS

Allele phenotype	ABCD	ABC	ABD	ACD	BCD	AB	AC	AD	BC	BD	CD
Number of plants	34	13	35	20	14	0	7	7	4	9	0
Simple	9	3	5	8	6	0	2	3	0	1	0
Semi-double	2	1	1	2	1	0	1	1	1	0	0
Double	23	9	29	10	7	0	4	2	3	8	0

Figure 4. Box-and-whiskers plot showing the distribution of data for number of petals among plants with different allele phenotypes of *Rosa x damascena* AGAMOUS in a segregating population of 143 plants obtained by self-pollination of *R. x damascena* f. trigintipetala. A, B, C and D designate the 4 different alleles of *R. x damascena* AGAMOUS scored by using the AGM2 primer pair (A = 147 bp, B = 153 bp, C = 155 bp and D = 163 bp).

controlling the double flower trait will be necessary in order to obtain a set of molecular markers for precise control of the trait in breeding programmes in *R. x damascena*.

Conclusions

The results obtained in this study clearly showed that the double flower trait in the tetraploid *R. x damascena* is controlled by a single copy dominant allele of the locus *Blfo/d6* previously mapped in the diploid *R. multiflora*. The results also indicated that the genetic background which determines the number of petals in the flowers of the tetraploid *R. x damascena* f. trigintipetala flowers is similar to that operating in the diploid *R. multiflora* and the teraploid *R. hybrida*. The study did not show the petal number of *R. x damascena* progeny plants to be influenced significantly by the allelic combination of the Class C flower homeotic gene AGAMOUS. The results from the present study offer direct application of molecular markers linked to the *Blfo/d6* locus in breeding programmes for improvement of the industrially cultivated *R. x damascena* and efficient early selection of desired double flower plants.

Disclosure statement

No potential conflict of interest was reported by the authors.

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