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Comparing plant morphology, fertility and secondary metabolites in Rosa hybrida cv Iceberg and its chromosome-doubled progenies

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ABSTRACT: In commercial breeding of ornamentals variation in plant morphology, alteration of secondary metabolites involved in production of flower color and aroma or increasing fertility are very important. In the present investigation morphological characteristics, pollen viability, pollen germination, seed setting and secondary metabolites content of *R. hybrida* cv Iceberg (Triploid) were compared with its chromosome-doubled progenies. Pollen viability was assayed by fluorescein diacetat. Pollen germination was determined using different concentrations of boric acid. Seed setting was compared in the two genotypes when they were allowed to open pollinate. Phenolic, anthocyanin, flavonoid, and carotenoid contents were compared after deriving the extract from petals, using a spectrophotometer. The aromatic compounds were extracted using hexane and the absolutes were analyzed by gas chromatography–mass spectrometry. Results indicated that some of morphological characteristics were significantly higher in the triploids than the hexaploids. However, diameter of the flowering stem, breadth/length ratio of the leaves and vase life of the flowers were significantly higher in the hexaploids than the triploids. The highest percentage of pollen germination was observed at 10 mg l^{-1} boric acid in both cultivars. The pollen viability and percentage of pollen germination were significantly higher in the hexaploids (30.54%, 34.41%) than the triploids (9.96%, 14.53%). Although, the total content of phenolic compounds were significantly higher in the hexaploids than the triploids, but there was not a significant difference in anthocyanin, flavonoid and carotenoid content between the two genotypes. In total, 16 chemical constituents were identified in the essential oil of triploid and hexaploid genotypes, from which some of the chemical constituents were significantly higher in the hexaploid than the triploid roses.
Keywords: Chromosome-Doubled, Pollen Germination, Pollen Viability, Seed Setting, *Rosa Hybrida*, Secondary Metabolites

INTRODUCTION

Roses are appreciated all over the world for their beauty and scent, and are by far the best selling cut flowers worldwide. Chromosome numbers in the genus *Rosa* are based on multiples of seven and range from 2n = 2x = 14 to 2n = 8x =56 (Darlington and Wylie, 1955). Most species are diploid, whereas most of the modern rose cultivars are tetraploid and usually interfertile (2n = 4x =28) (Smulders et al., 2011). Interspecific crosses between diploid species and tetraploid cultivars results in triploids. Triploid plants are often assumed to be sterile or semi-sterile and to have limited use in breeding programs. Chromosome doubling of the triploid roses is expected to produce hexaploids with higher levels of fertility. The new hexaploid plants may also possess other valuable characteristics which could make them notable individuals.

Polyploidization results in thicker leaves and stem, deeper green color, increased width/length ratio of leaves and more compact growth habit (Kermani et al., 2003; Allum et al., 2007). The larger and more heavily textured flowers and the longer or delayed flowering period associated with polyploidy can be of immense

significance in the breeding of ornamentals (Kumari and George, 2008). The polyploids in general are more tolerant to drought, cold, mutagens, herbicides and poor soils. The presence of additional alleles provides increased heterozygosity with higher levels of genetic and biochemical flexibility, which makes them adaptable to wider range of habitats and adverse climates (Kumari and George, 2008). In some cases polyploids have demonstrated greater resistance to pests and pathogens, greater nutrient uptake efficiency, better drought resistance, and superior cold tolerance. However, polyploidy may also result in reduced resistance to the stresses (Ranney, 2006).

Increasing the chromosome number and related gene dose may enhance the expression and concentration of certain secondary metabolites and defense chemicals (Kumari and George, 2008). However, this is not always the case and little is known about the relationship between gene dose, gene silencing, and expression of secondary metabolites (Ranney, 2006). According to Ranney (2006) a more promising approach would be to create allopolyploids between plants with diverse endogenous secondary metabolites. He suggested that unique and valuable characteristic of allopolyploids is that the secondary metabolites from the parental species are typically additive.

In most plant species artificial polyploidy generally enhances the growth vigor of determinate plant parts and may be favorable where vegetative organs and biomass constitute the economic product. Recently Dhooghe et al. (2011) reviewed the *in vitro* mitotic chromosome doubling of plants and its application in regard to the changes which occur in the chromosome-doubled progeny. They pointed out that some of these changes include: genetic adaptability and tolerance to environmental stresses, morphological and anatomical changes such as changes in leaves, flowers, stems and etc, exhibition of novel physiological and cultivation characteristics, demonstration of increased biomass and content of efficient compounds in medicinal plants and restoring fertility in interspecific hybrids.

In the present investigation in order to take advantage of the expected changes in the characteristics of the chromosome-doubled progeny, the morphological characteristics, pollen viability, pollen germination, seed setting and secondary metabolites content of *R. hybrida* cv Iceberg (triploid) and its chromosome-doubled progenies were evaluated. This is also the first report on comparison of chemical constituent between a rose and its chromosome-doubled (hexaploid) forms.

MATERIAL AND METHODS

Plant material

The own rooted triploid genotype *R. hybrida* cv Iceberg (the trade name of the rose genotype 'KORbin') and its chromosome-doubled genotype (from our previous work; Khosravi et al., 2007) were supplied by the Rose Germplasm Collection at the Agricultural Biotechnology Research Institute of Iran (ABRII). All of the chromosome doubled plants were originated from one of the chromosome-doubled plant (a clone), obtained from trifluralin treatments. As stated in Khosravi et al., 2007 chromosome doubling was assessed by flowcytometer and the mixoploids were excluded. All of the plants were transferred from *in vitro* conditions to the green house a year before and were studied when their second bloom was seen in the spring.

Morphological analysis

The morphological characteristics of one year *ex vitro* plants were evaluated as follow: total number of leaves per plant, total number of flowering stems per plant, total number of axillary branches/ main stem, plant height (cm), length of the flowering stem (cm), diameter of the flowering stem (cm) (using a vernier caliper), length of internodes (cm), leaflet breadth/length ratio, leaflet surface area (cm^2) (using a leaf area meter), number of petals, diameter of flower (cm) (using a vernier caliper when the flowers were four fifth open) and flower vase life (number of days before the flowers withered). Measurements of leaves were based on the means of five mid-stem leaves and breadth/width ratios were measured on the terminal leaflets. The data were recorded in the spring, when all the plants were one year old. The experiment was set according to the completely randomized design with four replications (each replication included 24 plants).

Pollen viability and germination

Flowers were collected at the stage of three-quarters-open bud (Richer et al., 2007) from one year *ex vitro* plants. Petals were removed, and the anthers were dried 2 h in a Petri dish at ambient temperature.

Pollen grain viability was assessed using fluorescein diacetate (FDA) which was dissolved in acetone at 10 mg ml^{-1}. Prior to each experiment, FDA was diluted in a 10% sucrose solution to a final concentration of 0.2 mg ml^{-1} (Ueda, 1994). Hydrated pollen was dipped in 250 µL of the FDA solution on a glass slide and kept in the dark for 5 min. The proportions of viable pollen were based on 100 pollen grains per plant and seven plants per genotype. Observations were made with a Nikon-E1000 fluorescent microscope using a violet filter combination.

In vitro pollen germination was studied using different concentrations of boric acid (0, 5, 10 and 15 mg l^{-1}) in 1% agar media and 25 mgl^{-1} sucrose. Semi-solid media were poured into Petri dishes with a thickness of about 1 cm and left to cool. Hydrated pollen were then sprinkled on the surface of the media and Petri dishes were placed in the dark at 22°C for 2 h. Samples were observed with a Nikon TE300 inverted microscope. The experiment was set according to the completely randomized factorial design with four replications (each replication included 50 pollen grains).

Hip and seed set

The *ex vitro* triploid and their hexaploid progenies were transferred to the garden (Rose germplasm collection at ABRII) in the early spring of the second year. The open pollination conditions were allowed; they were free to either self pollinate or cross pollinate. The hip and seed setting were compared at the end of autumn. For each genotype 25 hips were collected, the size of the hips was observed visually and photographs were taken. The hips were cut using a scalpel and seeds were collected.

Secondary metabolites content evaluation

Pigment extraction

Extracts were derived from freeze-dried petals (0.1 g) using 10 ml methanol (pH=2). The extract was then set in water bath at 60°C for 1 h, the mixture was centrifuged with 10000g for 20 min and the supernatant was used for further analysis to calculate total phenolics content, total anthocyanin content and total flavonoid content. For each analysis 5 samples were prepared and the mean was recorded.

Total phenolics content

Folin-Coicalteu reagent was used to determine the total phenolics (McDonald et al., 2001). 0.5 ml of the Folin-Coicalteu reagent was added to 0.5 ml of the petal extract and 0.5 ml of the standard gallic acid. Then 4 ml of 1M $NaCO_3$ was added to the solution and the mixture was left standing at room temperature for 15 min. The absorbance of total phenol was measured at 765 nm, with a UV/VIS spectrophotometer.

Total anthocyanin content:

Total anthocyanin content was estimated using pH differential assay (Rapisarda et al., 2000). An aliquot of petal extract (2 ml) was diluted up to 25 ml with pH 1 buffered solution (125 ml of 0.2 M potassium chloride and 375 ml of 0.2 M chloride acid). The second one was diluted with a pH 4.5 buffered solutions (400 ml of 1 M sodium acetate, 240 ml of 1 M chloride acid, and 360 ml of distilled water). Absorbance of the solutions was measured at 510 nm. Concentration of anthocyanin as cyanidin-3-glucoside was calculated by the equation:

$Cmg\ l^{-1} = (Abs_{pH\ 1} – Abs_{pH\ 4.5}) \times 484.82 \times 1000/24825 \times DF$.

The numbers of 484.82 and 24825 are the molecular mass and molar absorptivity (ζ) of cyanidin-3-glucoside at 510 nm, respectively and DF is the dilution factor.

Total flavonoid content

The aluminum chloride colorimetric method was used to determine the total flavonoid (Chang et al., 2002). The extract (0.5 ml) was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm with a UV/VIS spectrophotometer. Quercetin was used to make the calibration curve. Ten milligrams of quercetin was dissolved in 80% ethanol and then diluted to 0, 25, 50 and 100 µg ml^{-1}.

Total carotenoid content

For extraction of carotenoid, the fresh petals (1.0 g) were extracted with 20 ml of 80% acetone. The mixture was centrifuged with 937 g for 15 min. Then 1 ml of the supernatant was used for reading the absorbance of the reaction mixture at 440, 663 and 645 nm with a UV/VIS spectrophotometer and was calculated by the

equation described by Lichtenthaler and Wellburn (1985). In this analysis 5 samples were prepared and the mean was recorded.

mg carotenoid/g weight petal = (OD 490) – (OD663) (0.114) – (0.638) (OD645).

Essential oil constituents

Flowers of each genotype were collected between 6-8 am in the morning; 100 g of petals were soaked in hexane for 24h, then they were removed from the solvent. Distillation process was performed to recover solvent from concrete oil (organic solvent and rose oil) and this was done by rotary evaporator. The temperature of the rotary evaporator was set at 45°C. Absolute oil from concrete oil was recovered by adding 20 ml of absolute alcohol, the alcohol removes all the natural waxes present in the essential oil. The oil was filtered through a filter paper and absolute alcohol was removed by performing distillation process using a rotary evaporator (Sefidkon et al., 2006**)**. Different constituents of essential oil were determined using gas chromatography. In the present analysis the CP-3800 gas chromatograph, VF-5 with column length 30 m, and column thickness 25 µm was used. In all analysis the sample size was 1 µl and the carrier gas (helium) was kept at flow velocity of 1 ml per min. The initial column temperature and final column temperature were 60°C and 250°C respectively. The initial hold up time was 4 min and the ramp rate was 4°C per min.

A gas chromatography–mass spectrometry (GC/MS) analysis was performed on a Varian CP-3800 GC coupled with Varian 4000 (Ion trap) Mass system. The operating conditions were the same conditions as described above but the carrier gas was Helium Mass spectra were taken at 70 ev. Mass range was from m/z 35-400 amu.

The chemical composition of the essential oils were identified by calculation of their retention indices under temperature-programmed conditions for n-alkanes (C6–C24) and the oil on a VF-5 column under the same chromatographic conditions. The compounds were identified by comparison of their mass spectra with those of the internal reference mass spectra library (Wiley 7) or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those reported in the literature. For quantification purpose, relative area percentages obtained by FID were used without the use of correction factors.

Experimental design and statistical analysis

Data of morphology, pollen viability and secondary metabolites content were analyzed in a completely randomized design and experiment of pollen germination was analyzed in a factorial based on completely randomized design. Analysis of variance was performed and comparisons of means were conducted using least significant difference (LSD). All analyses were performed using SAS and MSTATC software. Differences were regarded as significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Morphological analysis

Statistical analysis of the morphological characters of hexaploid and triploid roses showed that total number of leaf (255.51 and 677.12), total number of flowering stems (1.66 and 5.66), length of internodes (3.77 and 4.88 cm), number of axillary branch of main stem (3.33 and 9.33), plant height (62.00 and 97.62 cm) and length of flowering stem (63.41 and 85.18 cm) were significantly less in hexaploid chromosome-doubled plants compared to their triploid progenitors (Table 1). Diameter of the flowering stem (0.50 and 0.37 cm), vase life of flower (13.00 and 5.80 day) and leaflet breadth/length ratio (0.64 and 0.48) were significantly greater in the hexaploid roses than triploids (Table 1). Although the leaflet surface area, diameter of flower and number of petals/flower were higher in the hexaploids, but the differences were not significant (Table 1). The color of the petals in the triploid flowers were whitish cream whereas the color of the petals in hexaploid flowers were light pink (Fig 1). Comparison of the leaves of hexaploid and triploid roses are shown in Fig 2.

Chromosome doubling only duplicates the number of existing chromosomes in a plant genome and new genetic material are not inserted into the plant. However, as it has been reviewed by Osborn et al. (2003) and Otto (2007) loss of duplicated genes, gene expression, alterations and epigenetic changes modulating gene expression are usually associated with chromosome doubling. These genetic changes may result in plants which are significantly different to their progenitors. Mitotic polyplodization induces changes in vegetative characters such as reduced height, thicker, darker green leaves with a more highly serrated margin, increased thickening of petiole and stem with correspondingly shortened nodes and wider crotch angles, features that have all been noted in roses (Kermani et al., 2003), cherry (James et al., 1987), Antirrhinum (Mahal et al., 1968), cotton (Mehetre et al., 2003)

and Spathiphyllum (Eeckhaut et al., 2004). Kermani et al., (2003) showed that internodes length and leaflet breath/length ratio were greater in hexaploid than triploid roses which is in agreement with the results of the present study.

In the present investigation, the different flower color of chromosome-doubled plants compared to their progenitor, could be due to the difference in concentration or composition of anthocyanin in petals. Table 2 illustrates that the anthocyanin content was higher in the hexaploids than the triploids, however, the difference was not significant. The anthocyanin compositions of the hexaploid and the triploid rose have not been analyzed. However, flower color also could be correlated with particular essential oils (Plummer et al., 1999). Deeper colored flowers have also been reported in carnation (Yamaguch, 1989) and cyclamen (Takamura and Miyajima, 1996) after mitotic polyplodization.

R. hybrida cv Iceberg has been used as a popular garden rose for many decades. The chromosome-doubled form of this plant with new and attractive morphological characteristics may also have good market potential.

Pollen viability and germination

The percentages of viable pollen grains were 30.54% in hexaploids and 9.96% in the triploid progenitors. Triploids have often lower fertility levels than plants with an even ploidy level due to the problems with pairing during meiosis and the resulting unbalanced gametes. Increase in percentage of pollen viability in chromosome doubled roses has been reported by a few authors. Kermani et al. (2003) and Allum et al. (2007) showed that pollen viability was lower in diploid and triploid roses compared to their tetraploid and hexaploid progenies. In contrast, Ishikawa et al. (1999) described that pollen viability was greater in tetraploid than diploid in Alstroemeria.
In the pollen tube, boron is involved in membrane and cell wall formation, H+-ATP_{ase} activity, pollen germination tube growth (Wang et al., 2003) and oscillation behavior (Holdaway-Clarke et al., 2003). Brewbaker and Kwack, (1963) showed that 10 mg l^{-1} boric acid was found to be essential for pollen germination of plants. In Picea meyeri, boron deficiency decreased pollen germination (Wang et al., 2003). In the present investigation the highest percentage of pollen germination was observed in the medium containing 10 mg l^{-1} boric acid in both genotypes. Fig 3 illustrates that the percentage of pollen germination was significantly greater in hexaploids (34.47%) than triploids (14.53%).

Fortescue and Turner (2004) stated that germination potential is defined as the ability of viable pollen to germinate under suitable germination conditions. In the present investigation the correlation observed between the percentages of pollen germination (14.53% and 34.47%) and pollen viability (9.96% and 30.54%) in the triploids and hexaploids indicates that *in vitro* pollen germination could be used as a tool to estimate pollen viability. The *in vitro* pollen germination has also been regarded as the most widely used method of testing pollen viability by Marcellán and Camadro (1996).

Hip and seed set

In roses the swollen receptacle is known as the hip and the fruit consisting of the seed and pericarp wrapping is called the achene. Since it is common to use the term seeds, throughout this paper we have employed the word ‘seeds’ instead of ‘achene’. In order for roses to produce hips they need two conditions. First: the rose is not a sterile cultivar, which would preclude it being able to produce seed. Second: the fertile rose is successfully pollinated, which could commence to hip/seed formation. In the present investigation the hip and seed set of *R. hybrida* cv Iceberg (triploid) were compared with its chromosome-doubled progenies. In general the hexaploid plants produced less hips compared to their triploid progenitor. This could be due to the fewer number of flowering stems of the hexaploids compared to the triploids (Table 1). As it is illustrated in Fig 4, hips from the hexaploid plants were smaller compared to the hips of triploids. Only a few hips (approximately $1/6^{th}$ of the total hips) of the triploids were of good size (Fig 4) which could produce seeds. Seed setting in the hexaploid plants was 0% but it was 16% (4 hipsfrom the 25 hips had ripened ahenes/seeds) in the triploids. In the four hips which contained seeds, two had two seeds each and two had one seed each (in total 6 seeds). Female fertility in flowering plants on rare occasions reaches its maximum; usually it is rare to obtain one hip per flower and one seed per ovule, especially in outcrossing species. Genetic and environmental factors contribute to the submaximal fertility in plant populations (Griffin and Barrett 2002). Griffin and Barrett (2002) suggested that hip and seed set are affecting by pollen limitation and resource limitation of seed set in Tillium graniflorum. In the present investigation the lack of seed setting in the hexaploids could be due to their morphological characteristics. For example the more tightly and profusely-petaled flower forms can be difficult for insects to pollinate well, which in turn can have a negative effect

on seed setting. Obviously it is expected that in controlled pollination conditions the rate of seed setting to be higher than when there are open pollination or self pollination conditions.

Assessment of secondary metabolites content

The results showed that amount of phenolic compounds were significantly higher in the hexaploids than the triploids, however, there was not a significant difference in anthocyanin, flavonoid and carotenoid content between the two genotypes (Table 2). Although there was not a significant difference in the total content of anthocyanin between triploid and hexaploid roses, however, because of the very clear difference in the flower color (Fig 1) it is possible that there were significant differences in the chromophores of anthocyanin content between the two genotypes. Tanaka et al. (2009) indicated that from the hundreds of anthocyanins which have been reported, all of them are based upon six common anthocyanidins; pelargonidin, cyanidin, peonidin, delphinidin, petunidin and malvidin. Nakamura et al. (2010) stated that orange/brick red petals tend to contain anthocyanins derived from pelargonidin, red/magenta petals contain anthocyanins derived from cyaniding or peonidin and blue/violet petals contain anthocyanins derived from delphinidin, petunidin or malvidin. Moreover according to Tanaka et al. (2009) the final flower color in those species in which color is derived from anthocyanin is determined by a combination of various factors. They stated that anthocyanin structure, type and concentration, co-existing compounds (co-pigments), metal ion type and concentration, pH of vacuoles, anthocyanin localization and shapes of surface cells all contribute to the final flower color.

The components identified through gas chromatography (GC/MS) in two genotypes are illustrated in Table 3which shows that the amount of components were different in *R. hybrida* cv Iceberg and its chromosome-doubled progenies. In total 16 components were identified in the essential oil of triploid and hexaploid genotypes. The major constituents were dihydroxy-B-ionol, geraniol and B-ionol. Dimethoxy toluene and Hexanol was observed only in triploids and beta-pinene, D-Germacrene and B-guaiene were detected in hexaploids.

Table 3 shows that percentages of some of the chemical constituents of essential oil in the hexaploids were higher than the triploid roses. Polyploidy can influence the physiological and biochemical activities such as net photosynthesis transpiration, enzyme activity, photosynthetic electron transport and isozyme expression. According to Dhawan and Lavania, 1996 induced polyploidy often cause gigantism leading to enhanced biomass production. They suggested that this potential can be utilized to obtain genetic improvement of plants which their vegetative organs and their biomass are economically important. An obvious support to such notion has been provided by Janaki-Ammal and Gupta (1966) where they reported a positive correlation for the concentration of essential oil present in the leaves of naturally occurring 2x, 4x, 6x cytotypes of lemon grass (Cymbopogon flexuosus). Also in vetiver grass (*Vetiveria zizanioides* L. Nash) tetraploids were vigorous with thicker and longer roots, and the percentage of essential oil increased from 0.98% to 1.4% in freshly harvested roots of tetraploid plants compared to the control (Lavania, 1988).

CONCLUSION

Present investigation is the first report on comparison of chemical constituent between a rose and its chromosome-doubled (hexaploid) progenies. The chemical constituent and flower color of the hexaploid rose were significantly different to its progenitor.

In the present work chromosome doubling has been presented as means of induction of valuable morphological changes which could be beneficial in flower industry. *R. hybrida* cv Iceberg has been used as a popular garden rose for many decades. The chromosome-doubled forms of this plant with new characteristics such as fewer number of leaves, fewer number of axillary branches (i.e. less vegetative growth), darker green leaves, thicker flowering stems, different flower color and different petal chemical constituents (oil composition) illustrates its potential for utilization as a new rose cultivar.

The percentage of pollen viability and pollen germination were significantly higher in the hexaploids than the triploids making this genotype to be more efficient in breeding programs. The new rose genotype with the suggested name ABRII has been introduced into our rose breeding program.

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Table 1. Morphological characteristics of hexaploid and triploid plants of *R. hybrida* cv Iceberg. Different letters show significant differences between the two genotype according to LSD (P ≤ 0.05%)

Characteristics (Average)	Hexaploid	Standard error	Triploid	Standard error
Total number of leaves	255.51[b]	42.93	677.12[a]	54.57
Total number of flowering stems	1.66[b]	0.33	5.66[a]	0.66
Number of axillary branches on main stem	3.33[b]	0.33	9.33[a]	1.45
Plant height (cm)	62.00[b]	4.41	97.62[a]	3.86
Length of flowering stem (cm)	63.41[b]	4.95	85.18[a]	3.06
Diameter of the flowering stem (cm)	0.50[a]	0.04	0.37[b]	0.03
Length of internodes (cm)	3.77[b]	1.05	4.88[a]	0.83
Leaflet breadth/length ratio	0.64[a]	0.05	0.48[b]	0.03
Leaflet surface area (cm^2)	12.65[a]	0.40	11.26[a]	0.60
Number of petals per flowers	33.40[a]	2.58	31.50[a]	1.34
Diameter of flower (cm)	5.41[a]	0.20	5.35[a]	0.33
Vase life of flower (day)	13.00[a]	0.70	5.80[b]	0.83

Table 2. Secondary metabolites content of hexaploid and triploid plants of *R. hybrida* cv Iceberg. Different letters show significant differences between the two genotype according to LSD (P ≤ 0.05%)

Secondary metabolites(mg/gdw)	Hexaploid	Standard error	Triploid	Standard error
Total phenolics content	2377.20[a]	20.80	2001.80[b]	32.69
Total anthocyanin content	11.16[a]	0.88	10.83[a]	1.23
Total flavonoid content	7.714[a]	0.28	6.94[a]	0.45
Total carotenoid content	0.02[a]	0.00	0.03[a]	0.00

Table 3. Comparison of chemical components of essential oil in the hexaploid and triploid plants of *R. hybrida* cv Iceberg. * ND means not identified

Components	Kovatas Index	Hexaploid (%constituents)	Triploid (%constituents)
Hexanal	803	0.61	7.37
Hexanol	852	-	4.41
Geraniol	1257	23.58	18.25
Dimethoxy toluene	1271	-	5.83
TheaspiraneA	1307	3.53	2.01
TheaspiraneB	1315	4.52	2.18
Beta-pinene	1323	2.00	-
Eugenol	1362	6.76	4.76
*ND	1396	1.57	-
B-ionol	1419	10.93	8.71
Dihydroxy-B-ionol	1451	26.52	21.55
*ND	1488	2.65	3.20
D-Germacrene	1491	6.92	-
*ND	1512	-	2.08
B-guaiene	1516	1.71	-
*ND	1607	2.14	3.71
Total	-	93.43	84.06

Figure 1. Flowers of *R. hybrida* cv Iceberg: (a) hexaploid and (b) triploid (Bars= 20 mm). Note the light pink petals in hexaploid and whitish cream petals in the triploid flower

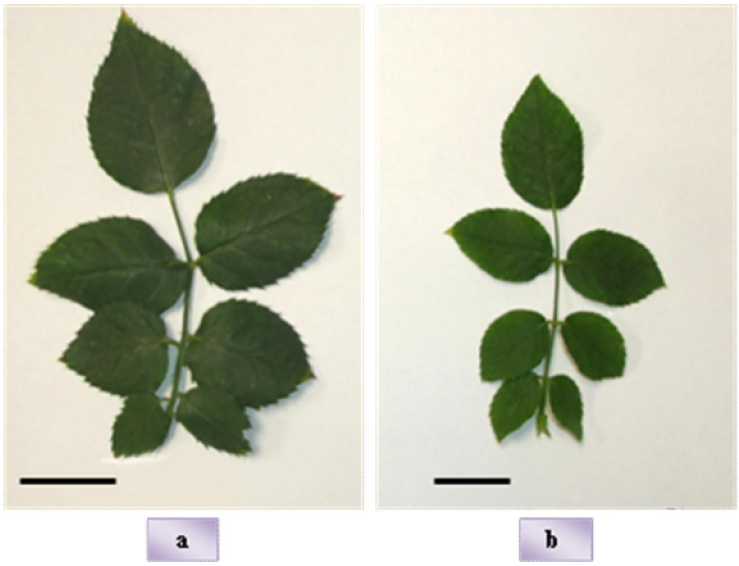

Figure 2. Leaves of *R. hybrida* cv Iceberg: (a) hexaploid and (b) triploid (Bars= 20 mm). Note the darker green leaves and the grater leaflet breadth/length ratio in the hexaploid plant compared to the triploid.

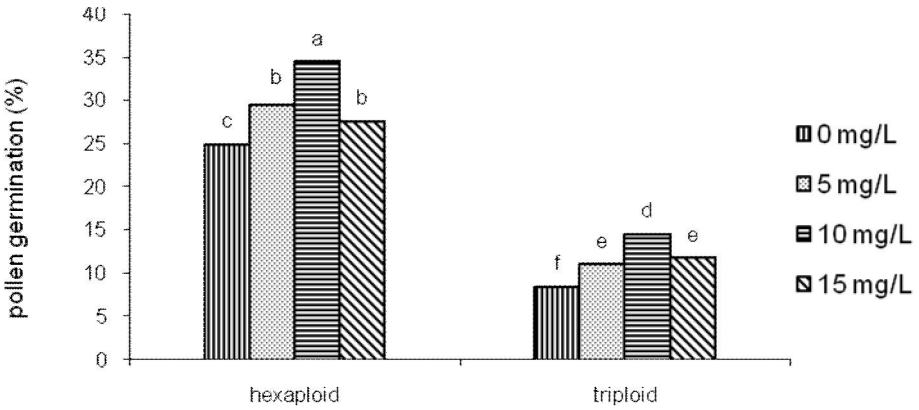

Figure 3. Effect of boric acid concentrations on percentage of pollen germination in the hexaploid and triploid plants of *R. hybrida* cv Iceberg. For each genotype, columns with the same letters are not significantly different at P ≤ 0.05

Figure 4. Hips of *R. hybrida* cv Iceberg: (a) hexaploid and (b) triploid (Bars= 20 mm). Note the larger size of the hips in the triploid plant.

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