A novel source of food – garden rose petals

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Abstract

Even though rose is the most widely grown ornamental, the garden rose market in western Europe has been declining for over 30 years. In the past roses were valued based on aesthetic characteristics, while petals and hips were considered waste. At the same time, a need for added value in garden roses production has arisen and trends for healthy nutrition and a need for new food sources became imperative. The rose petal compounds suddenly turned out to be a source for new product market combinations. In this study, two aspects of rose petals were considered: biochemical composition (amount of vitamin C, antioxidants, sugar and organic acids) and consumers' perception (taste and texture). Biochemical analysis revealed that rose petals contain a high amount of vitamin C and antioxidants, which indicates the potential value of roses as food. Additionally, the ratio between organic acids and sugars was positively correlated with taste perception and could be used as a marker for the selection of edible roses. The aim of the study was to test the effectiveness of a genome-wide association study (GWAS) for mapping QTLs related to biochemical compounds and morphological traits related to petal production. A set of 192 tetraploid cultivars/accessions was genotyped using the WagRhSNP Axiom SNP Array. PCA and Ward's clustering of genetic similarities indicated the presence of two main groups of cultivars/accessions. GWAS was performed by a linear mixed model approach, taking into consideration different corrections for population structure. All models detected QTLs for citric acid, antioxidant capacity and yield.

Keywords: genome-wide association study (GWAS), rose, taste, vitamin C, organic acids, antioxidants, sugar

INTRODUCTION

Roses are the most important ornamentals in today's global flower market and have been the center of attraction for consumers and breeders for hundreds of years. Modern roses (*Rosa hybrida*) have resulted from extensive hybridization of wild rose species. Despite the ornamental value of roses, the garden rose market in western Europe has been declining for over 30 years. In the past roses were valued based on aesthetic characteristics, while petals and hips mostly remained unused. At the same time when a need for added value in garden roses production arose, trends for healthy nutrition and a need for new food sources became imperative. The rose petal compounds suddenly turned out to be a source for new product market combinations.

The composition of rose petals (vitamins, antioxidants, phenols, carotenoids, etc.) makes them candidates as a new source of food. Rose petals are rich in vitamins, especially vitamin C, phenolic compounds, flavonoids, tannins, carotenoids, polysaccharides, anthocyanins and flavonoid glycosides. Rose petals were discovered to protect against cardiovascular and gastrointestinal diseases (Roman et al., 2013). In addition to antibacterial and antifungal properties, an antimutagenic effect of rose petals was confirmed. *R. rugosa* (Thunb.) is popular in Asian countries, where petals have been utilized in traditional medicine for the treatment of diarrhea, injuries, gastroenteritis, hepatitis, dysmenorrhea and blood circulation disorders, as well as pain management and hemostasis maintenance (Nowak et al., 2014). In spite of their potential, rose petals have not been extensively used as food. Till now rose petals have been used in wine and jam production, while their usage for fresh salads is

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not considered widely.

The main goals of this study were to detect nutritive valuable cultivars with high levels of biochemical compounds (vitamin C, sugars, antioxidants) that are at the same time tasty (based on data connected to the taste, texture, and perception of the food by different consumers). Selected cultivars preferably should be characterized by a high yield (number of petals, weight and size of petals, and number of flowers) and optimal production aspects (period of flowering). New trends in cultivation ask for eco-friendly (healthy, non-sprayed roses) and resource-saving (numerous petals, large number of flowers per season) cultivars. Important objectives of this study were the identification of good donors of traits related to nutrition and the development of tools for marker-assisted selection.

MATERIALS AND METHODS

Plant material

Initially, a panel of 312 cultivars was provided. To ensure a broad germplasm of natural/wild species, cultivars from different breeders and roses of different types were included in the study. Roses were grown in a greenhouse in Grubbenvorst (The Netherlands) under controlled conditions in 7.5-L pots. From each cultivar 20 plants were randomized in two blocks of 10 plants each.

After one year of growing, 220 genotypes from the initial set were selected for further experiments, based on 2 criteria: growth characteristics and disease resistance, provided that they were tetraploid. Additionally, some cultivars were excluded that were susceptible to disease and/or did not produce hips. This made them unsuitable for human intake and for this experiment. DNA was extracted from all cultivars included in experiment. A set of 192 cultivars was selected based on DNA quality and purity, and these were genotyped using the WagRhSNP Axiom SNP Array (Koning-Boucoiran et al., 2015). All 220 cultivars were phenotyped for biochemical, morphological, and taste-related traits.

Biochemical analysis

All biochemical analyses were performed in collaboration with BrightLab, Venlo in The Netherlands. Protocols for organic acids (malic, citric, and ascorbic) extraction were performed using ion-chromatography (IC) and high-performance liquid chromatography (HPLC), while antioxidant activity was measured by ferric reducing antioxidant power (FRAP) method. Sugar content was estimated by measuring °Brix value by refractometer.

Subsequently, the measurements were summarized as a single petal trait, which was organic acid per sugar content, defined as a sum of citric, malic and ascorbic acids divided by sugar °Brix value.

Morphological traits

Morphological traits connected to the yield were included in the experiment: flower diameter, fresh petal weight and fresh flower weight. From each cultivar/selection 20 plants (replicates) and from each plant 5 flowers were included in the experiment.

Yield estimation

Once every 2 weeks all buds and flowers were collected and counted in a period from April till the end of September.

Fragrance

Estimation of the fragrance was scored by sniffing flowers in the morning. Fragrance was scored on a 1-5 scale, where 1 was attributed to the absence of fragrance and 5 to the extremely fragrant flowers.

Taste-related traits

Taste-related traits are connected to the taste, texture, and perception of the food by different consumers. The aspects taken into consideration in this experiment were: 1) color suitability for food, 2) aroma/smell suitability for food, 3) mouthfeel (bite/afterbite), and 4) sweetness. All taste-related traits were graded from 0 to 3, where 3 is defined as most suitable. The experiment performed using a taste panel and was conducted in two consecutive years, 2015 and 2016.

Statistical analysis

The statistical analysis of GWAS and plotting were performed in R 3.3.2. R package GWASpoly (Rosyara et al., 2016) was used for QTL analysis. The package uses a linear mixed model approach with population structure correction. Four different models were used to find significant QTLs: 1) general model – allows the fixed effect for each genotype class to be arbitrary; 2) additive model – SNP effect is proportional to the dosage of the minor allele; 3) simplex dominant model – all three heterozygotes are equivalent to one of the homozygotes; as there are two homozygous classes, there are two non-equivalent simplex dominant parameterizations for each marker; and 4) duplex dominant model – two non-equivalent duplex dominant models for each marker.

An association study was performed to find a significant correlation between the molecular markers and the traits of interest among the genetically diverse rose genotypes, which included a structure analysis among the accessions based on the markers. Associated markers were mapped to the available genetic linkage map for tetraploid roses (Bourke et al., 2017).

The kinship matrix used in the mixed model is defined using the realized relationship model, based on membership probabilities. A diagnostic tool used for evaluating the models is the Q-Q plot of observed vs. expected -log(p) values. For every trait it was determined if its values follow a normal distribution by using frequency barplots and Shapiro-Wilk test with significance level $\alpha=0.05$. For the traits that did not follow a normal distribution transformation was performed, such as square root, log, or Box-Cox transformation.

For the determination of threshold values permutation tests and Bonferroni correction were used.

RESULTS AND DISCUSSION

Phenotypic characterization of morphological traits

Ascorbic acid amounts in petals varied between 0 and 0.55 mg g^{-1} fresh weight (FW). Evaluation of appropriateness of rose petals for use as food was conducted by comparison of the amount of ascorbic acid in petals and previous results for soft fruit (Giongo et al., 2010). The amount of ascorbic acid in rose petals was higher than in blueberry, raspberry, red current and strawberry, while blackcurrants have higher ascorbic acid concentrations. The range of malic acid was between 0.23 and 6.08 mg g^{-1} FW (Table 1), while the amount of citric acid in petals varied between 0.01 and 0.73 mg g^{-1} FW. Organic acids from petals have a role in protecting the gastrointestinal tract by expelling toxic compounds. Rose petals also act as a laxative and can prevent medical conditions such as: constipation, gastroenteritis, dysentery and diarrhea, and has a beneficial effect on the gastric mucosa and intestinal bacteria, urinary tract infections (clean bladder and kidneys) and female reproductive system (Nowak et al., 2014).

Table 1. Descriptive statistics for petal traits.

	Ascorbic acid (mg g^{-1} FW)	Mallic acid (mg g^{-1} FW)	Citric acid (mg g^{-1} FW)	FRAP mmol (Fe^{2+} kg^{-1} FW)
Minimum	0.00	0.23	0.01	29.45
Maximum	0.55	6.08	0.73	371.13
Mean	0.05	1.99	0.13	124.93
Standard Dev.	0.10	0.95	0.10	57.29

FW = fresh weight.

Antioxidant capacity was estimated by FRAP and fluctuated between 29.5 and 371.1 FRAP mmol Fe^{2+} kg^{-1} fresh weight (FW). In order to assess the potential for using petals as food, antioxidant capacity was analyzed for commercially available vegetables: lettuce, broccoli, cucumber, capsicum and tomato (1.55, 4.96, 0.31, 13.91, and 2.21 mmol Fe^{2+} kg^{-1} FW, respectively). The results indicated that rose petals are a valuable source of antioxidants. The best-rated cultivars for antioxidant activity were characterized by flowers of intensive dark red/purple color (Figure 1), which are conditioned by the presence of anthocyanins. This finding is in agreement with previous studies (Schulz et al., 2021). Anthocyanins are relatively strong natural antioxidants. They have recently caught the attention of scientists and the public because of their possible use in fighting the effects of aging and reducing the risk of cancer and cardiovascular disease through their antioxidant power.

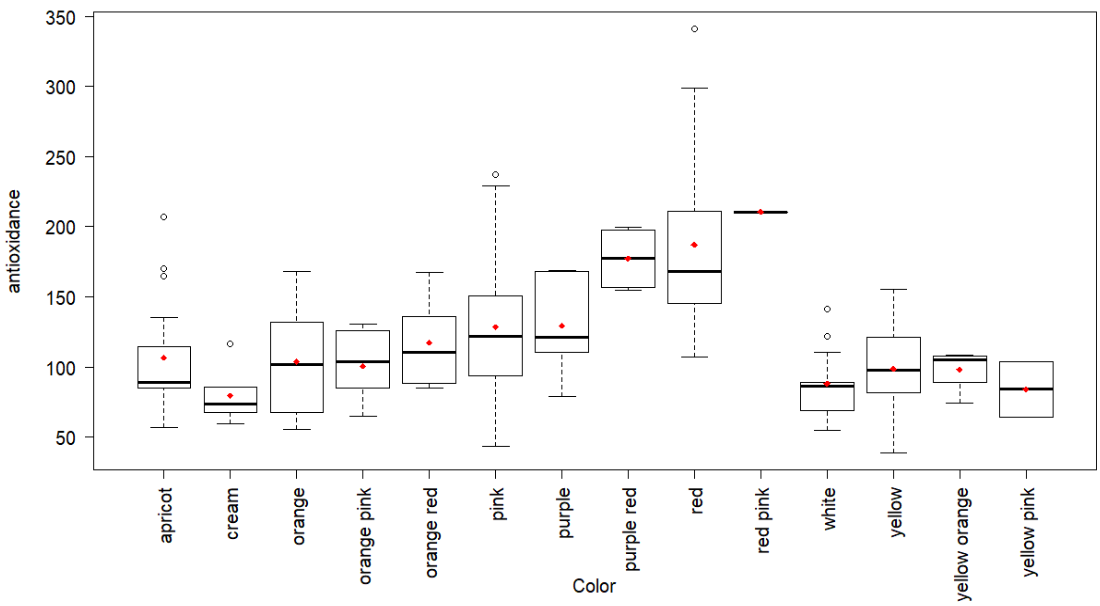

Figure 1. Distribution of cultivar mean values of antioxidant capacity in respect to color types defined.

The sugar content varies between 2 and 12.8 °Brix among genotypes. Phenotypic results indicate that the level of sugars is the most critical parameter for taste perception and that it is positively correlated with taste. Additionally, the ratio between organic acids (sum of malic, citric and ascorbic acid) and sugar level was directly correlated with taste, where higher relative sugar levels were associated with a higher taste score. However, some cultivars with a lower sugar content still scored high for taste. Also, cultivars with lower sugar content generally contained less organic acids, but their ratio of organic acids to sugar may still be high. This means that taste is a complex trait dependent on the biochemical composition and that the taste experience depended on sugar content as well as on the ratio between organic acids and sugar.

Practically, this information may help to select good tasting cultivars more efficiently. By measuring sugar and acid content, a preselection can be made of probably good tasting cultivars. Promising cultivars can then be subjected to a taste test for further selection.

Population structure

After a quality check and removing monomorphic markers, 10,786 SNPs were used for the GWAS analysis. Principal components analysis (PCA) and Ward's clustering indicated the presence of population structure, mainly related to origin and introgression (Figure 2). All cultivated roses grouped together, while three tetraploid wild species formed a separate cluster. Rose breeding programs seem to show a tendency toward using a limited gene pool and wild species can be valuable donors of traits.

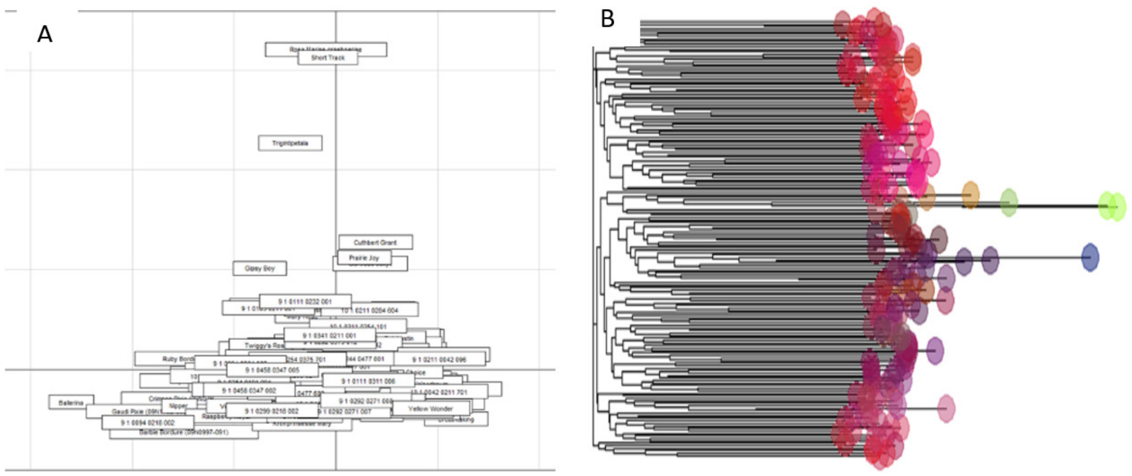

Figure 2. A) PCA based on 10,786 SNP markers with PC1 (explaining 9.15% of the variance) vs. PC2 (explaining 3.91% of the variance); B) Phylogram of the principal components retained, where every color corresponds to a respective principal component.

The association study for flower diameter revealed three QTL candidates: two on chromosome 2 and one on chromosome 1 (Table 2). These findings are partially overlapping with previous results. Yu et al. (2021) detected QTLs for flower diameter on chromosomes 2, 4 and 7. The main locus from this study, qfdia-2-2, was associated with SNPs located at the same region of chromosome 2 (103.47 cM), confirming that this region is responsible for inheritance of flower diameter.

Table 2. Results of the association study with respective QTL candidates.

	Models	QTLs	QTL score	Chromosome	Position (cM)
Flower diameter	1-dom-ref	K26_2683	5.14	2	88.22
	2-dom-alt	M15122_254	5.24	1	37.23
	2-dom-alt	K7396_324	6.04	2	103.08
Petal length	additive	M4500_727	4.93	5	6.46
Fragrance	1-dom-ref	K1346_882	5.25	7	48.17
Antioxidants	additive	K7634_1361	4.62	3	7.72
	additive	K1945_356	4.60	3	7.96
	additive	G57951_873	5.13	3	8.08
	additive	M10092_296	4.64	3	8.43
Yield	2-dom-ref	K1767_844	5.26	7	13.26
Citric acid	general	K1252_240	5.20	2	99.33
	additive	K1252_240	4.96	2	99.33
	2-dom-alt	K7396_324	5.03	2	103.08

GWAS analyses on petal length indicated a distinct peak at the beginning of chromosome 5 (6.46 cM). Coincidently, in a study of 95 garden rose genotypes (Schulz et al., 2021), a QTL for petal length was proposed and validated on chromosome 5.

Association with fragrance highlighted a single QTL located on chromosome 2 (94.55 cM). This finding partially parallels the study of Schulz et al. (2021), who also detected a major QTL on the terminal part of chromosome 2, in addition to putative minor QTLs on almost all chromosomes (except chromosome 6). Besides, Roccia et al. (2019) detected a QTL related to one of the scent components, geranyl acetate, on the same chromosome. For antioxidant capacity, we detected four markers with signals above the threshold. All four markers are located on chromosome 3 (from 7.72 to 8.43 cM). As these markers are located in a narrow chromosome region we assume that all four markers indicate the same QTL.

For the trait yield a QTL was detected on chromosome 7. Previously, chromosome 7 was highlighted as affecting the inheritance of vigor and biomass-related traits: stem width, leaf dry weight and chlorophyll content (Bourke et al., 2018).

For citric acid content we detected two significant SNPs on chromosome 2, separated by 4 cM. As these two markers are located in a narrow chromosome region, we assume that both markers indicate the same QTL.

A failure to detect QTLs for taste-related traits might indicate that a scale for estimation of taste should be based on a better reference systems and tasting panels should be organized in a better and more reproducible way. For other traits, a comparison of the results of this study with those of previous studies indicated that some QTLs had already been identified in other panels on the same chromosomes and regions as well, while some QTLs were identified in other positions. Differences in QTLs and QTL positions between our study and previous studies may be partly due to differences in the plant material. In our study the focus was on commercial rose cultivars, which means that some QTLs originating from more diverse germplasm could have been missed.

CONCLUSIONS

The results of this study indicate that rose petals are a nutritionally valuable novel food source. Rose petals provide more antioxidant capacity than most vegetables traditionally used in salads, while ascorbic acid content supports the use of rose petals as an alternative vitamin C source. Taste results indicate that flowers of many cultivars are not very tasteful for fresh consumption. Therefore, the ones that do have a pleasant taste, are very exclusive.

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