Rosa davurica Pall., a useful *Rosa* species for functional rose hip production with high content of antioxidants and multiple antioxidant activities in hydrophilic extract

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Abstract

Fruits of the genus *Rosa* plants are called rose hips. The common hips of *R. canina* are well known as a rich source of antioxidants like ascorbic acid and polyphenols. To investigate availability in the hips in *Rosa* spp., wild *Rosa* hips originating from East Asia, i.e. *R. acicularis*, *R. davurica*, *R. multiflora* and *R. rugosa* were evaluated in terms of the content of antioxidants and antioxidant ability in the hydrophilic extracts. The hips from *R. glauca* originating from south Europe and its interspecific hybrids ('Kitaayaka' and 'Consared'), and purchased *R. canina* hips were also examined. In addition to the colorimetric detections of DPPH and ORAC, ESR–ST methods were employed for evaluating antioxidant ability, which can determine scavenging activities against naturally–occurring ROS i.e. superoxide anion radical ($^{\bullet}O_2^-$), hydroxyl radical ($HO^{\bullet}$), alkoxyl radical ($RO^{\bullet}$) and singlet oxygen ($^1O_2$), individually. The hips of *R. davurica* and 'Consared' showed quite high values in both the total content of ASA plus DHA (40.8–103.1 g/kg DW) and total polyphenols (119.2–161.5 g quercetin eq./kg DW) regardless of the years collected. They also had high antioxidant activities against each radical compared to other rose hips, and thus their antioxidant ability seems multiple. Both ASA and polyphenols could scavenge radicals of $ROO^{\bullet}$ and $^1O_2$, since significant correlations ($P < 0.05$) were confirmed. However, polyphenols might have greater contribution to the antioxidant activities, because the correlation coefficients were higher in total polyphenols than ASA. *R. davurica* can be one of the useful genetic resources for breeding cultivars which will bear antioxidant–rich rose hips, since 'Consared' is a progeny of *R. davurica* × *glauca*.

Keywords

Ascorbic acid; ESR–ST; polyphenol; radical scavenging activity; ROS; *Rosa* spp.

45 Abbreviations: DPPH, 2,2-diphenyl-1-picrylhydrazyl; ORAC, oxygen radical
46 absorbance capacity; ESR–ST, electron spin resonance–spin trapping; ROS, reactive
47 oxygen species; ASA, L–ascorbic acid; DHA, dehydroascorbic acid.
48

1. Introduction

Plants of the genus *Rosa*, including more than 100 species widely dispersed from the northern hemisphere, have fruits, namely rose hips, especially of dog rose (*Rosa canina*), that are sometimes used for making into jam, syrup and tea. It is also well known that rose hips are rich sources of antioxidants like ASA, polyphenols and carotenoids (Cunja et al., 2016; He et al., 2016; Tabaszewska and Najgebauer-Lejko, 2020). Perspective on utilizing rose hips for making functional foods has been discussed (Fan et al., 2014). Nagatomo et al. (2015) demonstrated that the fruit extract of *Rosa canina* could inhibit obesity of rats when mixed with feeds, and therefore functional roles of rose hip phytochemicals in diet attracted the attention of researchers. In Hokkaido Japan, there are some wild *Rosa* spp. i.e. *R. rugosa*, the official flower designated by the prefecture in 1978, *R. acicularis*, *R. davurica* and *R. multiflora*, however these fruits have not been widely utilized for foodstuffs. In this study, the native *Rosa* spp. were evaluated in terms of the content of antioxidants and antioxidant activities in rose hips to clarify their availability for fruit production and/or genetic resources on breeding cultivars which can bear antioxidant–rich rose hips.

On the evaluation of antioxidant activities different kinds of methods have been utilized with their own principle (Shahidi and Ambigaipalan, 2015), since there were many ROS like superoxide anion radical ($^{\bullet}O_2^-$), hydroxyl radical ($HO^{\bullet}$), alkoxyl radical ($RO^{\bullet}$), peroxyl radical ($ROO^{\bullet}$), non–radical hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$) related to the oxidation of biomolecules in living organisms, and a kind of antioxidant phytochemical can only scavenge specific types of ROS and is not universal against all ROS. Thus, it is very difficult to evaluate antioxidant activity of a foodstuff using a single index. ESR–ST method has a great advantage in that antioxidant activity against a specific ROS can be detected by the method when being combined with an appropriate spin trapping reagent. Tumbas et al. (2012) tried first to determine the

antioxidant activities against $^{\bullet}O_2^-$ and $HO^{\bullet}$ in *Rosa canina* hips using ESR-ST with an ordinary spin trapping reagent, namely 3,4-dihydro-2,2-dimethyl-2H-pyrrole 1-oxide (DMPO). By using a novel and powerful spin trapping reagent of 2– (5,5–dimethyl–2–oxo–2λ5–[1,3,2] dioxaphosphinan–2–yl) –2–methyl–3,4–dihydro–2*H*–pyrrole 1–oxide (CYPMPO) (Kamibayashi et al., 2006), the procedures of ESR–ST have been established specifically for $^{\bullet}O_2^-$ (Prolla and Mehlhorn, 1990), $HO^{\bullet}$ (Kameya and Ukai, 2012) and $RO^{\bullet}$ (Ukai et al., 2009). A similar protocol has also been established for $^1O_2$ (Jung and Min, 2009) without CYPMPO and/or DMPO. So, we employed the above ESR–ST procedures to clarify antioxidant activity of the wild rose hips against individual ROS, respectively. We also employed DPPH and ORAC methods that were used commonly for evaluation of antioxidant activity, to compare the values with those obtained by ESR–ST methods, and examine correlation of each antioxidant activity with the content of antioxidants.

2. Materials and Methods

2.1. Reagents

For the analyses of ASA, DHA and polyphenols: 2,4–dinitrophenylhydrazine (DNP) was purchased from Kishida Chemical (Osaka, Japan), metaphosphoric acid, thiourea, sulfuric acid and sodium carbonate from Fujifilm Wako Pure Chemical (Osaka, Japan), 2,6–dichloroindophenol (DCIP) from Merck (Darmstadt, Germany), 'Folin–Denis' reagent from Sigma–Aldrich Chemical (St. Louis, MO, USA), and quercetin from Kanto Chemical (Tokyo, Japan). For the antioxidant activity analyses: phosphate buffer (pH 7.4), morpholinoethanesulfonic acid (MES), 2,2'–azobis (2–amidinopropane) dihydrochloride (AAPH), ethylenediaminetetraacetic acid (EDTA)–2Na, H_2O_2, pterin, N,N,N',N'-tetramethyl-1,4-benzenediamine (TMPD), diethylenetriaminepentaacetic acid (DTPA), glycine and riboflavin from Fujifilm Wako Pure Chemical (Osaka, Japan),

2,2–diphenyl–1–picrylhydrazyl (DPPH) and fluorescein sodium from Sigma–Aldrich Chemical (St. Louis, MO, USA), 2– (5,5–dimethyl–2–oxo–2λ5–[1,3,2] dioxaphosphinan–2–yl) –2–methyl–3,4–dihydro–2*H*–pyrrole 1–oxide (CYPMPO) from Radical Research (Hino, Japan). For the standards: 6–hydroxy–2,5,7,8–tetramethylchroman–2–carboxylic acid (Trolox) was purchased from Sigma–Aldrich Chemical (St. Louis, MO, USA), and ASA, *α*–lipoic acid and glutathione (GSH) from Fujifilm Wako Pure Chemical (Osaka, Japan). Methanol and ethanol were all HPLC grade.

2.2. Plant material

Matured fruits (rose hips) were collected from 18–year–old shrubs of five wild *Rosa* species originating from East Asia, i.e. *R. acicularis* Lindl. (1.06 g fresh weight (FW)/fruit, 63.9 % in water content), *R. davurica* Pall. (0.83 g FW/fruit, 71.4% in water content), *R. multiflora* Thunb. (0.20 g FW/fruit, 65.5% in water content), *R. rugosa* Thunb. (2.11 g FW/fruit, 76.0% in water content), and *R. rugosa* Thunb. f. *plena* Byhouwer (3.26 g FW/fruit, 69.4% in water content), grown in the experimental farm, composed of brown lowland soil without fertilization, in Mikasa of the Forestry Research Institute of Hokkaido on October 2, 2013. Twenty–forty fruits were collected from 4 different shrubs (5–10 fruits/shrub depending on the fruit size in each species). To compare the component of antioxidants and antioxidant activities in rose hips of East Asian *Rosa* spp. to those of European, fruits of *R. glauca* Pourret (0.99 g FW/fruit, 63.7% in water content) originating from south Europe (as control), and interspecific hybrid cultivars of ‘Kitaayaka’ (*R. glauca* × *rugosa*) (1.25 g FW/fruit, 68.3% in water content) and ‘Consared’ (*R. davurica* × *glauca*) (0.76 g FW/fruit, 66.1% in water content) bred both for floriculture by the Forestry Research Institute of Hokkaido were also collected from 18–year–old shrubs grown in the same experimental farm at the

same time with the same manner. Fruits were collected again from the above–mentioned shrubs on September 28, 2017 for clarifying yearly variations. Seasonal changes in air temperature in 2017 were similar to those in 2013, however the amounts of water precipitation from January through March and from June through July in 2017 were less and more than those in 2013, respectively. Dried fruits of *R. canina* L., namely common rose hip in a narrow sense, grown commercially in Republic of Chile were purchased from a Japanese importer as a reference plant material. All above genus *Rosa* plants (Suppl. 1) are categorized into subgenus *Rosa*. In more detail, *R. canina* and *R. glauca* are classified into section *Caninae*, *R. acicularis*, *R. rugosa* and *R. davurica* are classified into section *Rosae*, and *R. multiflora* is classified into section *Synstyllae* in the 10 different sections of subgenus *Rosa* (Nomura, 2010; Smulders et al, 2011). Distribution on some of the species was described in the literature (Smulders et al, 2011). After being harvested, raw fruits excluding seeds were quickly frozen in liquid nitrogen, lyophilized, ground into powder and then stored at –30˚C for subsequent analyses.

2.3. Quantification of antioxidants

2.3.1. Ascorbic acid

Determination of ASA and DHA followed the DNP method established by Roe *et al.* (1948). For quantifying total concentration of ASA plus DHA, triplicates of 5 mg of the lyophilized sample were extracted with 1 mL of 5% metaphosphoric acid in a 1.5 mL taper plastic tube with lid by shaking for 3 h using a laboratory shaker. After centrifugation at 12,000*g* for 10 min, the supernatant was collected. A 20 μL solution of 0.03% DCIP, a 40 μL solution of 5% metaphosphoric acid supplemented with 2% thiourea, and a 40 μL solution of 2% DNP were added to the 40 μL of extracts or ASA standards in order into a 96–well plate (P96F03N; As One, Osaka, Japan), and mixed.

After incubation at 37˚C for 3 h, a 100 μL solution of 85%(v/v) sulfuric acid was added to each well, mixed, cooled by placing the microplate on crushed ice for 30 min, and the absorbance was read at 520 nm using a microplate reader (Powerscan HT; DS Pharma Biomedicals, Osaka, Japan). When quantifying DHA concentration in an extract excluding ASA, triplicates of 5 mg of the lyophilized sample were extracted with 1 mL of 5% metaphosphoric acid supplemented with 2% thiourea in a 1.5 mL taper plastic tube. A 20 μL solution of 5% metaphosphoric acid was added to the extracts instead of 0.03% DCIP. The concentration of ASA in an extract was calculated by subtracting the DHA concentration from the total concentration of ASA plus DHA in the same extract. Standard curve was calculated from the values (n = 3) on 5 graded concentrations.

2.3.2. Total polyphenols

Total polyphenols were determined according to the Folin–Denis colorimetric method (Folin and Denis, 1915). Triplicates of 5 mg of the lyophilized sample were extracted with the 1 mL of 80%(v/v) methanol in a 1.5 mL taper plastic tube with lid by shaking for 3 h using a laboratory shaker. After centrifugation at 12,000*g* for 10 min, the supernatant was collected. The 75 μL solution of 50% Folin–Denis' reagent and 75 μL of a 5% sodium carbonate solution were added to the 150 μL of extracts or quercetin standards in order into a 96–well plate (As One), mixed, left to stand on the bench for 60 min, and the absorbance was read at 700 nm using the microplate reader. Total polyphenols were estimated as the μmol quercetin equivalent of a sample using the standard curve of quercetin which was calculated from the values (n = 3) on 5 graded concentrations.

2.4. Antioxidant activity for ROS

2.4.1. DPPH radical scavenging activity

Analysis using artificial DPPH radical (DPPH•) was carried out as described previously (Sharma and Bhat, 2009). Triplicates of 5 mg of the lyophilized sample were extracted with the 1 mL of 80%(v/v) ethanol in the same manner for total polyphenols. One hundred and fifty μL solution of DPPH (400 μM in ethanol): MES buffer (pH 6.0, 200 mM): 20%(v/v) ethanol = 1:1:1 (v/v/v) were added to the 50 μL of the extracts or the standards into a 96–well plate (As One). The mixture was left to stand at room temperature for 20 min, then the absorbance was read at 520 nm in the microplate reader. DPPH• scavenging activity was estimated as the μmol Trolox equivalent (TE) of a sample using the standard curve of Trolox which was calculated from the values (n = 3) on 5 graded concentrations.

2.4.2. ORAC method

Analysis was carried out as described by Watanabe et al. (2012). Triplicates of 5 mg of the lyophilized sample were extracted with the 1 mL solution of methanol:distilled water:acetic acid, 90:9.5:0.5 v/v/v (MWA) in the same manner for total polyphenols. ORAC method can determine antioxidant activity for naturally–occurring peroxyl radical (ROO•). A 115 μL solution of fluorescein (110.7 nM) and a 50 μL solution of AAPH (31.7 mM) were added to the 35 μL of extracts, Trolox standards or a blank into a 96–well plate (Falcon® 353072; Corning, Glendale, AZ, USA). After covering the plate with a film (NJ–500; Takara Bio, Otsu, Japan), the fluorescence intensity (excitation at 485 nm, emission at 530 nm) was monitored at 37 ˚C every two min for a total of 90 min using the microplate reader. The net area under the curve (AUC) was calculated by subtracting the AUC for the blank from the reagents or standards. The ORAC value was estimated as the μmol TE of a sample using the standard curve of Trolox which was calculated from the values (n = 3) on 5 graded concentrations.

205 *2.4.3. ESR–spin trapping method*

206 Triplicates of 5 mg of the lyophilized sample were extracted with the 1 mL MWA
207 solution in the same manner for total polyphenols.

208 Superoxide anion radical ($^{\bullet}O_2^-$)–scavenging assay was carried out as described by
209 Prolla and Mehlhorn (1990): 50 μL aliquots of the extracts, the standards or a blank
210 were added to 20 μL of 200 μM riboflavin (precursor/sensitizer reagent), 100 μL of 10
211 mM CYPMPO, 20 μL of 10 mM EDTA, 20 μL of 0.1 mM glycine and 50 μL of 100
212 mM phosphate buffer (pH 7.4) into an ESR disposable flat cell with a plastic syringe
213 (RDC-60-S, Flashpoint Co., Ltd., Ome, Japan).

214 Hydroxyl radical (HO$^{\bullet}$)–scavenging assay was carried out as described by Kameya
215 and Ukai (2012) and Kameya et al. (2014): 50 μL aliquots of the extracts, the standards
216 or a blank were added to 50 μL of 1%(v/v) H_2O_2 (precursor/sensitizer reagent), 20 μL of
217 10 mM CYPMPO, 30 μL of 10 mM DTPA and 50 μL of 100 mM phosphate buffer (pH
218 7.4) into an ESR flat cell.

219 Alkoxyl radical (RO$^{\bullet}$)–scavenging assay was carried out as described by Ukai et al.
220 (2009): 50 μL aliquots of the extracts, the standards or a blank were added to 50 μL of 4
221 mM AAPH (precursor/sensitizer reagent), 20 μL of 10 mM CYPMPO and 80 μL of 100
222 mM phosphate buffer (pH 7.4) into an ESR flat cell.

223 Singlet oxygen ($^1O_2$)–scavenging assay was carried out as described by Jung and
224 Min (2009): 40 μL aliquots of the extracts, the standards or a blank were added to 50 μL
225 of 0.6 mM pterin (precursor/sensitizer reagent), 50 μL of 100 mM TMPD, 20 μL of 15
226 mM DTPA and 40 μL of 100 mM phosphate buffer (pH 7.4) into an ESR flat cell.

227 In these cases, the *α*–lipoic acid, ASA, glutathione (GSH) and GSH were used as the
228 standard scavengers for $^{\bullet}O_2^-$, HO$^{\bullet}$, RO$^{\bullet}$ and $^1O_2$, respectively. The reason why the same
229 standard reagent was not used is that the solubility of a reagent in each ESR–ST system
230 was quite different. The ESR flat cell was set in an ESR cavity, and was then irradiated

for 5 sec (20 sec in case of $^{\bullet}O_2^-$) with ultraviolet rays for producing radicals. At this time, the ESR spectrum was immediately measured using an X–band ESR spectrometer (JES–RE1X, JEOL, Tokyo, Japan) with a 100 kHz field modulation. The spectrometer conditions were as follows: resonance field, 3521 G; field modulation width, 1.0 G; microwave power, 6 mW; light source, 200 W medium pressure mercury/xenon arc lamp (LC–8, Hamamatsu Photonics K.K., Hamamatsu, Japan); UV irradiation intensity for photolysis, 2.78 mW/cm^2 (LC–8, Hamamatsu Photonics K.K., Hamamatsu, Japan) measured by a UV intensity meter (Cole–Parmer International, IL, USA); the band–pass filter, G–533 (HOYA, Tokyo, Japan). The analysis of adducted signal was carried out as described by Kameya et al. (2014). The scavenging activities were estimated as the mmol standard equivalent of a sample using the standard curve which was calculated from the values (n = 3) on 5 graded concentrations.

2.5. Statistical analyses of data

Two wells of a microplate were used for each determination of a sample extract or a standard solution and the average value was used for subsequent calculation. Results were represented as average ± SE (n = 3). Data were analyzed statistically using analysis of variance (ANOVA) followed by Tukey's multiple range test. Yearly variations were analyzed using Student's t–test. Correlations between content of antioxidants and each antioxidant activity were examined.

3. Results

3.1. Content of antioxidants

The content of ASA, DHA and polyphenols in rose hips collected in 2013 and 2017 were shown in Table 1. Total content of ASA (reduced form) plus DHA (oxidized form) was statistically ($P < 0.05$) higher in rose hips of *R. davurica* and 'Consared' in 2013,

and of ‘Consared’ in 2017, whereas lower in rose hips of *R. multiflora* and *R. rugosa* f. *plena* in 2013, of *R. multiflora* in 2017, and of *R. canina* (reference) than those of *R. glauca* (control). In these cases, ASA occupied a higher percentage of total than DHA. Yearly variations were statistically significant ($P < 0.05$) in rose hips of *R. acicularis*, *R. davurica*, *R. rugosa* and the control.

Total content of polyphenols was statistically ($P < 0.05$) higher in rose hips of *R. davurica* and ‘Consared’ in 2013, and of *R. davurica*, *R. multiflora* and ‘Consared’ in 2017, whereas lower in rose hips of *R. multiflora* and *R. rugosa* f. *plena* in 2013, of *R. acicularis* and ‘Kitaayaka’ in 2017, and of *R. canina* (reference) than those of the control. No significant yearly variation was confirmed by ANOVA in total content of polyphenols in the hips examined.

3.2. Antioxidant activities

Antioxidant activities in rose hips evaluated using colorimetric methods i.e. DPPH and ORAC, and ESR-ST methods are shown in Table 2. On the ESR–ST detections, independent and characteristic ESR adduct signals could be obtained against individual ROS examined (Fig. 1). The signals were gained first from the center of the chart and spread to both right and left sides almost symmetrically. So, we used the distance of the first two peaks (top and bottom) labelled with an asterisk in the figure as a measure for calculation. Antioxidant activities of the sample extracts were calculated using each standard curve.

The DPPH$^{\bullet}$ scavenging activity was statistically ($P < 0.05$) higher in hips of *R. davurica* and ‘Consared’ in 2013, and of *R. davurica*, *R. multiflora* and ‘Consared’ in 2017, whereas lower in hips of *R. acicularis* and *R. rugosa* in 2013, and of *R. canina* (reference) than those of the control. No significant yearly variation was confirmed by ANOVA in the DPPH$^{\bullet}$ scavenging activity of the hips examined. The ROO$^{\bullet}$ scavenging

283 activity (ORAC value) was statistically ($P < 0.05$) higher in hips of *R. davurica* and
284 'Consared' in 2013 and 2017, whereas lower in other hips than those of the control.
285 Yearly variations were statistically ($P < 0.05$) in *Rosa* spp. grown in the farm
286 other than 'Kitaayaka'.
287 In the cases of ESR-ST detections, yearly variations could not be determined, since
288 some of the samples collected in 2017 were exhausted. The $^{\bullet}O_2^-$ scavenging activity
289 was statistically ($P < 0.05$) higher in hips of 'Kitaayaka' and 'Consared' than that of the
290 control, but no significant difference could be confirmed between the other species and
291 the control. The $HO^{\bullet}$ and $RO^{\bullet}$ scavenging activities were statistically ($P < 0.05$) lower in
292 hips of *R. rugosa* and *R. canina* than that of the control, but no significant difference
293 could be confirmed between the other species and the control. The $^1O_2$ scavenging
294 activities in hips of *R. acicularis*, *R. multiflora*, *R. rugosa* f. *plena*, 'Kitaayaka' and *R.*
295 *canina* were statistically ($P < 0.05$) lower than that of the control, but no significant
296 difference could be confirmed between the other species and the control.
297
298 *3.3. Correlation between content of antioxidants and antioxidant activity*
299 Correlations between ASA content and individual antioxidant activity are shown in
300 Fig. 2. In these cases, DHA content is excluded since DHA has no antioxidant effect,
301 and furthermore the glutathione–ascorbic acid cycle will not be available *in vitro*.
302 Correlation coefficient ($r = 0.533–0.746$) was statistically significant ($P < 0.05$)
303 between ASA content and antioxidant activity against $DPPH^{\bullet}$, $ROO^{\bullet}$, $HO^{\bullet}$ and $^1O_2$.
304 Correlations between total content of polyphenols and individual antioxidant activity
305 are shown in Fig. 3. Correlation coefficient ($r = 0.835–0.932$) was statistically
306 significant ($P < 0.01$) between total polyphenols and antioxidant activity against $DPPH^{\bullet}$,
307 $ROO^{\bullet}$ and $^1O_2$.
308

4. Discussion

On the content of antioxidants in rose hips, Cunja et al. (2016) reported that the total content of ascorbic acid in *R. canina* hips (common hips belonging to section *Caninae*) was 18.4 g/kg DW, and the range was from 2.4 to 47.1 g/kg DW in rose hips from selected species/cultivars. Roman et al. (2013) demonstrated that average amounts of vitamin C in natural *R. canina* hips collected in Transylvania ranged from 1.1 to 3.6 g/kg frozen pulp. Ercişli (2007) pointed out that vitamin C level was estimated to be 3–40 g/kg DW depending upon species, genotype, and environmental factors. Total content of ASA plus DHA (3.7 g/kg DW) of our purchased *R. canina* hips was at similar or lower level in comparison to the above values, which may be due to destruction of ASA and DHA during the drying process and/or storage period (Tabaszewska and Najgebauer-Lejko, 2020). Total values of ASA plus DHA (47.3 g/kg DW in 2013, and 32.2 g/kg DW in 2017) in *R. glauca* hips (control), which was also classified into section *Caninae*, were at high level in the above range of vitamin C. Among East Asian *Rosa* hips, total values of ASA plus DHA of the species classified into section *Rosae* (*R. acicularis*, *R. davurica*, *R. rugosa*, and *R. rugosa* f. *plena*) ranged from 31.8 to 103.1 g/kg DW, which could be converted into 9.7–29.5 g/kg FW by calculation depending on the water content of each sample. Ercişli and Eşitken (2004) showed that the fruits of twelve promising rose hip genotypes selected from 10,000 seedling shrubs of *R. dumalis, R. canina, R. pulverulanta* and *R. montana* collected in the Erzurum province of Turkey contained 10.74–25.57 g ascorbic acid/kg FW. The range of ASA plus DHA content in our native rose hips categorized into section *Rosae* is very close to this range. Furthermore, the values are greater than that (4.1–4.4 g/kg FW) of sea buckthorn fruits (Gutzeit et al., 2008) and that (2.2 g/kg FW) of guava fruits (Standard Tables of Food Composition in Japan, 2015), and correspond to that (17.0 g/kg FW) of acerola fruits (Standard Tables of Food Composition in Japan, 2015).

335 Thus, the content of ASA plus DHA in the hips of section *Rosae* is said to be quite high.
336 By contrast, *R. multiflora* hips classified into section *Synstyllae* may be poor in the
337 content of ASA plus DHA. Among the all samples examined, since the content of ASA
338 plus DHA in the hips of *R. davurica* in 2013, and of 'Consared' in 2013 and 2017 were
339 statistically ($P < 0.05$) higher than that of the control, and greater than the standard
340 values in vitamin C of the fruits which are well known to have rich content of vitamin
341 C, they could be useful as foodstaffs containing vitamin C at quite high level. Yearly
342 variations confirmed in rose hips of *R. acicularis*, *R. davurica*, *R. rugosa* and *R. glauca*
343 might be due to degree of maturity of the fruits (Uggla et al., 2005). From another point
344 of view a climate condition such as water precipitation during growth of the hips might
345 have relation with the content of ASA plus DHA. Furthermore, since shrub age interacts
346 with ecological conditions, the observed differences might be due to such interaction.
347 In case of total polyphenols, Cunja et al. (2016) reported that *R. canina* hips had 5.6
348 g/kg DW and the range of the total phenols was 3.0–44.7 g/kg DW in rose hips from
349 selected species/cultivars. The value (33.2 g quercetin eq./kg DW) of our purchased *R.*
350 *canina* hips was in the same ballpark. The values of total polyphenols in *R. glauca* hips
351 (control) were higher than that of *R. canina* hips mentioned by Cunja et al. (2016), even
352 if both species were classified into section *Caninae*. Among the all samples examined,
353 the content of total polyphenols in *R. davurica* and 'Consared' ranged from 119.2 to
354 161.5 g quercetin eq./kg DW in both years, which were statistically ($P < 0.05$) higher
355 than those of the control and might be greater than that (37.6–78.5 g/kg DW) of black
356 chokeberry (*Aronia melanocarpa*) fruits which are known as a polyphenol–rich small
357 fruits (Kulling and Rawel, 2008).
358 Antioxidant activities of fruits has been evaluated using many methods, but it is
359 difficult to investigate the accuracy of the procedure used in an experiment and compare
360 the results with those obtained by other researchers, because experimental conditions

and/or samples used for the determination are sometimes not the same as those written in literatures. However, since the ORAC values of our matured black chokeberry fruits collected in the experimental farm were 847.1 mmol TE/kg DW (170.1 mmol TE/kg FW) in 2018 and 960.9 mmol TE/kg DW (188.9 mmol TE/kg FW) in 2019, which was very close to the values (158.2–160.2 mmol TE/kg FW) in the review on *Aronia* (Kulling and Rawel, 2008), our experiments on the ORAC determination seemed to be performed precisely. From this point of view, the ORAC values (2487.4–3933.3 mmol TE/kg DW) of *R. davurica* and 'Consared' are said to be quite high and they have outstandingly strong scavenging ability against $ROO^{\bullet}$. Similarly, they also showed high scavenging activities against $DPPH^{\bullet}$ regardless of the collection year and against naturally–occurring ROS ($^{\bullet}O_2^-$, $HO^{\bullet}$ and $^1O_2$).

On the roles of ASA and polyphenols related to the antioxidant activities in rose hips, both could scavenge radicals of $ROO^{\bullet}$ and $^1O_2$, since significant ($P < 0.01$, 0.05) correlations were confirmed (Figs. 2 and 3). However, polyphenols might have greater contribution to these antioxidant activities, because the correlation coefficients were higher in total polyphenols than ASA. This was also pointed out in the reports on utilization of fruit pulps of citrus (Ramful et al., 2011) and peach (Liu et al., 2015). However, it might be possible that the difference in the activities between ASA and polyphenols was caused by difference in extraction solution (5% metaphosphoric acid for ASA, 80% methanol for total polyphenols, 80% ethanol for DPPH, and MWA for naturally–occurring ROS). To confirm the effects of extraction solution, ASA was extracted from the lyophilized powder of *R. davurica* and 'Consared' hips by 80% ethanol or MWA first, evaporated and re-extracted by 5% metaphosphoric acid. Then ASA content was compared with that extracted from the same material by 5% metaphosphoric acid directly. As a result, the recovery of ASA content was 88-93% (Suppl. 2). Thus, it seems probable that the effect of extraction solution might be small.

On the other hand, ASA and polyphenols might have no scavenging ability against $^{\bullet}O_2^-$ and RO$^{\bullet}$. The significant correlation ($P < 0.05$) between the ASA content and the HO$^{\bullet}$ scavenging activity seems reasonable, since ASA has been employed as a standard antioxidant reagent in the HO$^{\bullet}$ scavenging assay using ESR–ST (Kameya and Ukai, 2012). The antioxidant roles of polyphenols in hips of each *Rosa* species should further be investigated since they are composed of various phytochemicals like anthocyanin, flavonol, ellagic acid, catechin, etc., and component of polyphenols might be different.

To compare the results between plant materials, we represented all values related to the content of antioxidants and antioxidant activities as a percent of the maximum value of each evaluation system and plotted them on a radar chart (Fig. 4). As a result, it was clearly demonstrated that rose hips of *R. davurica* and 'Consared' had high values in all parameters related to the antioxidant role, and thus their antioxidant ability may be multiple against different kinds of ROS. Rose hips with high antioxidant ability will be useful for making antimicrobial food additives (Yi et al, 2007). The *R. davurica* hips have been used as a traditional Chinese medicine (Kuang et al., 1989), which may be due to their strong and multiple antioxidant activities. Furthermore, the fact that 'Consared' had been bred from *R. davurica* by crossing with *R. glauca* indicates that *R. davurica* would be one of the useful genetic resources, as a mother plant, for breeding cultivars which can bear antioxidant–rich rose hips.

Acknowledgments

We thank Prof. Dr. Jun Watanabe (Obihiro University of Agriculture and Veterinary Medicine) for technical assistance on the ORAC determination.

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Legends of Figures

Fig. 1. Examples of individual adduct signal in ESR–ST analyses against radicals of $^{\bullet}O_2^-$ (a), $HO^{\bullet}$ (b), $RO^{\bullet}$ (c) and $^1O_2$ (d). Asterisk shows peaks which were used to evaluate the magnitude of wave, as distance between the peaks (top and bottom).

Fig. 2. Correlation between the content of ASA, shown in Table 1, and the antioxidant activities shown in Table 2. $^{**}P < 0.01$; $^{*}P < 0.05$ (Pearson's correlation coefficient test, $n = 16$ for $DPPH^{\bullet}$ and $ROO^{\bullet}$ including data in both 2013 and 2017, $n = 9$ for $^{\bullet}O_2^-$, $HO^{\bullet}$, $RO^{\bullet}$ and $^1O_2$ in 2013 only).

Fig. 3. Correlation between the content of total polyphenols, shown in Table 1, and antioxidant activities shown in Table 2. The plot of *R. canina* is hidden behind the plot of 'Kitaayaka' in the panel of $^1O_2$. $^{**}P < 0.01$ (Pearson's correlation coefficient test, $n = 16$ for $DPPH^{\bullet}$ and $ROO^{\bullet}$ including data in both 2013 and 2017, $n = 9$ for $^{\bullet}O_2^-$, $HO^{\bullet}$, $RO^{\bullet}$ and $^1O_2$ in 2013 only).

Fig. 4. Radar charts representing species/cultivar differences in multiple characters related to the antioxidant ability of fruits in the genus *Rosa*. The values show percentages of the maximum value in each character.

Highlights

Content of ascorbic acid and polyphenols was compared in nine *Rosa* species/cultivars.

Antioxidant activities against naturally-occurring ROS were determined using ESR–ST.

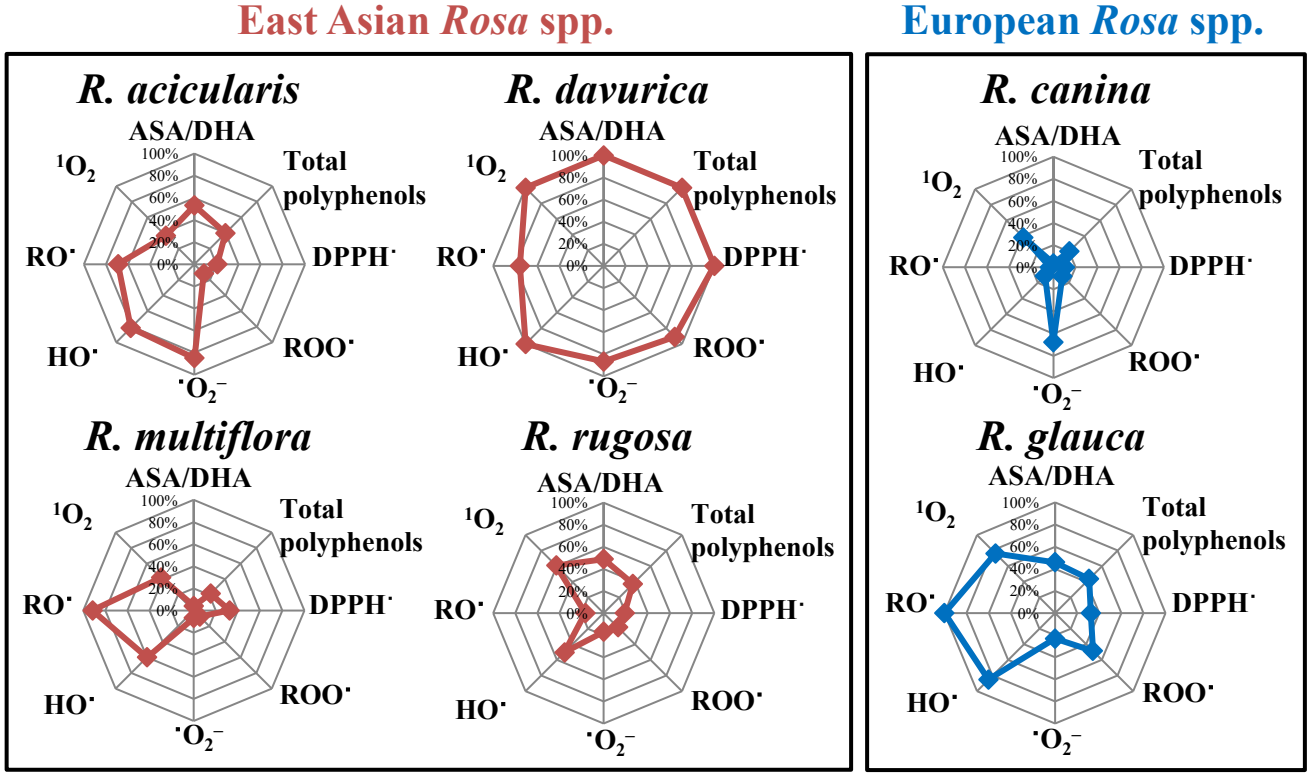
Hips from *R. davurica* had quite high content of antioxidants and antioxidant activities.

Hips from ‘Consared’, a progeny of *R. davurica*, also had high antioxidant abilities.

Content of ascorbic acid and polyphenols correlated with the activities scavenging $ROO^{\cdot}$ and $^{1}O_{2}$.

Rosa davurica Pall., a useful _Rosa_ species for functional rose hip production with high content of antioxidants and multiple antioxidant activities in hydrophilic extract

Arisa Osada, Kentaro Horikawa, Youichi Wakita, Hideo Nakamura, Mitsuko Ukai, Hanako Shimura, Yutaka Jitsuyama, Takashi Suzuki*



Species differences in antioxidant abilities of rose hips

Table 1. Species differences in the content of ascorbic acid (ASA), dehydroascorbic acid (DHA), and polyphenols in rose hips collected in 2013 and 2017.

Spieces/cultivar	Content of ASA and DHA (g/kg DW)						Total polyphenols (g quercetin eq./kg DW)	
Year:	2013			2017			2013	2017
	ASA	DHA	Total	ASA	DHA	Total		
East Asian								
R. acicularis	44.1 ± 1.0 b	10.6 ± 0.2 de	54.8 ± 1.2 bc	36.5 ± 4.0 ab	2.4 ± 0.5 **	38.8 ± 3.7 b *	64.2 ± 0.3 c	35.0 ± 1.9 f
R. davurica	84.6 ± 2.0 a	18.5 ± 0.7 a	103.1 ± 2.0 a	36.9 ± 3.1 ab **	3.9 ± 1.8 **	40.8 ± 1.9 b **	161.5 ± 4.2 a	139.2 ± 1.8 a
R. multiflora	0.6 ± 0.4 e	3.6 ± 0.3 g	4.2 ± 0.6 e	3.2 ± 1.3 c	3.5 ± 1.6	6.8 ± 0.8 c	34.8 ± 1.5 d	100.2 ± 2.6 c
R. rugosa	41.2 ± 1.6 bc	9.3 ± 0.4 ef	50.4 ± 1.3 bc	36.6 ± 3.4 ab	5.6 ± 1.9 *	42.2 ± 1.6 b *	60.3 ± 1.2 c	80.3 ± 2.6 d
R. rugosa f. *plena*	24.0 ± 0.6 d	7.8 ± 0.1 f	31.8 ± 0.7 d	– [z]	–	–	41.7 ± 0.8 d	–
European								
R. glauca (Cont.)	33.7 ± 1.5 c	13.7 ± 0.2 bc	47.3 ± 1.3 c	28.5 ± 5.9 b	3.8 ± 1.6 **	32.2 ± 4.4 b *	70.4 ± 1.0 c	79.3 ± 1.4 d
Hybrid								
'Kitaayaka'	39.8 ± 1.4 bc	14.6 ± 0.2 b	54.4 ± 1.4 bc	39.3 ± 4.7 ab	3.4 ± 1.1 **	42.8 ± 4.2 b	64.0 ± 2.0 c	49.4 ± 2.3 e
'Consared'	46.4 ± 4.3 b	12.4 ± 0.9 cd	58.8 ± 5.1 b	54.2 ± 5.2 a	4.3 ± 2.1 *	58.5 ± 3.1 a	138.9 ± 4.2 b	119.2 ± 2.8 b
R. canina [y]	1.2 ± 0.2 e	2.4 ± 0.1 g	3.7 ± 0.1 e	1.2 ± 0.2 c	2.4 ± 0.1	3.7 ± 0.1 c	33.2 ± 0.8 d	33.2 ± 0.8 f

ANOVA	ASA	DHA	Total	Total polyphenols
Spieces/cultivar (S)	**	**	**	**
Year (Y)	**	**	**	ns
S × Y	**	**	**	**

Data represent average ± SE of three independent experiments.
[z] No material of *R. rugosa* f. *plena* was available in 2017 and the data in 2013 were excluded from ANOVA.
[y] Rose hips of *R. canina* were purchased via importer as a reference and the data were excluded from ANOVA.
Different alphabets indicate significant differences between materials in the same year ($P < 0.05$, Tukey's multiple range test). Where no alphabet is labelled, differences are not significant at 5% level (Tukey's test).
$^{**}P < 0.01$; $^{*}P < 0.05$ (Student's t–test) vs 2013 in the same species/cultivar.
In the results of ANOVA: **, $P < 0.01$; ns, not significant at 5% level.

Table 2. Species differences in the radical scavenging activities against DPPH•, ROO•, $^{\bullet}O_2^-$, HO•, RO• and $^1O_2$ in rose hips collected in 2013 and 2017.

Spieces/cultivar	Radical scavenging activities							
	DPPH• (mmol TE/kg DW)		ROO• (mmol TE/kg DW)		$^{\bullet}O_2^-$ (mol α–lipoic acid eq./kg DW)	HO• (mol ASA eq./kg DW)	RO• (mol GSH eq./kg DW)	$^1O_2$ (mol GSH eq./kg DW)
Year:	2013	2017	2013	2017	2013	2013	2013	2013
East Asian								
R. acicularis	365.4 ± 8.6 de	531.7 ± 93.5 bc	478.3 ± 5.1 d	423.1 ± 19.0 e *	253.6 ± 44.4 ab	676.6 ± 13.8 abc	83.9 ± 8.0 a	63.5 ± 2.7 c
R. davurica	1763.9 ± 89.1 a	1200.5 ± 66.4 a	3576.6 ± 105.4 a	2487.4 ± 53.3 a **	258.2 ± 38.6 ab	830.8 ± 83.6 a	92.2 ± 8.2 a	174.2 ± 19.0 a
R. multiflora	571.6 ± 20.6 c	1185.5 ± 98.6 a	303.0 ± 27.6 d	694.8 ± 21.7 d **	20.4 ± 12.7 c	497.5 ± 70.6 bc	111.3 ± 11.2 a	73.0 ± 4.9 c
R. rugosa	335.2 ± 12.8 de	778.7 ± 98.0 b	716.6 ± 5.2 cd	943.1 ± 2.5 c **	50.3 ± 11.8 c	417.7 ± 13.9 cd	20.3 ± 0.8 b	106.0 ± 2.6 bc
R. rugosa f. *plena*	413.9 ± 27.5 cd	– [z]	694.2 ± 15.2 cd	–	25.0 ± 7.0 c	589.9 ± 20.7 abc	94.4 ± 8.2 a	79.8 ± 9.1 c
European								
R. glauca (Cont.)	572.6 ± 24.2 c	731.9 ± 66.5 b	1904.9 ± 62.4 b	1626.0 ± 10.5 b *	69.2 ± 14.6 bc	707.2 ± 58.8 ab	121.5 ± 12.9 a	132.9 ± 7.1 ab
Hybrid								
'Kitaayaka'	446.3 ± 7.6 cd	578.5 ± 88.7 b	1061.1 ± 87.9 c	867.6 ± 25.3 c	291.4 ± 78.6 a	659.6 ± 92.0 abc	80.3 ± 6.9 a	63.1 ± 5.6 c
'Consared'	1511.8 ± 56.0 b	1177.3 ± 40.2 a	3933.3 ± 220.4 a	2605.3 ± 71.3 a **	298.3 ± 12.1 a	694.5 ± 69.5 abc	81.9 ± 9.8 a	128.0 ± 15.1 ab
R. canina [y]	184.0 ± 18.7 e	184.0 ± 18.7 c	451.7 ± 13.2 d	451.7 ± 13.2 e	201.5 ± 61.7 abc	90.3 ± 4.3 d	3.6 ± 0.4 b	66.8 ± 6.2 c

ANOVA	DPPH•	ROO•	$^{\bullet}O_2^-$	HO•	RO•	$^1O_2$
Spieces/cultivar (S)	**	**	**	**	**	**
Year (Y)	ns	**				
S × Y	**	**				

Data represent average ± SE of three independent experiments.
[z] No material of *R. rugosa* f. *plena* was available in 2017 and the data in 2013 were excluded from ANOVA.
[y] Rose hips of *R. canina* were purchased via importer as a reference and the data were excluded from ANOVA.
Different alphabets indicate significant differences between materials in the same year ($P < 0.05$, Tukey's multiple range test).
$^{**}P < 0.01$; $^{*}P < 0.05$ (Student's t–test) vs 2013 in the same species/cultivar.
In the results of ANOVA: **, $P < 0.01$; ns, not significant at 5% level.

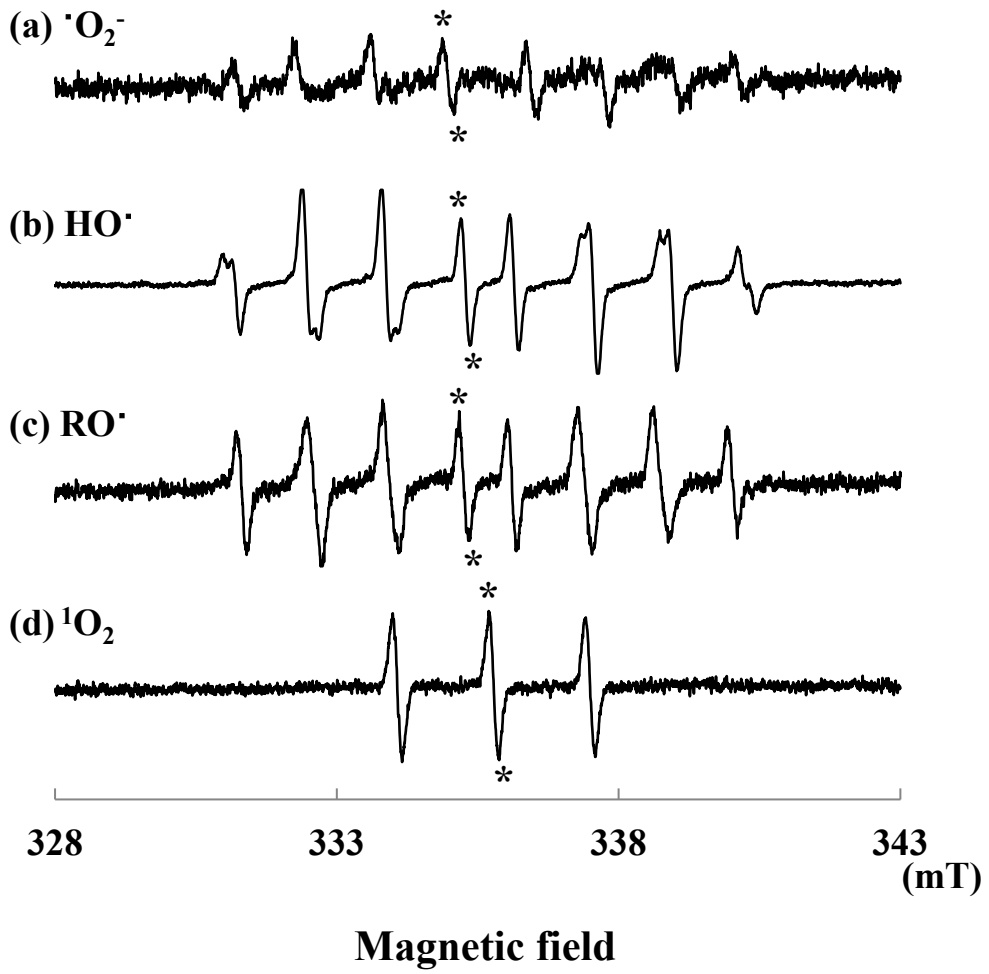


Fig. 1

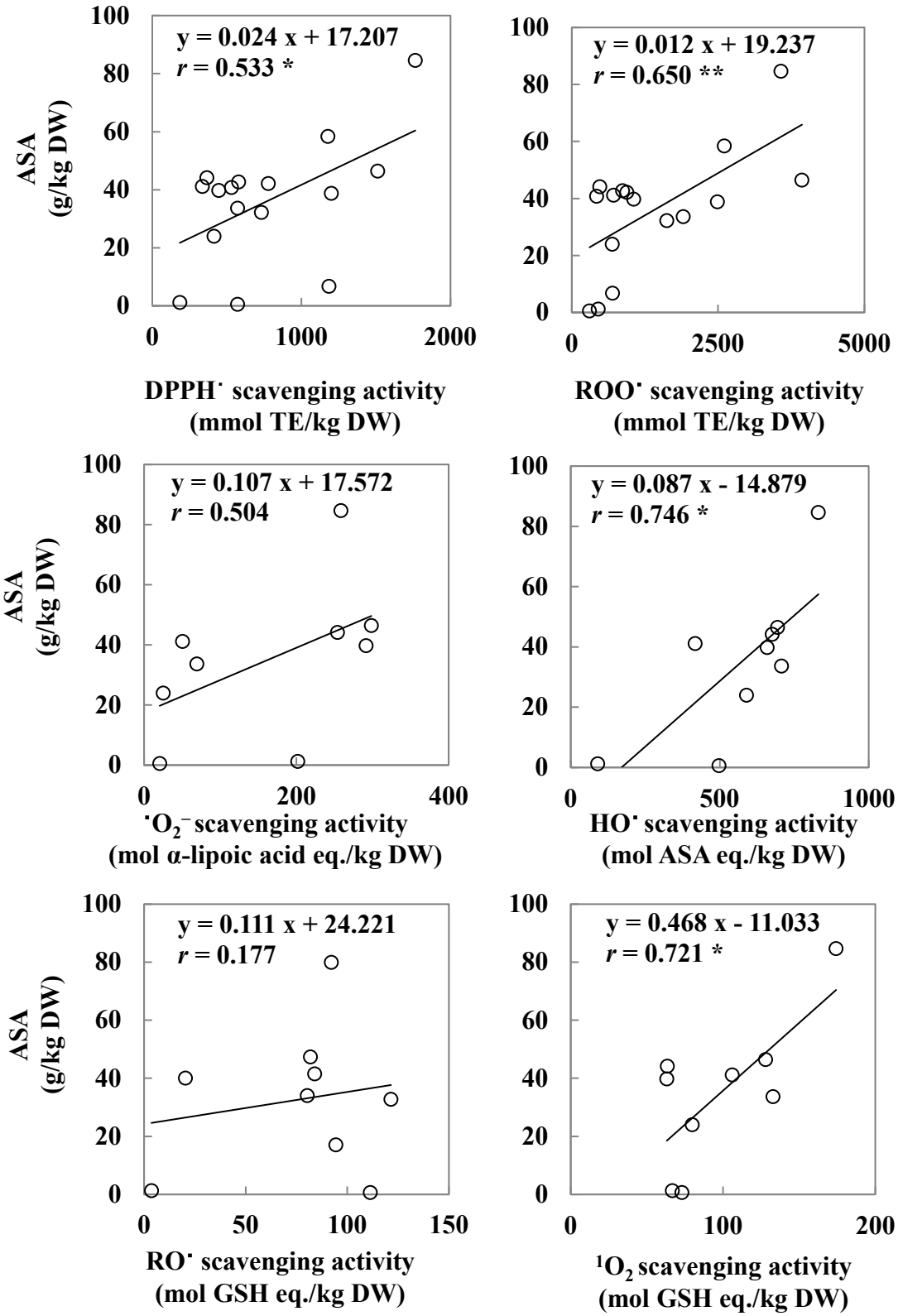

y = 0.024 x + 17.207
r = 0.533 *
ASA (g/kg DW)
DPPH· scavenging activity (mmol TE/kg DW)
y = 0.012 x + 19.237
r = 0.650 **
ROO· scavenging activity (mmol TE/kg DW)
y = 0.107 x + 17.572
r = 0.504
·O2− scavenging activity (mol α-lipoic acid eq./kg DW)
y = 0.087 x - 14.879
r = 0.746 *
HO· scavenging activity (mol ASA eq./kg DW)
y = 0.111 x + 24.221
r = 0.177
RO· scavenging activity (mol GSH eq./kg DW)
y = 0.468 x - 11.033
r = 0.721 *
1O2 scavenging activity (mol GSH eq./kg DW)

Fig. 2

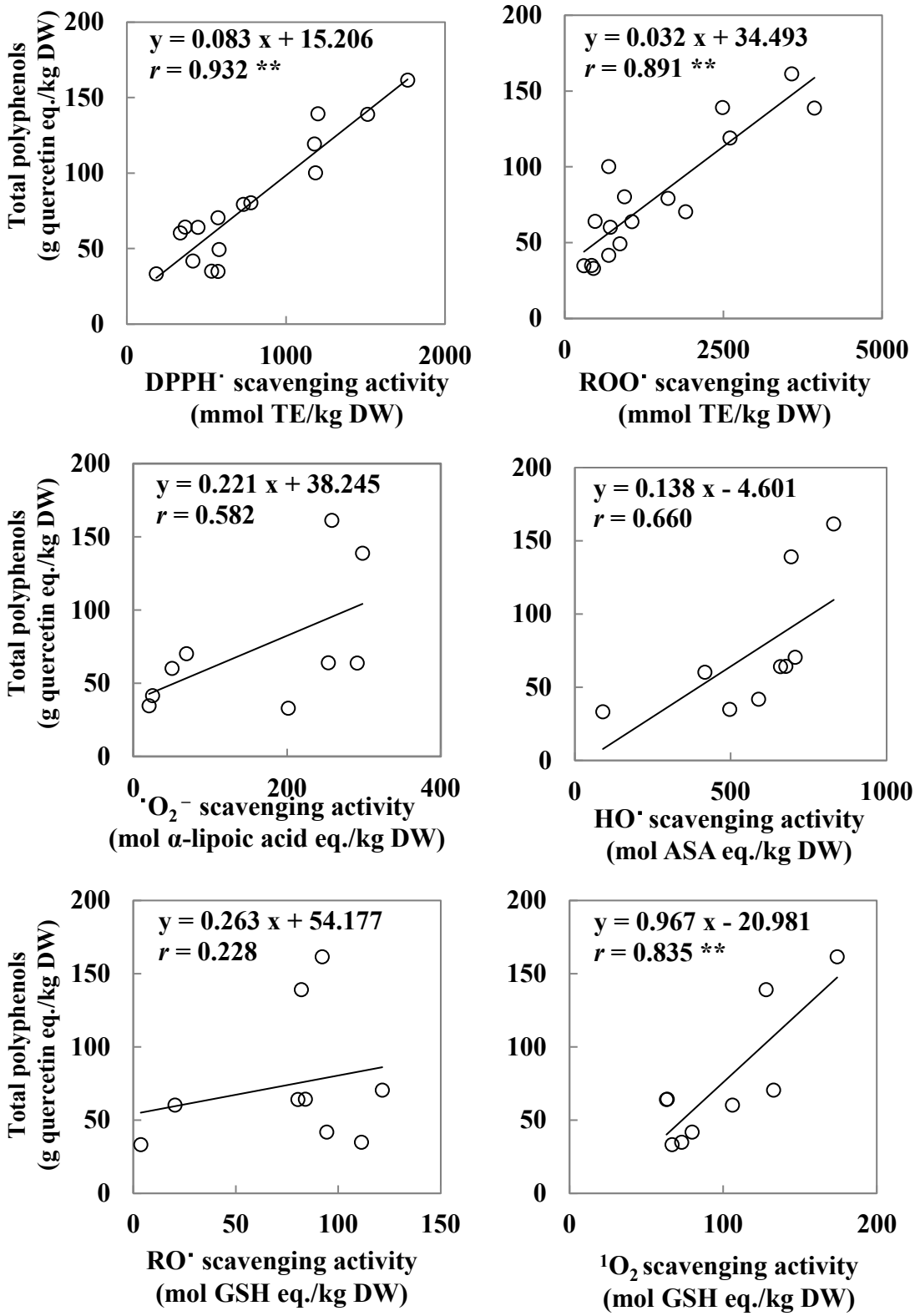

y = 0.083 x + 15.206
r = 0.932 **
Total polyphenols (g quercetin eq./kg DW)
DPPH· scavenging activity (mmol TE/kg DW)
y = 0.032 x + 34.493
r = 0.891 **
ROO· scavenging activity (mmol TE/kg DW)
y = 0.221 x + 38.245
r = 0.582
·O2− scavenging activity (mol α-lipoic acid eq./kg DW)
y = 0.138 x - 4.601
r = 0.660
HO· scavenging activity (mol ASA eq./kg DW)
y = 0.263 x + 54.177
r = 0.228
RO· scavenging activity (mol GSH eq./kg DW)
y = 0.967 x - 20.981
r = 0.835 **
1O2 scavenging activity (mol GSH eq./kg DW)

Fig. 3

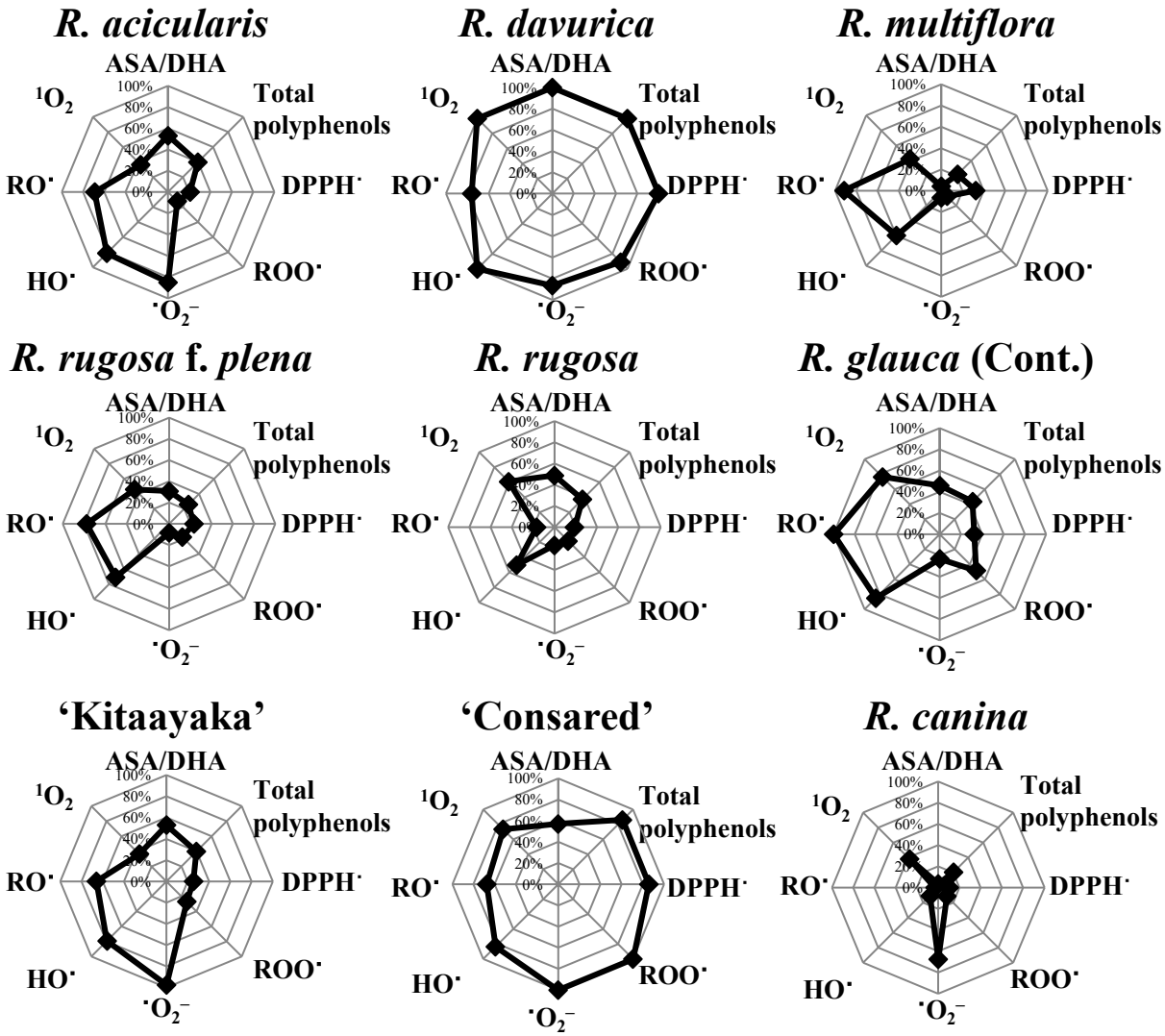

R. acicularis
R. davurica
R. multiflora
R. rugosa f. plena
R. rugosa
R. glauca (Cont.)
'Kitaayaka'
'Consared'
R. canina
ASA/DHA
Total polyphenols
DPPH·
ROO·
·O2−
HO·
RO·
1O2
100%
80%
60%
40%
20%
0%

Fig. 4