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

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18

19 **Abstract**

20 Fruits of the genus *Rosa* plants are called rose hips. The common hips of *R. canina*
21 are well known as a rich source of antioxidants like ascorbic acid and polyphenols. To
22 investigate availability in the hips in *Rosa* spp., wild *Rosa* hips originating from East
23 Asia, i.e. *R. acicularis*, *R. davurica*, *R. multiflora* and *R. rugosa* were evaluated in terms
24 of the content of antioxidants and antioxidant ability in the hydrophilic extracts. The
25 hips from *R. glauca* originating from south Europe and its interspecific hybrids
26 ('Kitaayaka' and 'Consared'), and purchased *R. canina* hips were also examined. In
27 addition to the colorimetric detections of DPPH and ORAC, ESR–ST methods were
28 employed for evaluating antioxidant ability, which can determine scavenging activities
29 against naturally–occurring ROS i.e. superoxide anion radical ($\cdot\text{O}_2^-$), hydroxyl radical
30 ($\text{HO}\cdot$), alkoxyl radical ($\text{RO}\cdot$) and singlet oxygen ($^1\text{O}_2$), individually. The hips of *R.*
31 *davurica* and 'Consared' showed quite high values in both the total content of ASA plus
32 DHA (40.8–103.1 g/kg DW) and total polyphenols (119.2–161.5 g quercetin eq./kg
33 DW) regardless of the years collected. They also had high antioxidant activities against
34 each radical compared to other rose hips, and thus their antioxidant ability seems
35 multiple. Both ASA and polyphenols could scavenge radicals of $\text{ROO}\cdot$ and $^1\text{O}_2$, since
36 significant correlations ($P < 0.05$) were confirmed. However, polyphenols might have
37 greater contribution to the antioxidant activities, because the correlation coefficients
38 were higher in total polyphenols than ASA. *R. davurica* can be one of the useful genetic
39 resources for breeding cultivars which will bear antioxidant–rich rose hips, since
40 'Consared' is a progeny of *R. davurica* \times *glauca*.

41

42 **Keywords**

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

44

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

49 **1. Introduction**

50 Plants of the genus *Rosa*, including more than 100 species widely dispersed from
51 the northern hemisphere, have fruits, namely rose hips, especially of dog rose (*Rosa*
52 *canina*), that are sometimes used for making into jam, syrup and tea. It is also well
53 known that rose hips are rich sources of antioxidants like ASA, polyphenols and
54 carotenoids (Cunja et al., 2016; He et al., 2016; Tabaszewska and Najgebauer-Lejko,
55 2020). Perspective on utilizing rose hips for making functional foods has been discussed
56 (Fan et al., 2014). Nagatomo et al. (2015) demonstrated that the fruit extract of *Rosa*
57 *canina* could inhibit obesity of rats when mixed with feeds, and therefore functional
58 roles of rose hip phytochemicals in diet attracted the attention of researchers. In
59 Hokkaido Japan, there are some wild *Rosa* spp. i.e. *R. rugosa*, the official flower
60 designated by the prefecture in 1978, *R. acicularis*, *R. davurica* and *R. multiflora*,
61 however these fruits have not been widely utilized for foodstuffs. In this study, the
62 native *Rosa* spp. were evaluated in terms of the content of antioxidants and antioxidant
63 activities in rose hips to clarify their availability for fruit production and/or genetic
64 resources on breeding cultivars which can bear antioxidant-rich rose hips.

65 On the evaluation of antioxidant activities different kinds of methods have been
66 utilized with their own principle (Shahidi and Ambigaipalan, 2015), since there were
67 many ROS like superoxide anion radical ($\cdot\text{O}_2^-$), hydroxyl radical ($\text{HO}\cdot$), alkoxyl radical
68 ($\text{RO}\cdot$), peroxy radical ($\text{ROO}\cdot$), non-radical hydrogen peroxide (H_2O_2) and singlet
69 oxygen ($^1\text{O}_2$) related to the oxidation of biomolecules in living organisms, and a kind of
70 antioxidant phytochemical can only scavenge specific types of ROS and is not universal
71 against all ROS. Thus, it is very difficult to evaluate antioxidant activity of a foodstuff
72 using a single index. ESR-ST method has a great advantage in that antioxidant activity
73 against a specific ROS can be detected by the method when being combined with an
74 appropriate spin trapping reagent. Tumbas et al. (2012) tried first to determine the

75 antioxidant activities against $\cdot\text{O}_2^-$ and $\text{HO}\cdot$ in *Rosa canina* hips using ESR-ST with an
76 ordinary spin trapping reagent, namely 3,4-dihydro-2,2-dimethyl-2H-pyrrole 1-oxide
77 (DMPO). By using a novel and powerful spin trapping reagent of 2-(5,5-dimethyl-2-
78 oxo-2λ5-[1,3,2] dioxaphosphinan-2-yl)-2-methyl-3,4-dihydro-2H-pyrrole 1-oxide
79 (CYPMPO) (Kamibayashi et al., 2006), the procedures of ESR-ST have been
80 established specifically for $\cdot\text{O}_2^-$ (Prolla and Mehlhorn, 1990), $\text{HO}\cdot$ (Kameya and Ukai,
81 2012) and $\text{RO}\cdot$ (Ukai et al., 2009). A similar protocol has also been established for $^1\text{O}_2$
82 (Jung and Min, 2009) without CYPMPO and/or DMPO. So, we employed the above
83 ESR-ST procedures to clarify antioxidant activity of the wild rose hips against
84 individual ROS, respectively. We also employed DPPH and ORAC methods that were
85 used commonly for evaluation of antioxidant activity, to compare the values with those
86 obtained by ESR-ST methods, and examine correlation of each antioxidant activity
87 with the content of antioxidants.

88

89 **2. Materials and Methods**

90 *2.1. Reagents*

91 For the analyses of ASA, DHA and polyphenols: 2,4-dinitrophenylhydrazine (DNP)
92 was purchased from Kishida Chemical (Osaka, Japan), metaphosphoric acid, thiourea,
93 sulfuric acid and sodium carbonate from Fujifilm Wako Pure Chemical (Osaka, Japan),
94 2,6-dichloroindophenol (DCIP) from Merck (Darmstadt, Germany), 'Folin-Denis'
95 reagent from Sigma-Aldrich Chemical (St. Louis, MO, USA), and quercetin from
96 Kanto Chemical (Tokyo, Japan). For the antioxidant activity analyses: phosphate buffer
97 (pH 7.4), morpholinoethanesulfonic acid (MES), 2,2'-azobis (2-amidinopropane)
98 dihydrochloride (AAPH), ethylenediaminetetraacetic acid (EDTA)-2Na, H_2O_2 , pterin,
99 N,N,N',N'-tetramethyl-1,4-benzenediamine (TMPD), diethylenetriaminepentaacetic acid
100 (DTPA), glycine and riboflavin from Fujifilm Wako Pure Chemical (Osaka, Japan),

101 2,2-diphenyl-1-picrylhydrazyl (DPPH) and fluorescein sodium from Sigma-Aldrich
102 Chemical (St. Louis, MO, USA), 2-(5,5-dimethyl-2-oxo-2λ5-[1,3,2]
103 dioxaphosphinan-2-yl)-2-methyl-3,4-dihydro-2H-pyrrole 1-oxide (CYPMPO) from
104 Radical Research (Hino, Japan). For the standards: 6-hydroxy-2,5,7,8-
105 tetramethylchroman-2-carboxylic acid (Trolox) was purchased from Sigma-Aldrich
106 Chemical (St. Louis, MO, USA), and ASA, α-lipoic acid and glutathione (GSH) from
107 Fujifilm Wako Pure Chemical (Osaka, Japan). Methanol and ethanol were all HPLC
108 grade.

109

110 2.2. Plant material

111 Matured fruits (rose hips) were collected from 18-year-old shrubs of five wild *Rosa*
112 species originating from East Asia, i.e. *R. acicularis* Lindl. (1.06 g fresh weight
113 (FW)/fruit, 63.9 % in water content), *R. davurica* Pall. (0.83 g FW/fruit, 71.4% in water
114 content), *R. multiflora* Thunb. (0.20 g FW/fruit, 65.5% in water content), *R. rugosa*
115 Thunb. (2.11 g FW/fruit, 76.0% in water content), and *R. rugosa* Thunb. f. *plena*
116 Byhouwer (3.26 g FW/fruit, 69.4% in water content), grown in the experimental farm,
117 composed of brown lowland soil without fertilization, in Mikasa of the Forestry
118 Research Institute of Hokkaido on October 2, 2013. Twenty-four fruits were collected
119 from 4 different shrubs (5–10 fruits/shrub depending on the fruit size in each species).
120 To compare the component of antioxidants and antioxidant activities in rose hips of East
121 Asian *Rosa* spp. to those of European, fruits of *R. glauca* Pourret (0.99 g FW/fruit,
122 63.7% in water content) originating from south Europe (as control), and interspecific
123 hybrid cultivars of ‘Kitaayaka’ (*R. glauca* × *rugosa*) (1.25 g FW/fruit, 68.3% in water
124 content) and ‘Consared’ (*R. davurica* × *glauca*) (0.76 g FW/fruit, 66.1% in water
125 content) bred both for floriculture by the Forestry Research Institute of Hokkaido were
126 also collected from 18-year-old shrubs grown in the same experimental farm at the

127 same time with the same manner. Fruits were collected again from the above–
128 mentioned shrubs on September 28, 2017 for clarifying yearly variations. Seasonal
129 changes in air temperature in 2017 were similar to those in 2013, however the amounts
130 of water precipitation from January through March and from June through July in 2017
131 were less and more than those in 2013, respectively. Dried fruits of *R. canina* L.,
132 namely common rose hip in a narrow sense, grown commercially in Republic of Chile
133 were purchased from a Japanese importer as a reference plant material. All above genus
134 *Rosa* plants (Suppl. 1) are categorized into subgenus *Rosa*. In more detail, *R. canina* and
135 *R. glauca* are classified into section *Caninae*, *R. acicularis*, *R. rugosa* and *R. davurica*
136 are classified into section *Rosae*, and *R. multiflora* is classified into section *Synstylae* in
137 the 10 different sections of subgenus *Rosa* (Nomura, 2010; Smulders et al, 2011).
138 Distribution on some of the species was described in the literature (Smulders et al,
139 2011). After being harvested, raw fruits excluding seeds were quickly frozen in liquid
140 nitrogen, lyophilized, ground into powder and then stored at -30°C for subsequent
141 analyses.

142

143 2.3. Quantification of antioxidants

144 2.3.1. Ascorbic acid

145 Determination of ASA and DHA followed the DNP method established by Roe *et*
146 *al.* (1948). For quantifying total concentration of ASA plus DHA, triplicates of 5 mg of
147 the lyophilized sample were extracted with 1 mL of 5% metaphosphoric acid in a 1.5
148 mL taper plastic tube with lid by shaking for 3 h using a laboratory shaker. After
149 centrifugation at 12,000g for 10 min, the supernatant was collected. A 20 μL solution of
150 0.03% DCIP, a 40 μL solution of 5% metaphosphoric acid supplemented with 2%
151 thiourea, and a 40 μL solution of 2% DNP were added to the 40 μL of extracts or ASA
152 standards in order into a 96–well plate (P96F03N; As One, Osaka, Japan), and mixed.

153 After incubation at 37°C for 3 h, a 100 µL solution of 85%(v/v) sulfuric acid was added
154 to each well, mixed, cooled by placing the microplate on crushed ice for 30 min, and the
155 absorbance was read at 520 nm using a microplate reader (Powerscan HT; DS Pharma
156 Biomedicals, Osaka, Japan). When quantifying DHA concentration in an extract
157 excluding ASA, triplicates of 5 mg of the lyophilized sample were extracted with 1 mL
158 of 5% metaphosphoric acid supplemented with 2% thiourea in a 1.5 mL taper plastic
159 tube. A 20 µL solution of 5% metaphosphoric acid was added to the extracts instead of
160 0.03% DCIP. The concentration of ASA in an extract was calculated by subtracting the
161 DHA concentration from the total concentration of ASA plus DHA in the same extract.
162 Standard curve was calculated from the values ($n = 3$) on 5 graded concentrations.

163

164 2.3.2. Total polyphenols

165 Total polyphenols were determined according to the Folin–Denis colorimetric
166 method (Folin and Denis, 1915). Triplicates of 5 mg of the lyophilized sample were
167 extracted with the 1 mL of 80%(v/v) methanol in a 1.5 mL taper plastic tube with lid by
168 shaking for 3 h using a laboratory shaker. After centrifugation at 12,000g for 10 min,
169 the supernatant was collected. The 75 µL solution of 50% Folin–Denis’ reagent and 75
170 µL of a 5% sodium carbonate solution were added to the 150 µL of extracts or quercetin
171 standards in order into a 96–well plate (As One), mixed, left to stand on the bench for
172 60 min, and the absorbance was read at 700 nm using the microplate reader. Total
173 polyphenols were estimated as the µmol quercetin equivalent of a sample using the
174 standard curve of quercetin which was calculated from the values ($n = 3$) on 5 graded
175 concentrations.

176

177 2.4. Antioxidant activity for ROS

178 2.4.1. DPPH radical scavenging activity

179 Analysis using artificial DPPH radical (DPPH[•]) was carried out as described
180 previously (Sharma and Bhat, 2009). Triplicates of 5 mg of the lyophilized sample were
181 extracted with the 1 mL of 80%(v/v) ethanol in the same manner for total polyphenols.
182 One hundred and fifty µL solution of DPPH (400 µM in ethanol): MES buffer (pH 6.0,
183 200 mM): 20%(v/v) ethanol = 1:1:1 (v/v/v) were added to the 50 µL of the extracts or
184 the standards into a 96–well plate (As One). The mixture was left to stand at room
185 temperature for 20 min, then the absorbance was read at 520 nm in the microplate
186 reader. DPPH[•] scavenging activity was estimated as the µmol Trolox equivalent (TE) of
187 a sample using the standard curve of Trolox which was calculated from the values ($n =$
188 3) on 5 graded concentrations.

189

190 2.4.2. ORAC method

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

204

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 ($\cdot\text{O}_2^-$)–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 ($\text{HO}\cdot$)–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 Alkoxy radical ($\text{RO}\cdot$)–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 ($^1\text{O}_2$)–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 $\cdot\text{O}_2^-$, $\text{HO}\cdot$, $\text{RO}\cdot$ and $^1\text{O}_2$, 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

231 for 5 sec (20 sec in case of $\cdot\text{O}_2^-$) with ultraviolet rays for producing radicals. At this
232 time, the ESR spectrum was immediately measured using an X-band ESR spectrometer
233 (JES-RE1X, JEOL, Tokyo, Japan) with a 100 kHz field modulation. The spectrometer
234 conditions were as follows: resonance field, 3521 G; field modulation width, 1.0 G;
235 microwave power, 6 mW; light source, 200 W medium pressure mercury/xenon arc
236 lamp (LC-8, Hamamatsu Photonics K.K., Hamamatsu, Japan); UV irradiation intensity
237 for photolysis, 2.78 mW/cm² (LC-8, Hamamatsu Photonics K.K., Hamamatsu, Japan)
238 measured by a UV intensity meter (Cole-Parmer International, IL, USA); the band-pass
239 filter, G-533 (HOYA, Tokyo, Japan). The analysis of adducted signal was carried out as
240 described by Kameya et al. (2014). The scavenging activities were estimated as the
241 mmol standard equivalent of a sample using the standard curve which was calculated
242 from the values ($n = 3$) on 5 graded concentrations.

243

244 2.5. Statistical analyses of data

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

251

252 3. Results

253 3.1. Content of antioxidants

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

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

262 Total content of polyphenols was statistically ($P < 0.05$) higher in rose hips of *R.*
263 *davurica* and 'Consared' in 2013, and of *R. davurica*, *R. multiflora* and 'Consared' in
264 2017, whereas lower in rose hips of *R. multiflora* and *R. rugosa* f. *plena* in 2013, of *R.*
265 *acicularis* and 'Kitaayaka' in 2017, and of *R. canina* (reference) than those of the
266 control. No significant yearly variation was confirmed by ANOVA in total content of
267 polyphenols in the hips examined.

268

269 3.2. Antioxidant activities

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

278 The DPPH' scavenging activity was statistically ($P < 0.05$) higher in hips of *R.*
279 *davurica* and 'Consared' in 2013, and of *R. davurica*, *R. multiflora* and 'Consared' in
280 2017, whereas lower in hips of *R. acicularis* and *R. rugosa* in 2013, and of *R. canina*
281 (reference) than those of the control. No significant yearly variation was confirmed by
282 ANOVA in the DPPH' scavenging activity of the hips examined. The ROO' 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 significant ($P < 0.05$) 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 $\cdot\text{O}_2^-$ 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 $\text{HO}\cdot$ and $\text{RO}\cdot$ 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 $^1\text{O}_2$ 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 ($r = 0.533\text{--}0.746$) was statistically significant ($P < 0.05$)
303 between ASA content and antioxidant activity against $\text{DPPH}\cdot$, $\text{ROO}\cdot$, $\text{HO}\cdot$ and $^1\text{O}_2$.
304 Correlations between total content of polyphenols and individual antioxidant activity
305 are shown in Fig. 3. Correlation coefficient ($r = 0.835\text{--}0.932$) was statistically
306 significant ($P < 0.01$) between total polyphenols and antioxidant activity against $\text{DPPH}\cdot$,
307 $\text{ROO}\cdot$ and $^1\text{O}_2$.

308

309 4. Discussion

310 On the content of antioxidants in rose hips, Cunja et al. (2016) reported that the total
311 content of ascorbic acid in *R. canina* hips (common hips belonging to section *Caninae*)
312 was 18.4 g/kg DW, and the range was from 2.4 to 47.1 g/kg DW in rose hips from
313 selected species/cultivars. Roman et al. (2013) demonstrated that average amounts of
314 vitamin C in natural *R. canina* hips collected in Transylvania ranged from 1.1 to 3.6
315 g/kg frozen pulp. Ercişli (2007) pointed out that vitamin C level was estimated to be 3–
316 40 g/kg DW depending upon species, genotype, and environmental factors. Total
317 content of ASA plus DHA (3.7 g/kg DW) of our purchased *R. canina* hips was at
318 similar or lower level in comparison to the above values, which may be due to
319 destruction of ASA and DHA during the drying process and/or storage period
320 (Tabaszewska and Najgebauer-Lejko, 2020). Total values of ASA plus DHA (47.3 g/kg
321 DW in 2013, and 32.2 g/kg DW in 2017) in *R. glauca* hips (control), which was also
322 classified into section *Caninae*, were at high level in the above range of vitamin C.
323 Among East Asian *Rosa* hips, total values of ASA plus DHA of the species classified
324 into section *Rosae* (*R. acicularis*, *R. davurica*, *R. rugosa*, and *R. rugosa* f. *plena*) ranged
325 from 31.8 to 103.1 g/kg DW, which could be converted into 9.7–29.5 g/kg FW by
326 calculation depending on the water content of each sample. Ercişli and Eşitken (2004)
327 showed that the fruits of twelve promising rose hip genotypes selected from 10,000
328 seedling shrubs of *R. dumalis*, *R. canina*, *R. pulverulanta* and *R. montana* collected in
329 the Erzurum province of Turkey contained 10.74–25.57 g ascorbic acid/kg FW. The
330 range of ASA plus DHA content in our native rose hips categorized into section *Rosae*
331 is very close to this range. Furthermore, the values are greater than that (4.1–4.4 g/kg
332 FW) of sea buckthorn fruits (Gutzeit et al., 2008) and that (2.2 g/kg FW) of guava fruits
333 (Standard Tables of Food Composition in Japan, 2015), and correspond to that (17.0
334 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 foodstuffs 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

361 and/or samples used for the determination are sometimes not the same as those written
362 in literatures. However, since the ORAC values of our matured black chokeberry fruits
363 collected in the experimental farm were 847.1 mmol TE/kg DW (170.1 mmol TE/kg
364 FW) in 2018 and 960.9 mmol TE/kg DW (188.9 mmol TE/kg FW) in 2019, which was
365 very close to the values (158.2–160.2 mmol TE/kg FW) in the review on *Aronia*
366 (Kulling and Rawel, 2008), our experiments on the ORAC determination seemed to be
367 performed precisely. From this point of view, the ORAC values (2487.4–3933.3 mmol
368 TE/kg DW) of *R. davurica* and ‘Consared’ are said to be quite high and they have
369 outstandingly strong scavenging ability against ROO[•]. Similarly, they also showed high
370 scavenging activities against DPPH[•] regardless of the collection year and against
371 naturally–occurring ROS ([•]O₂⁻, HO[•] and ¹O₂).

372 On the roles of ASA and polyphenols related to the antioxidant activities in rose
373 hips, both could scavenge radicals of ROO[•] and ¹O₂, since significant ($P < 0.01, 0.05$)
374 correlations were confirmed (Figs. 2 and 3). However, polyphenols might have greater
375 contribution to these antioxidant activities, because the correlation coefficients were
376 higher in total polyphenols than ASA. This was also pointed out in the reports on
377 utilization of fruit pulps of citrus (Ramful et al., 2011) and peach (Liu et al., 2015).
378 However, it might be possible that the difference in the activities between ASA and
379 polyphenols was caused by difference in extraction solution (5% metaphosphoric acid
380 for ASA, 80% methanol for total polyphenols, 80% ethanol for DPPH, and MWA for
381 naturally–occurring ROS). To confirm the effects of extraction solution, ASA was
382 extracted from the lyophilized powder of *R. davurica* and ‘Consared’ hips by 80%
383 ethanol or MWA first, evaporated and re-extracted by 5% metaphosphoric acid. Then
384 ASA content was compared with that extracted from the same material by 5%
385 metaphosphoric acid directly. As a result, the recovery of ASA content was 88-93%
386 (Suppl. 2). Thus, it seems probable that the effect of extraction solution might be small.

387 On the other hand, ASA and polyphenols might have no scavenging ability against $\cdot\text{O}_2^-$
388 and $\text{RO}\cdot$. The significant correlation ($P < 0.05$) between the ASA content and the $\text{HO}\cdot$
389 scavenging activity seems reasonable, since ASA has been employed as a standard
390 antioxidant reagent in the $\text{HO}\cdot$ scavenging assay using ESR–ST (Kameya and Ukai,
391 2012). The antioxidant roles of polyphenols in hips of each *Rosa* species should further
392 be investigated since they are composed of various phytochemicals like anthocyanin,
393 flavonol, ellagic acid, catechin, etc., and component of polyphenols might be different.

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

406

407 **Acknowledgments**

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409 Medicine) for technical assistance on the ORAC determination.

410

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519

520 **Legends of Figures**

521

522 Fig. 1. Examples of individual adduct signal in ESR–ST analyses against radicals of
523 $\cdot\text{O}_2^-$ (a), $\text{HO}\cdot$ (b), $\text{RO}\cdot$ (c) and $^1\text{O}_2$ (d). Asterisk shows peaks which were used to
524 evaluate the magnitude of wave, as distance between the peaks (top and bottom).

525

526 Fig. 2. Correlation between the content of ASA, shown in Table 1, and the antioxidant
527 activities shown in Table 2. $**P < 0.01$; $*P < 0.05$ (Pearson's correlation coefficient
528 test, $n = 16$ for DPPH' and ROO' including data in both 2013 and 2017, $n = 9$ for
529 $\cdot\text{O}_2^-$, $\text{HO}\cdot$, $\text{RO}\cdot$ and $^1\text{O}_2$ in 2013 only).

530

531 Fig. 3. Correlation between the content of total polyphenols, shown in Table 1, and
532 antioxidant activities shown in Table 2. The plot of *R. canina* is hidden behind the
533 plot of 'Kitaayaka' in the panel of $^1\text{O}_2$. $**P < 0.01$ (Pearson's correlation coefficient
534 test, $n = 16$ for DPPH' and ROO' including data in both 2013 and 2017, $n = 9$ for
535 $\cdot\text{O}_2^-$, $\text{HO}\cdot$, $\text{RO}\cdot$ and $^1\text{O}_2$ in 2013 only).

536

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

540

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.

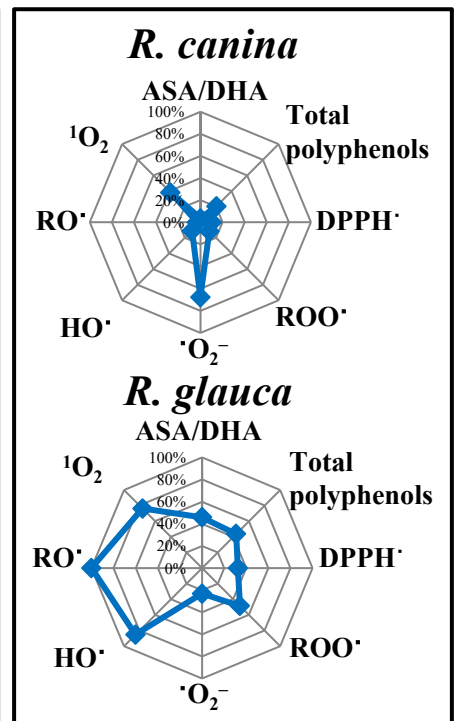
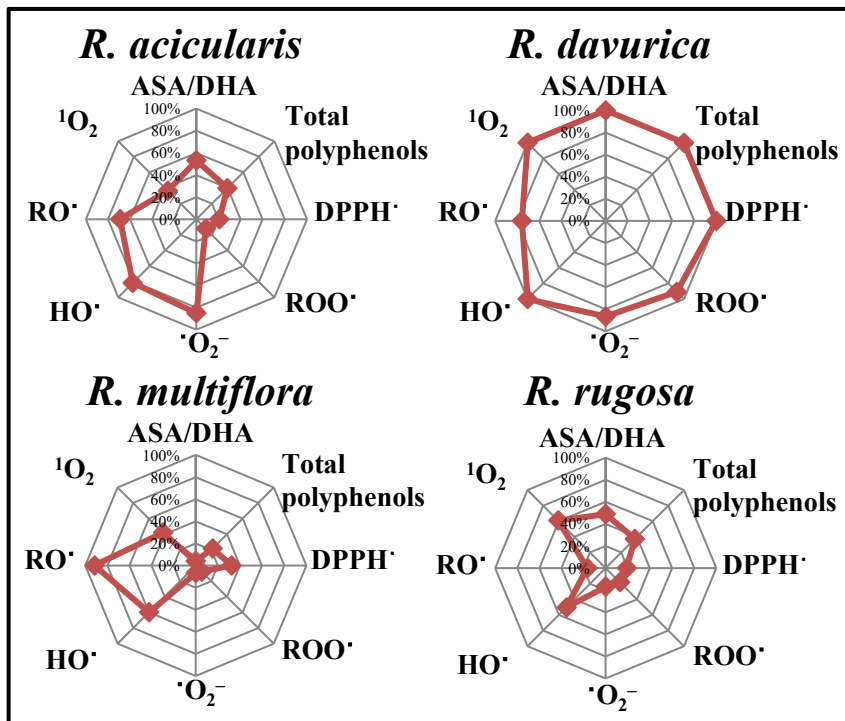
Content of ascorbic acid and polyphenols correlated with the activities scavenging ROO^\cdot and $^1\text{O}_2$.

***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*

East Asian *Rosa* spp.

European *Rosa* spp.



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.

Species/cultivar	Content of ASA and DHA (g/kg DW)						Total polyphenols (g quercetin eq./kg DW)	
	2013			2017			2013	2017
	ASA	DHA	Total	ASA	DHA	Total		
East Asian								
<i>R. acicularis</i>	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
<i>R. davurica</i>	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
<i>R. multiflora</i>	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
<i>R. rugosa</i>	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
<i>R. rugosa</i> f. <i>plena</i>	24.0 ± 0.6 d	7.8 ± 0.1 f	31.8 ± 0.7 d	– ^z	–	–	41.7 ± 0.8 d	–
European								
<i>R. glauca</i> (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
<i>R. canina</i> ^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	
Species/cultivar (S)		**	**	**			**	
Year (Y)		**	**	**			ns	
S × Y		**	**	**			**	

Data represent average ± SE of three independent experiments.

^zNo material of *R. rugosa* f. *plena* was available in 2017 and the data in 2013 were excluded from ANOVA.

^yRose 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[•], [•]O₂⁻, HO[•], RO[•] and ¹O₂ in rose hips collected in 2013 and 2017.

Species/cultivar	Radical scavenging activities															
	DPPH [•] (mmol TE/kg DW)		ROO [•] (mmol TE/kg DW)		[•] O ₂ ⁻ (mol α -lipoic acid eq./kg DW)	HO [•] (mol ASA eq./kg DW)	RO [•] (mol GSH eq./kg DW)	¹ O ₂ (mol GSH eq./kg DW)								
	Year:	2013	2017	2013	2017	2013	2013	2013	2013							
East Asian																
<i>R. acicularis</i>	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
<i>R. davurica</i>	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
<i>R. multiflora</i>	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
<i>R. rugosa</i>	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
<i>R. rugosa</i> f. <i>plena</i>	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																
<i>R. glauca</i> (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
<i>R. canina</i> ^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 [•]		[•] O ₂ ⁻	HO [•]	RO [•]	¹ O ₂								
Species/cultivar (S)	**		**		**	**	**	**								
Year (Y)	ns		**													
S × Y	**		**													

Data represent average ± SE of three independent experiments.

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Different alphabets indicate significant differences between materials in the same year ($P < 0.05$, Tukey's multiple range test).

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In the results of ANOVA: **, $P < 0.01$; ns, not significant at 5% level.

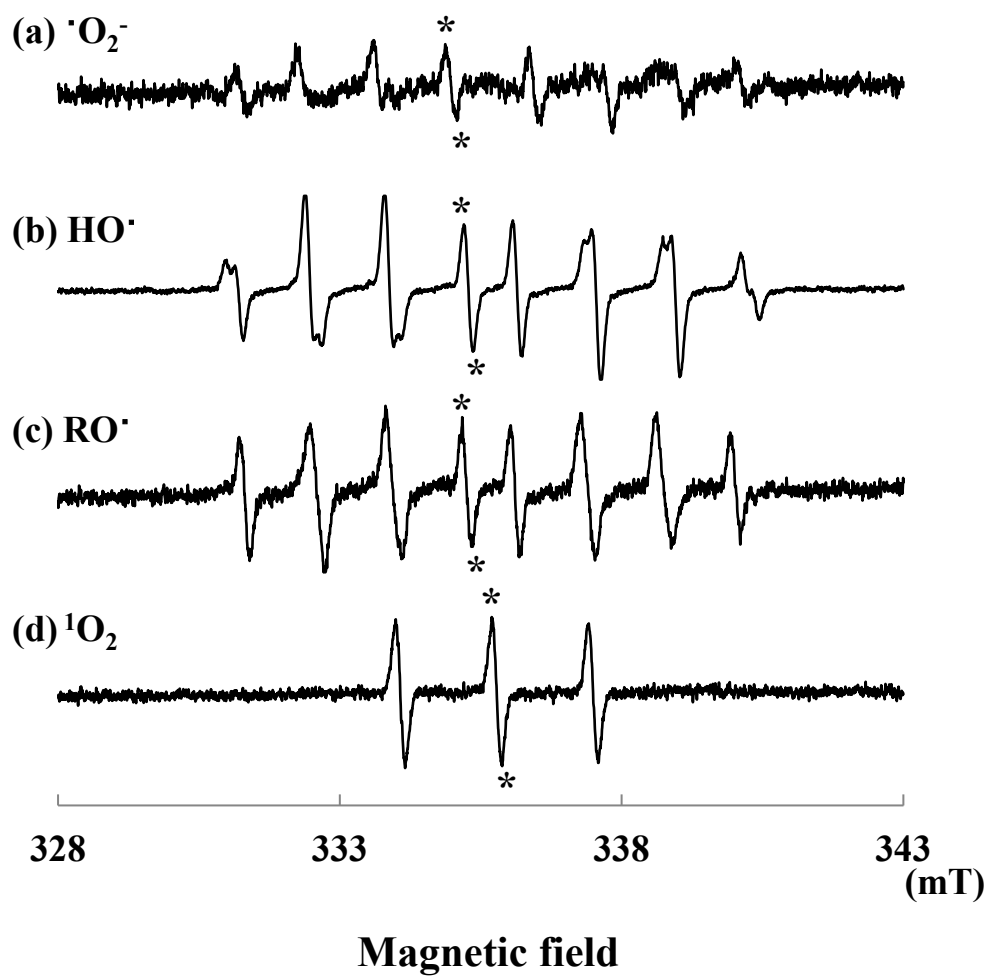


Fig. 1

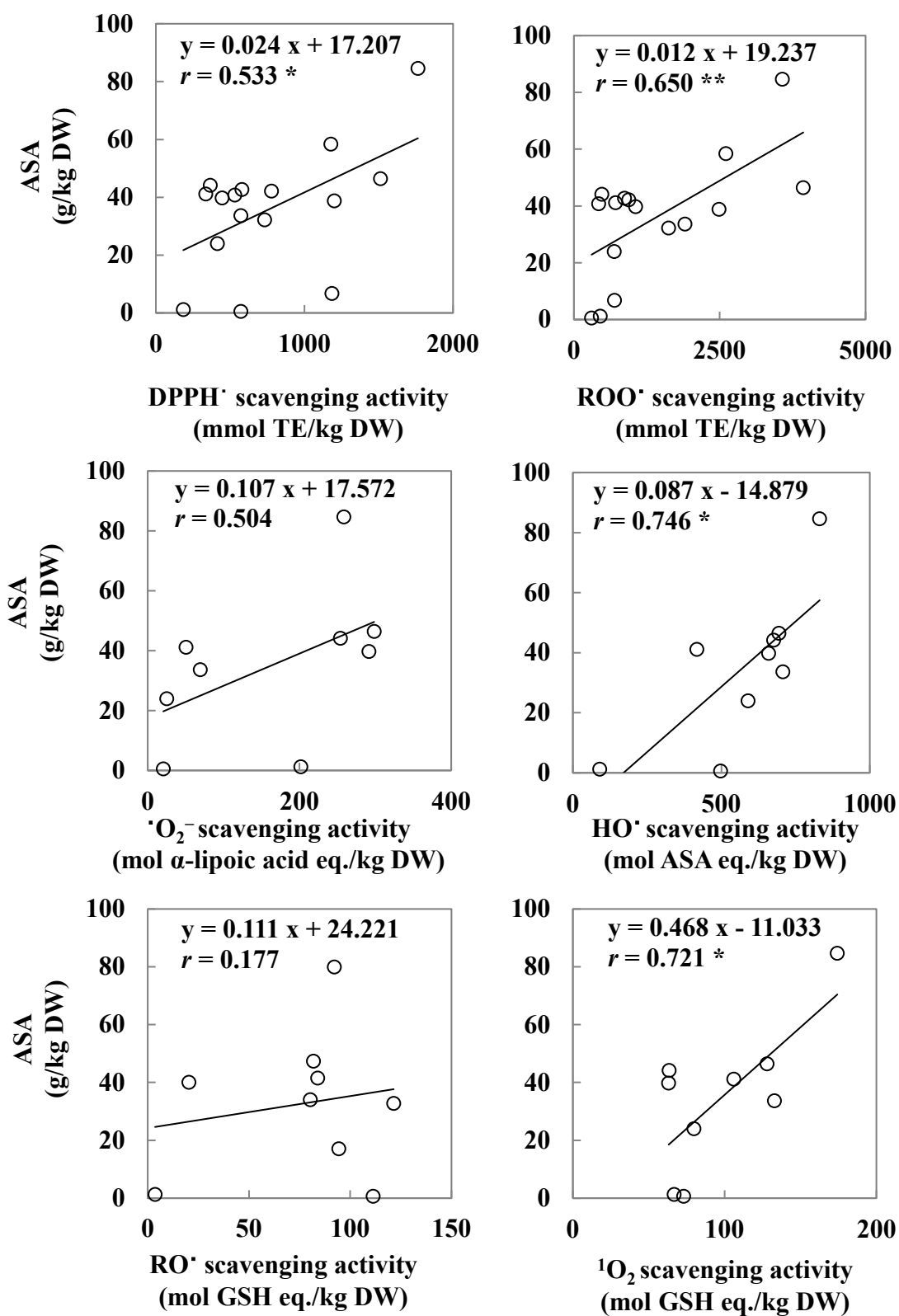


Fig. 2

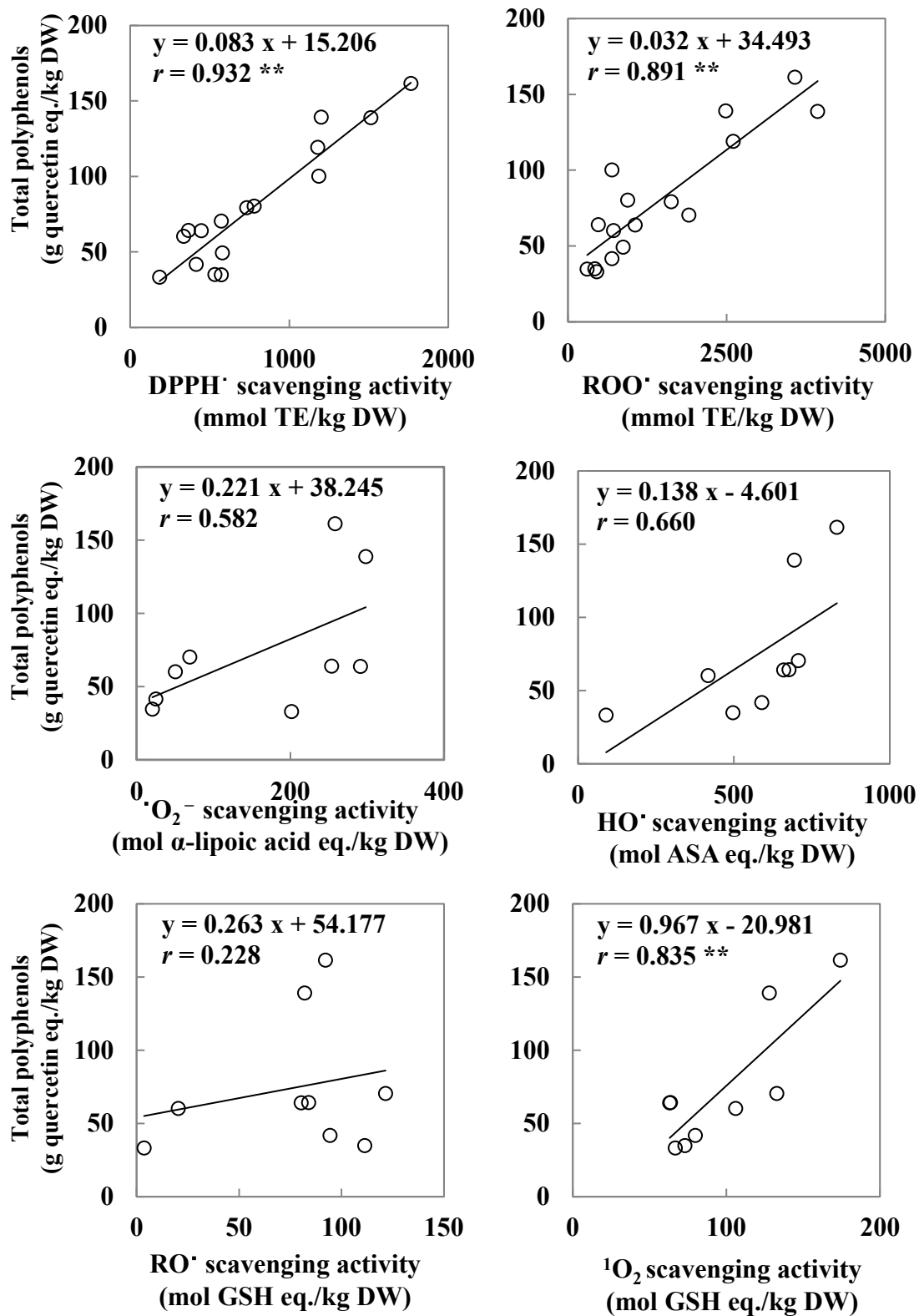


Fig. 3

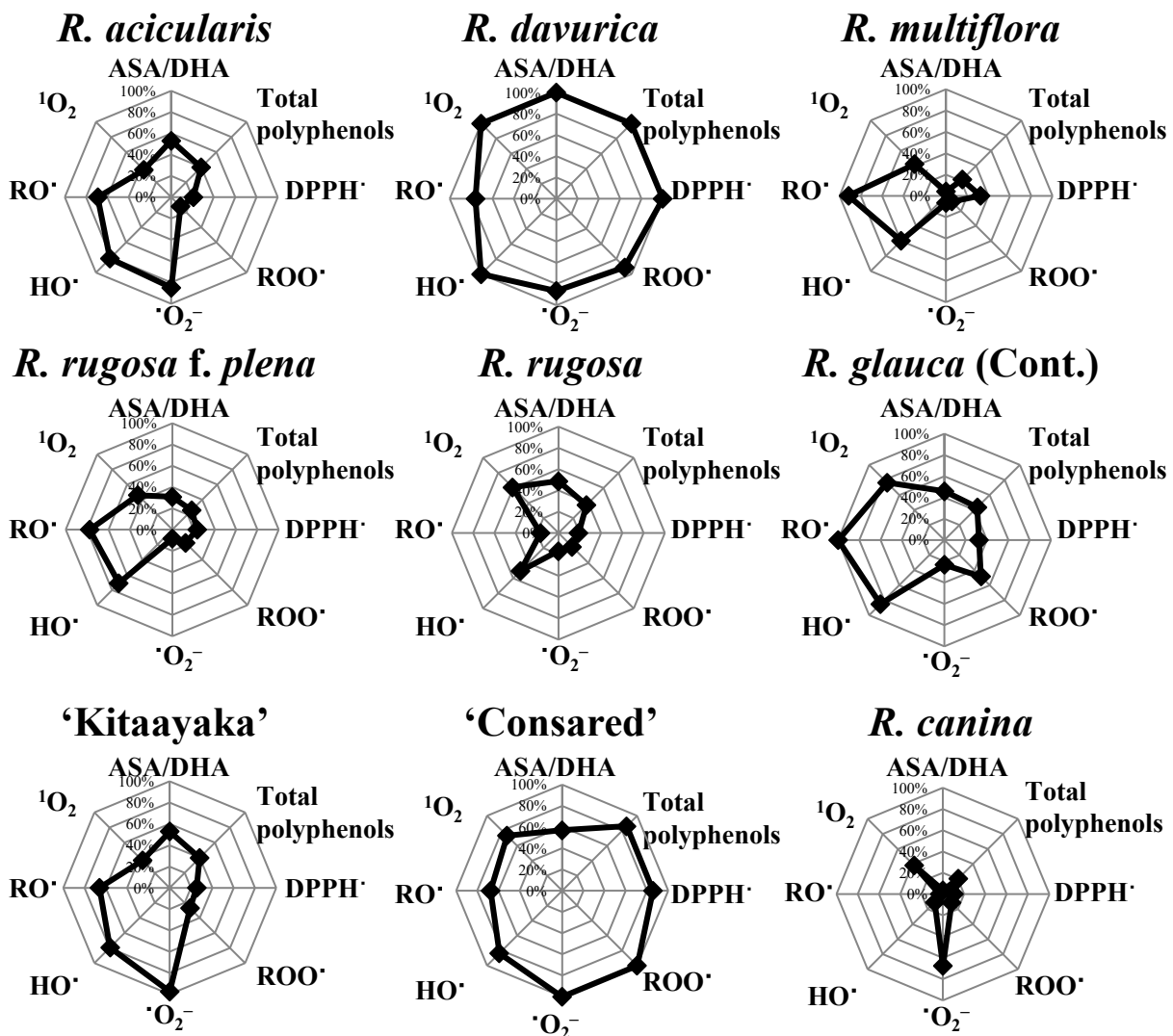


Fig. 4