Vegetative Growth and Fruit Quality of ‘Ruby Roman’ Grapevines Grafted on Two Species of Rootstock and Their Tetraploids

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The growth and berry quality of ‘Ruby Roman’ (Vitis labruscana) grapevines grafted on two species of rootstock, ‘Kober 5BB’ [‘5BB(2×)’] (V. berlandieri × V. riparia, a semi-dwarf rootstock) and ‘Hybrid Franc’ [‘HF(2×)’] (V. rupestris × V. vinifera, a vigorous rootstock), and their colchicine-induced autotetraploids [‘5BB(4×)’ and ‘HF(4×)’] were investigated through 2 years of pot cultivation followed by 2 years of ground cultivation. During the nursery stage, the survival rate and root and shoot growth of the grafted cuttings in the two diploid rootstocks were obviously greater than in their corresponding tetraploids. During subsequent cultivation in pots and in the ground, the grapevines grafted on ‘5BB(4×)’ had less shoot growth (weight and length), shorter internode length, and in some cases smaller stem diameter than those grafted on ‘5BB(2×)’.

However, in contrast with ‘5BB’, there was no significant difference in total vine growth between ‘HF(2×)’ and ‘HF(4×)’ during pot cultivation, and the total shoot length and weight in the ‘HF(4×)’ grapevine was greater than in ‘HF(2×)’ during ground cultivation in 2014. After 2 years of pot cultivation, the root growth of the ‘HF’ (diploid and tetraploid) rootstocks was more vigorous than that of the ‘5BB’ (diploid and tetraploid) rootstocks. In addition, the proportions of the thin roots (diameters <1 mm) in the two diploid rootstocks [‘5BB(2×)’ and ‘HF(2×)’] were greater than those in the two tetraploid rootstocks [‘5BB(4×)’ and ‘HF(4×)’]. In contrast, the proportions of the thick roots (diameters 1–2 mm, 2–5 mm, and >5 mm) in the two diploid rootstocks were less than those in the two tetraploid rootstocks. Furthermore, the berries of ‘Ruby Roman’ harvested from the grapevines grafted on ‘5BB(4×)’ exhibited a much deeper skin coloration than those harvested from the other grapevines.

Key Words: berry skin coloration, colchicine-induced autotetraploid, grape, tree vigor.

Introduction

In Japan, most of the large-berry table grape cultivars are tetraploid. ‘Ruby Roman’ is a new red table grape cultivar selectively bred from seedlings of ‘Fujiminori’, a tetraploid black table grape obtained from progeny of Ikawa Selection 682 × ‘Pioné’ (Yamada and Sato, 2016). ‘Ruby Roman’ has some excellent characteristics, such as bright ruby red berries that are extremely large such that the fresh weight exceeds 20 grams, nearly twice the weight of ‘Kyoho’ berries, a popular and representative large-berry table grape in Japan. Since it was first marketed in 2008, ‘Ruby Roman’ has become a famous cultivar and has attracted public attention every year with its high price. In 2015, the most expensive cluster set a record of ¥1 million ($8200). However, because there are some problems with ‘Ruby Roman’ cultivation, such as coloration defects, fruit cracking, small berries, disease, and in particular, poor coloration, only approximately 40% of harvested clusters are allowed to enter the market every year.

Most of the large-berry tetraploid grape cultivars are interspecific hybrids descended from Vitis vinifera and V. labruscana; they usually have no tolerance to grape phylloxera (Daktulosphaira vitifoliae), one of the most destructive grape pests worldwide. Accordingly, in
viticulture, vines grafted on rootstocks are generally used instead of own-rooted grapevines in order to prevent phylloxera. Resistant rootstock species, such as ‘Teleki’ strains (Vis. berlandieri × V. riparia) including ‘8B’, ‘Kober 5BB’ (‘5BB’), and ‘5C’, are generally used (Uehara, 1995). Aside from pest resistance, these rootstocks also provide the beneficial characteristics of high berry quality, early ripening, and wide soil adaptability. Uehara (1995) described ‘5BB’ as a semi-dwarf rootstock with vigorous growth and some scion overgrowth. ‘5BB’ has a shallow-growing root system, and good drought tolerance, but less humidity tolerance than Teleki 8B and 5C. Because ‘5BB’ rootstock is relatively easier to root and propagate than Teleki 8B, it is currently the most widely utilized rootstock in viticulture in Japan. To date, all ‘Ruby Roman’ grapevines have been grafted on ‘5BB’ rootstocks. Therefore, we selected ‘5BB’ rootstock as one of our test materials. Another rootstock species used in this study is ‘Hybrid Franc’ (Vis. rupestris × V. vinifera), described as a typical vigorous rootstock without scion overgrowth. ‘Hybrid Franc’ was originally introduced to Japan as the wine cultivar ‘French Hybrid’, later used as a rootstock. Today, in Japan, almost all of the large-berry tetraploid grape cultivars are cultivated by grafting them on diploid rootstocks. However, when tetraploid cultivars are grafted on these diploid rootstocks, the vines show vigorous growth and often flower shattering (Motosugi et al., 2007). In general, excessively vigorous cane growth induces poor berry coloration and reduces berry quality (Okamoto et al., 1991). To improve the quality of tetraploid grape cultivars, many cultural practices aim to suppress excessive cane growth through such means as enlargement of the canopy, light pruning (long cane pruning), decreased nitrogen application, fruit cluster thinning, shoot tipping, and application of chemical growth regulators. It has been reported (Imai, 2001; Imai et al., 1987; Okamoto and Imai, 1989) that controlling grapevine vigor by restricting the root zone could improve fruit quality. Thus, controlling the vigor of tetraploid grape cultivars with appropriate dwarfing rootstocks can be expected to improve berry coloration and quality.

Motosugi et al. (2002) developed a series of colchicine-induced tetraploid grape rootstocks and observed that tetraploid rootstocks have shorter and thicker roots and shorter shoots, which result in more compact vines than the original diploid rootstocks. Moreover, ‘Kyoho’ grapevines grafted on the colchicine-induced ‘Couderc 3309’ (Vis. riparia × V. rupestris) and ‘Riparia Gloire de Montpelier’ (Vis. riparia Michx.) autotetraploid rootstocks showed weaker growth, and the berries exhibited much deeper skin coloration than those grafted on the corresponding diploid rootstocks (Motosugi et al., 2007). In the present study, we investigated the grapevine growth and berry quality of ‘Ruby Roman’ scions grafted on the rootstocks ‘5BB’ and ‘Hybrid Franc’ (‘HF’) and their corresponding colchicine-induced tetraploid rootstocks.

**Materials and Methods**

**Grafting and management of grafted cuttings**

Scions of ‘Ruby Roman’ were collected from dormant canes of mature grapevines planted in the vinyl greenhouse of the Ishikawa Sand Dune Agricultural Experiment Station in February 2011. The canes of ‘5BB’ (‘5BB(2×)’) and ‘HF’ (‘HF(2×)’) rootstock and their colchicine-induced autotetraploids (‘5BB(4×)’ and ‘HF(4×)’) were simultaneously collected from the grapevines planted on the Kyoto Prefectural University Farm. The collected dormant canes were placed in vinyl bags and kept at 4°C until grafting. Scions were cut into approximately 5-cm lengths containing one bud, and rootstocks were cut into approximately 20-cm lengths with all buds removed. The cuttings were grafted using an omega-cut grafting machine (Wagner Pflanzen-Technik GmbH, Friedelsheim, Germany) on March 25, 2011. The grafted region was sealed by rapid dipping in melted paraffin, and the basal end of the rootstock was dipped in Oxyberon SL (Bayer Crop Science, Tokyo, Japan) to accelerate rooting. The grafted cuttings were placed upright in boxes filled with growth medium (sawdust 3; perlite 1; vermiculite 1) and kept in a greenhouse at 30°C and 80% relative humidity in order to promote root development and germination.

After two months of nursery incubation, the budding and rooting rates of the grafted cuttings were evaluated. The survival rate was represented by the percentage of grafted cuttings with simultaneous budding from the scion and rooting from the rootstock against the total of grafted cuttings. The number and total length of the primary roots growing directly from the rootstock were recorded, as well as the shoot length of the budding scion.

On May 31, 2011, 12 vigorous grafted plantlets for each type of rootstock were planted into 60 L polyethylene pots (50 cm in diameter, 30 cm in height) filled with sand and bark compost (2:1), and 20 g of readily available fertilizer (N:P:K = 16:10:14) was scattered on the pot surface. The pots were kept in a vinyl greenhouse.

**Cultivation management and the investigation of growth of the grafted grapevines in pot cultivation**

In the first growing season (2011), a single shoot derived from the ‘Ruby Roman’ scion was trained vertically to 1.5 m in height followed by subsequent horizontal trellising (Fig. 1). During the growing season, the lateral shoots were repeatedly pruned every two weeks at the first node, leaving only one leaf. All the pruned shoots were weighed, and the internode length and number of nodes were recorded. On July 21, August 19, and November 4, the length, number of nodes, and stem diameter at the second internode above the graft union of the primary shoot were recorded. In
November 2011, the primary shoot was pruned at 2 m in length, providing 50 cm vine as the fruiting mother shoot. The pruned vines were weighed. The weight of the live vines after pruning was calculated by extrapolation given the length and weight of the pruned shoots. The total length and weight of shoots grown during the whole growing season were calculated based on all of the pruned lateral shoots, pruned canes, and the live vines after pruning.

In April 2012, all of the germinating buds from the vertical vine were removed, and six new shoots from the horizontal vine were trained on the trellis at approximately 10-cm intervals (Fig. 1). All the shoots were top-pruned when they grew to 2.5 m. All lateral shoots were repeatedly pruned at the first node in the same manner as the previous year. The weight, length, and node number of all the pruned shoots were recorded. After defoliation in November, all canes were pruned to two nodes (spur-pruning). The weight, length, node number, and stem diameter at the basal internode of all the pruned vines were measured. The total length and weight of shoots grown during the whole growing season were calculated by the total of all pruned lateral shoots and pruned vines.

Cultivation management and the investigation of growth of the grafted grapevines in ground cultivation

In April 2013, the grafted grapevines were transplanted into the ground in the vinyl greenhouse. Nine grapevines for each type of rootstock were planted at 0.7-m intervals (Fig. 1). Six new shoots that budded and grew from the spurs for each grapevine were trained on the trellis and allowed to grow freely on the trellis without top-pruning during the growing season. All lateral shoots were repeatedly pruned as described above. Water was irrigated to keep the soil moist. No fertilizer was applied except 20 g of readily available fertilizer at transplanting. After defoliation in autumn, the vines were spur-pruned in the same manner as the previous year. The weight, length, and node number of all the pruned lateral shoots and vines pruned in autumn, and the stem diameter at the basal internode of the pruned vines were measured.

In 2014 and 2015, six new shoots were again allowed to grow on each grapevine, but they were top-pruned once they reached 2 m (Fig. 1). Other management, including lateral shoot pruning, irrigation, and fertilization, was conducted in the same manner as the previous year. In 2014, the growth of shoots during the whole growing season was also investigated as described above. Before the bloom, one flower cluster on each shoot was left and shaped in the conventional way, leaving the bottom 3.5 cm. At full bloom on May 25, 2014 and May 19, 2015, the flower clusters were dipped in a solution of 25 mg·L⁻¹ gibberellic acid (GA₃) and 5 mg·L⁻¹ forchlorfenuron (Kyowa Hakko Bio Co., Ltd., Tokyo, Japan) to induce seedlessness. Ten days after full bloom, the flower clusters were again treated with 25 mg·L⁻¹ GA₃ (second GA treatment). Two weeks after blooming, the clusters were thinned to leave two clusters on each grapevine, and the berries were thinned to leave 25 to 30 berries on each cluster. All clusters were then covered with special transparent plastic bags (‘Ruby Roman’ club, Ishikawa, Japan). The fruit clusters were harvested on August 25, 2014 and August 17, 2015. Pest management during the growth period was performed according to the cultivation guide for ‘Ruby Roman’ grapes edited by the ‘Ruby Roman’ workshop of Ishikawa Prefecture.

Investigation of root growth of grafted grapevines

In April 2013, three grapevines for each type of rootstock were removed from pots and the roots were washed to remove all soil. All roots were then separated and grouped by diameter into the following classes: <1 mm, 1–2 mm, 2–5 mm, and >5 mm. The roots were dried in an 80°C oven for 3 days, at which time the dry weight
of the roots was measured. For each diameter class of each type of rootstock, the correlation between dry weight and length was evaluated using dry roots of 10, 20, 30, 40, and 50 cm in length, and the root lengths for each group were estimated based on a calibration curve, except for the >5 mm diameter class where the roots were actually measured.

Investigation of berry quality and skin coloration

On August 25, 2014 and August 17, 2015, all the clusters were harvested, ten clusters for each rootstock were used to evaluate berry quality. The cluster weight, berry number per cluster, stem weight, and color chart values (using a special color index sheet for ‘Ruby Roman’) were evaluated. Afterwards, five berries in each cluster were randomly collected and used to measure the berry diameter, total soluble solid (TSS) content, and the acidity of the juice. TSS (“°Brix” was measured with a hand refractometer, and acidity was measured by titration with 0.1 N NaOH to a phenolphthalein endpoint. Titratable acidity (TA) content was expressed as g tartaric acid per 100 mL juice. For the analysis of anthocyanin content, three berries were randomly selected from each cluster (30 berries for each type of grapevine) and divided into five repetitions (six berries per repetition).

The development of berries at different growth stages was also investigated. 15 berries for each type of grapevine were collected from different clusters and randomly divided into three samples (replicates) on June 6, 16, and 24, July 4, 10, 14, and 24, and August 5 and 15, 2014. 30 berries were randomly collected and divided into five replicates on June 8, 18, 25, and 29, July 1, 3, 6, 10, 17, and 28, and August 7, 2015. The color chart value, berry diameter, berry weight, TSS content, and the acidity of the juice were evaluated.

Total anthocyanin content in the berry skin was measured as described by Shiraishi et al. (2007), with slight modification. One skin disc was collected from each berry with a cork borer (Φ = 8 mm). Anthocyanins were extracted in 50% (v/v) aqueous acetic acid for 24 h at 4°C in the dark, followed by filtration through a 0.45-μm PVDF filter (EMD Millipore, Darmstadt, Germany). The absorbance of the extract at 520 nm was measured with a spectrophotometer (Biospec-1600; Shimadzu, Kyoto, Japan). The total anthocyanin concentration was expressed as nmol·cm⁻² skin of equivalent of cyanidin 3-glucoside chloride standard (TOKIWA PHYTOCHEMICAL CO., LTD., Chiba, Japan).

Statistics

A one-way analysis of variance by Tukey’s test was conducted to detect the differences among the four types of rootstock. A two-way analysis of variance (ANOVA) was performed to analyze the significance of the main effects and interactions of the rootstocks and the ploidies.

Results

Budding and rooting in the nursery stage

After two months of nursery incubation, the germination and shoot growth of the scions on each rootstock differed in appearance. The shoots grafted on the two diploid rootstocks ['5BB(2×)' and 'HF(2×)'] were more vigorous than those grafted on the corresponding tetraploid rootstocks ['5BB(4×)' and 'HF(4×)'] (Fig. 2A). More normal plantlets with roots and shoots were observed on the grafted cuttings of the diploid rootstocks (Fig. 2B), whereas some abnormal plantlets, which rooted from the scion but not the rootstock, were observed in the grafted cuttings of the tetraploid rootstocks (Fig. 2C). The rooting rates for the ‘5BB(4×)’ and ‘HF(4×)’ rootstocks were 33% and 51%, respectively, both lower than those of their diploids [89% for ‘5BB(2×)’ and 95% for ‘HF(2×)’] (Table 1). The percentages for budding from the scion grafted on the two tetraploid rootstocks were also lower than those of the diploid rootstocks, particularly on the ‘5BB(4×)’ rootstock (49%). The survival rates of grafted cuttings for the diploid rootstocks were approximately 80% [82% for ‘5BB(2×)’, 79% for ‘HF(2×)’], double the rate of the tetraploid rootstocks [33% for ‘5BB(4×)’ and 40% for ‘HF(4×)’] (Table 1). The number and total length of primary roots in ‘5BB(2×)’ and ‘HF(2×)’, which had grown directly from the rootstock, were greater than...
those in ‘5BB(4×)’ and ‘HF(4×)’. Of the two diploid rootstocks, ‘5BB(2×)’ had more primary roots than ‘HF(2×)’, but root length in ‘HF(2×)’ was greater than in ‘5BB(2×)’. The shoot lengths of the scions grafted on the diploid rootstocks were also greater than those grafted on the tetraploid rootstocks. The shoot length of the scions in ‘HF(2×)’ was longer than that in ‘5BB(2×)’. Whether between the diploid and tetraploid rootstocks or between ‘5BB’ and ‘HF’ rootstocks, there were significant differences in the growth of both roots and shoots.

Growth of grafted grapevines in pot cultivation

In the first growing season (2011), most of the shoots from ‘Ruby Roman’ scions grew to over 2 m. The development of shoots grafted on ‘5BB(2×)’ was the fastest among the four types of rootstock and the shoots grafted on ‘5BB(4×)’ were the slowest (Fig. 3). Shoot growth was faster on the diploid rootstocks than on the corresponding tetraploid rootstocks. The total shoot length grown during the whole growing season, including the pruned lateral shoots and primary shoots on the ‘5BB(2×)’ rootstock was greater than that of ‘5BB(4×)’ (Table 2). The fresh weight of the total shoots on ‘5BB(2×)’ was the heaviest, whereas that on ‘5BB(4×)’ was the lightest among the four types of rootstock. However, there were no significant differences between the diploid and tetraploid ‘HF’ rootstocks in the total length and fresh weight of the shoots. The internode length and stem diameter of shoots in the ‘5BB(4×)’ rootstock also tended to have the lowest values.

In 2012, because all of the shoots were top-pruned at 2.5 m, there were no significant differences detected in total shoot length among the four types of rootstock. However, in the ‘5BB’ rootstocks, the shoot weight and internode length of scions grafted on ‘5BB(4×)’ were less than those on ‘5BB(2×)’. No difference was observed between ‘HF(2×)’ and ‘HF(4×)’ in shoot growth. On the other hand, the stem diameter between the two types of rootstock (‘5BB’ and ‘HF’) differed significantly when using a two-way ANOVA (Table 2).

Growth of grafted grapevines in ground cultivation

In 2013, two weeks after transplanting the grafted grapevines into the ground, the vine buds began to germinate. Because the transplanting was performed in April, root growth was relatively late, therefore, most of the shoots continued to grow until late July. Germination of buds on ‘5BB(2×)’ was later than on the other three rootstocks. On July 25, the number of shoots that grew to over 3 m long was more than 80%, with the exception of ‘5BB(4×)’. The longest shoot (4.9 m) was...
observed on the grapevine grafted on the ‘HF(4×)’ rootstock. The total length and weight of shoots on the ‘5BB(4×)’ rootstock grown during the whole growing season, including the pruned lateral shoots and grafted primary vines, were the lowest, and no significant difference was observed among ‘5BB(2×)’, ‘HF(2×)’, and ‘HF(4×)’ (Table 2). The internode length of the vines grafted on ‘5BB(4×)’ was also less than on ‘HF(2×)’ and ‘HF(4×)’, whereas the stem diameter of shoots on ‘HF(4×)’ was greater than those on ‘5BB(4×)’ (Table 2). According to the results of a two-way ANOVA, there were differences in length and weight of total shoots among rootstocks and among ploidies. Moreover, the internode length and stem diameter on the ‘HF’ rootstocks were greater than those on the ‘5BB’ rootstocks.

In the second year after transplanting (2014), because all the shoots were top-pruned at 2 m in order to improve the light conditions for the clusters, the differences in shoot length and weight were primarily the result of the pruned lateral shoots. On the ‘5BB(4×)’ rootstock, the total shoot length, as well as the total shoot weight, internode length, and stem diameter were the least, and the total shoot length on ‘HF(4×)’ was the greatest among the four types of rootstock. In the ‘5BB’ rootstocks, the vegetative growth of vines grafted on the diploid was more vigorous than that of the tetraploid; however, in contrast, in the ‘HF’ rootstocks, the vegetative growth of vines grafted on the tetraploid was more vigorous than that of the diploid (Table 2).

Table 2. Vegetative growth of ‘Ruby Roman’ grapevines grafted on the four types of rootstock in pot and in ground cultivation.

| Rootstock | Ploidy | Cultivation method | Year | Total shoot length (cm) | Total shoot fresh weight (g) | Internode length (cm) | Stem diameter (mm) |
|-----------|--------|--------------------|------|-------------------------|----------------------------|----------------------|------------------|
| 5BB       | 2×     | pot               | 2011 | 753.7 a                 | 310.2 a                    | 6.47 a               | 10.61 a          |
|           | 4×     |                   |      | 425.8 c                 | 134.3 c                    | 5.16 b               | 6.27 b           |
| HF        | 2×     |                   |      | 613.5 ab                | 222.2 b                    | 5.87 ab              | 9.33 a           |
|           | 4×     |                   |      | 538.2 bc                | 195.3 bc                   | 6.12 a               | 8.95 a           |
| ANOVA     |        |                   |      |                         |                            |                      |                  |
| Rootstock | NS     | NS                |      | NS                      | NS                         | NS                   | NS               |
| Ploidy    | **     | **                |      | **                      | **                         | **                   | **               |
| Interaction | **     | **                |      | **                      | **                         | **                   | **               |
| 5BB       | 2×     | pot               | 2012 | 2490.9 a                | 1346.2 a                   | 8.38 a               | 10.82 a          |
|           | 4×     |                   |      | 2254.2 a                | 1126.8 b                   | 8.07 b              | 10.20 a          |
| HF        | 2×     |                   |      | 2355.1 a                | 1197.6 ab                  | 8.22 a              | 11.22 a          |
|           | 4×     |                   |      | 2285.5 a                | 1193.5 ab                  | 8.19 ab             | 11.35 a          |
| ANOVA     |        |                   |      |                         |                            |                      |                  |
| Rootstock | NS     | NS                |      | NS                      | NS                         | NS                   | *                |
| Ploidy    | *      | *                 |      | *                       | NS                         | NS                   | NS               |
| Interaction | NS    | NS                |      | NS                      | NS                         | NS                   | NS               |
| 5BB       | 2×     | ground            | 2013 | 4844.9 a                | 2595.4 a                   | 8.90 ab              | 12.38 ab         |
|           | 4×     |                   |      | 3253.1 b                | 1598.4 b                   | 8.44 b              | 12.27 b          |
| HF        | 2×     |                   |      | 4810.9 a                | 2616.0 a                   | 9.14 a              | 12.47 ab         |
|           | 4×     |                   |      | 5195.5 a                | 2851.4 a                   | 9.25 a              | 13.08 a          |
| ANOVA     |        |                   |      |                         |                            |                      |                  |
| Rootstock | **     | **                |      | **                      | **                         | **                   | *                |
| Ploidy    | **     | **                |      | NS                      | NS                         | NS                   | NS               |
| Interaction | **    | **                |      | NS                      | NS                         | NS                   | NS               |
| 5BB       | 2×     | ground            | 2014 | 4309.6 b                | 2827.1 ab                  | 11.62 a             | 12.32 a          |
|           | 4×     |                   |      | 2640.0 c                | 1661.8 c                   | 10.69 b             | 11.31 b          |
| HF        | 2×     |                   |      | 4007.1 b                | 2533.3 b                   | 11.04 ab            | 12.30 a          |
|           | 4×     |                   |      | 5068.6 a                | 3282.1 a                   | 11.22 ab            | 12.53 a          |
| ANOVA     |        |                   |      |                         |                            |                      |                  |
| Rootstock | **     | **                |      | **                      | NS                         | NS                   | NS               |
| Ploidy    | NS     | NS                |      | NS                      | NS                         | NS                   | *                |
| Interaction | **    | **                |      | NS                      | NS                         | NS                   | NS               |

z All of the shoots grown in the whole growing season containing all the lateral shoots pruned every two weeks and primary new shoots.

y Different letters indicate significant differences among the four types of rootstock at P<0.05 by Tukey’s test (n = 12 in 2011 and 2012, n = 9 in 2013 and 2014).

x **, *, and NS indicate significance at P<0.01, 0.05, and no significance, respectively.
Root growth in pot cultivation

After two growing seasons in pot cultivation (2013), the roots of the grafted grapevines were investigated. Figure 4A shows the appearance of the roots of the four types of rootstock. It could be observed that the root growth of the ‘HF’ rootstocks, including ‘HF(2×)’ and ‘HF(4×)’, were more vigorous than those of the ‘5BB’ rootstocks. The roots of the three investigated ‘5BB(4×)’ grapevines were notably less than those of the other rootstocks. The length of the longest primary root in each dissected grapevine was measured, and those of the ‘HF(2×)’ grapevines were 250, 247, and 241 cm, respectively, they were longer than those of ‘HF(4×)’ (206, 204, and 199 cm), followed by ‘5BB(2×)’ (155, 146, and 145 cm), and ‘5BB(4×)’ (149, 141, and 137 cm). Although the ‘HF(2×)’ rootstock had the longest primary root, the number of primary roots in ‘HF(2×)’ was fewer than in ‘HF(4×)’ (data not shown).

The total root dry weight of the ‘HF’ rootstocks was higher than that of the ‘5BB’ rootstocks (Table 3). There was no difference in total root dry weight between the diploid and the tetraploid in both ‘5BB’ and ‘HF’; however, the total root length of ‘5BB(2×)’ was longer than in ‘5BB(4×)’. There was no difference in the total root length between ‘HF(2×)’ and ‘HF(4×)’. Total root length between the two rootstock species (‘5BB’ and ‘HF’), as well as between the two ploidies (diploid and tetraploid), was different according to the results of a two-way ANOVA (Table 3). In other words, the ‘HF’ rootstocks had a longer total root length than the ‘5BB’ rootstocks, whereas the diploid rootstocks had a longer total root length than the tetraploid rootstocks.

In order to examine the structure of the roots, we divided all roots into the following four groups based on diameter class: <1 mm, 1–2 mm, 2–5 mm, and >5 mm (Fig. 4B). It was found that between the diploid and the tetraploid, the ratio of root length to total root length was different for each group. The proportion of the length of thin roots with a diameter <1 mm in the two diploids [80.5% for ‘5BB(2×)’ and 81.4% for ‘HF(2×)’] was greater than in the two tetraploids [75.8% for ‘5BB(4×)’ and 78.0% for ‘HF(4×)’]. In contrast, the proportion of thick roots with diameters of 1–2 mm, 2–5 mm, and >5 mm in the two diploids [15.3%,
3.9%, and 0.3% for ‘5BB(2×)’, respectively; 14.1%, 4.1%, and 0.4% for ‘HF(2×)’ was lower than in the two tetraploids [18.4%, 4.9%, and 0.9% for ‘5BB(4×)’; 15.9%, 5.3%, and 0.8% for ‘HF(4×)’] (Table 3). In the <1 mm diameter class, the dry weight and root length of the ‘HF’ rootstocks were higher than those of the ‘5BB’ rootstocks. Moreover, the root length of the diploids was longer than that of the tetraploids according to the results of a two-way ANOVA.

Berry quality and berry skin coloration

During the berry development period in 2014 and 2015, berry weight, sugar content (TSS content), titratable acidity content (TA content), and total anthocyanin content were periodically measured. The average berry weight of the two types of ‘5BB’ tended to be higher than that of the two types of ‘HF’ rootstock, especially in 2015 (Fig. 5A). The veraison, at which point most berries had softened, occurred almost simultaneously in all of the types of rootstock: July 10 in 2014 and July 6 in 2015 (arrows in Fig. 5). TSS contents began to increase before veraison, whereas TA contents rapidly decreased after veraison. TSS content in the ‘5BB(4×)’ berries seemed to remain at a higher level than in the other berries (Fig. 5B); however, the TA content showed an opposite trend (Fig. 5C). Total anthocyanin content in the ‘5BB(4×)’ berry skin was consistently highest during the whole development period, in both 2014 and 2015. The anthocyanin content in the ‘5BB(2×)’ berry skin was less than in ‘HF(4×)’ and ‘HF(2×)’ at harvest in 2014; however, it was consistently higher than in the two types of ‘HF’ berry skin in 2015 (Fig. 5D).

Figure 6 shows the appearance of grape clusters from
the four types of grapevine in 2014. Only the clusters of ‘5BB(4×)’ began to redden on July 14. Ten days afterward, on July 24, most of the ‘5BB(4×)’ clusters had reddened further, whereas the other three types of berries had just begun to redden.

The berry qualities at harvest in 2014 and 2015 are presented in Table 4. The average cluster weight and berry number per cluster in ‘5BB’ grapevines were greater than those in ‘HF’ grapevines. More cracked berries were observed in ‘HF’ clusters. As a result, the berry number per cluster in the two types of ‘HF’ grapevines were less than in the two types of ‘5BB’ grapevines, despite the berries being thinned to approximately the same degree in early development. No differences were observed in cluster weight and berry number between the diploid and tetraploid in either ‘5BB’ or ‘HF’. Although there were no significant differences in berry weight among the four types of grapevine, a two-way ANOVA showed that the berry weight of ‘5BB’ was higher than that of ‘HF’ in both 2014 and 2015, and the berries of ‘5BB’ were bigger than those of ‘HF’ in 2015. The color chart value and total anthocyanin content of ‘5BB(4×)’ berries were significantly higher than the other types of berries in both 2014 and 2015. In 2015 in particular, differences in skin color among the four types of grape berries were further pronounced; ‘5BB(4×)’ berries had the highest anthocyanin content, followed by ‘5BB(2×)’, ‘HF(4×)’, and ‘HF(2×)’. In 2015, there were significant differences in the color chart value and anthocyanin content among rootstocks and ploidies according to the results of a two-way ANOVA, in brief, the berries on the ‘5BB’ rootstocks had deeper coloration than on the ‘HF’ rootstocks, whereas the berries on the tetraploid rootstocks had deeper coloration than on the diploid rootstocks. The TSS content of the ‘5BB(4×)’ berries was higher than that of the other types of berries in 2014. The juice acidity of grapes on ‘HF’ rootstocks was higher than that on ‘5BB’ rootstocks in both 2014 and 2015.

![Fig. 6. Comparison of the coloration of ‘Ruby Roman’ berries grafted on the four types of rootstock. Pictures show the representative grape clusters on July 14 (upper) and 24 (lower), 2014.](image)

| Rootstock | Ploidy | Year | Cluster weight (g) | Berry number per cluster | Berry weight (g) | Berry diameter (mm) | Color chart value | Total anthocyanin content (nmol·cm⁻²) | TSS content (%Brix) | TA content (g/100 mL) |
|-----------|--------|------|-------------------|-------------------------|-----------------|-------------------|------------------|--------------------------|-------------------|---------------------|
| 5BB       | 2×     | 2014 | 562.2 a             | 24.0 a                  | 23.2 a           | 32.6 a            | 6.5 b            | 28.16 c                  | 17.1 b            | 0.33 b               |
|           | 4×     |      | 529.9 ab            | 23.0 ab                 | 22.8 a           | 32.2 a            | 8.0 a            | 45.69 a                  | 18.3 a            | 0.31 b               |
| HF        | 2×     |      | 467.3 b             | 21.0 b                  | 21.7 a           | 32.2 a            | 6.7 b            | 35.26 b                  | 16.8 b            | 0.35 ab              |
|           | 4×     |      | 425.8 bc            | 20.0 b                  | 20.5 a           | 31.4 a            | 6.6 b            | 33.46 b                  | 16.5 b            | 0.38 a               |
| ANOVA     |        |      | Rootstock           | **                   | **               | NS               | NS               | NS                       | NS               | **                  |
|           |        |      | Ploidy             | NS                    | NS               | NS               | NS               | NS                       | NS               | NS                  |
|           |        |      | Interaction         | NS                    | NS               | NS               | NS               | NS                       | NS               | NS                  |
| 5BB       | 2×     | 2015 | 536.3 a             | 24.0 a                  | 21.2 a           | 32.7 a            | 7.0 ab           | 31.99 b                  | 16.6 a            | 0.38 c               |
|           | 4×     |      | 479.6 ab            | 23.0 ab                 | 20.4 a           | 32.5 a            | 7.9 a            | 40.83 a                  | 17.5 a            | 0.39 bc              |
| HF        | 2×     |      | 443.8 b             | 22.0 ab                 | 19.3 a           | 31.7 ab           | 5.1 c            | 16.78 d                  | 16.5 a            | 0.43 ab              |
|           | 4×     |      | 436.0 b             | 22.0 b                  | 19.5 a           | 31.3 b            | 6.6 bc           | 22.88 c                  | 17.1 a            | 0.43 a               |
| ANOVA     |        |      | Rootstock           | **                   | *                | **               | **               | NS                       | NS               | **                  |
|           |        |      | Ploidy             | NS                    | NS               | NS               | NS               | NS                       | NS               | NS                  |
|           |        |      | Interaction         | NS                    | NS               | NS               | NS               | NS                       | NS               | NS                  |

Different letters indicate significant differences among the four types of rootstock at $P<0.05$ by Tukey’s test ($n = 10$, except $n = 5$ for total anthocyanin content).

**, *, and NS indicate significance at $P<0.01$, 0.05, and no significance, respectively.
Discussion

Rootstocks may affect the growth of a scion vine by influencing its water potential, nutrient status, vigor, or stress tolerance, or by interactions between rootstock and scion cultivars, leading to changes in berry quality (Chou and Li, 2014). In this study, we investigated the effects of rootstocks on the vegetative growth and berry quality of ‘Ruby Roman’ grapevines by grafting them on to two rootstock species and their corresponding tetraploids. Although the growth of scions grafted on the vigorous rootstock ‘HF(2×)’ in the early nursery stage showed more vigor than those grafted on ‘5BB(2×)’, a semi-dwarf rootstock, vine growth on ‘5BB(2×)’ was not significantly different from those grafted on the ‘HF(2×)’ rootstock during the 4-year study period. These results indicate that rootstock vigor is not the only factor in determining scion vigor; compatibility between the rootstock and scion is also important.

Polyploid (triploid, tetraploid, or hexaploid) plants have been proposed for use as dwarfing rootstocks to control scion vigor in fruit tree crops. In citrus, many tetraploid somatic hybrid rootstocks (allotetraploids) have been commercially used for reducing tree size (Grosser and Chandler, 2000, 2003). Prassinos et al. (2009) reported that some dwarf cherry rootstocks were triploid. Webster (1996) found that the sweet cherry scion cultivar ‘Merchant’ grew less vigorously on a hexaploid than on a triploid ‘Colt’ rootstock. Polyploid organisms often show increased vigor and, in some cases, outperform their diploid relatives in several respects (Sattler et al., 2016) because polyploidization of chromosomes usually causes an increase in cell size. Consequently, polyploid individuals may exhibit larger organs (roots, leaves, fruits, etc.) than the corresponding diploid does. However, increased cell size does not always lead to an overall increase in the size of the whole plant. For example, tetraploid rootstocks have thicker and shorter roots with fewer thin roots than their diploid relatives; the total superficial area of roots, a principal factor dictating root absorbency, is smaller in tetraploids than in diploids, hence the reason why tetraploids can be used as dwarf rootstocks. In the present study, the ratios of the length of thick roots (with diameters of 1–2 mm, 2–5 mm, and >5 mm) to total root length were higher in the two tetraploid rootstocks than in their original diploid rootstocks. In contrast, the ratios of the length of thin roots (with diameters <1 mm) to total root length were lower in the tetraploids than in the diploids. Consequently, the ratios of total root dry weight to total root length in the diploids were less than in the tetraploids (Table 3).

Shoot and root growth of the grapevines grafted on ‘5BB(4×)’ consistently exhibited the lowest values among the four types of rootstock. However, the grapevines grafted on ‘HF(4×)’ did not show a decrease in vegetative growth compared to ‘HF(2×)’; rather, they sometimes showed the opposite effect. For example, in 2013, the longest vine growth (4.9 m) was observed on ‘HF(4×)’, and there were greater values in total shoot length and weight on ‘HF(4×)’ though significant differences were not observed among ‘5BB(2×)’, ‘HF(2×)’, and ‘HF(4×)’. Moreover in 2014, ‘HF(4×)’ grapevines had greater total shoot length and weight than ‘HF(2×)’ grapevines. Furthermore, internode length and stem diameter values for ‘HF(4×)’ in 2013 and 2014 were greater than those for ‘HF(2×)’, but not to a significant degree. It is not clear why the two tetraploids exhibited different effects on the regulation of scion vigor. At least, in the early nursery stage, the two tetraploid grafted cuttings were similar, and showed fewer roots, shoots, and a lower survival rate than their corresponding diploids. The different regulation effects of ‘5BB(4×)’ and ‘HF(4×)’ on scion vigor might be caused by the quantity of roots. The total root dry weight and length of the ‘HF(4×)’ rootstock were, respectively, 1.7 and 1.5 times greater than those of the ‘5BB(4×)’ rootstock. Based on these results, we considered whether an autotetraploid rootstock does not always reduce scion vigor, but in some cases may be able to promote scion growth. Although the mechanisms by which rootstocks regulate scion vigor remain unclear (Basile et al., 2003), root absorbance of water and nutrients is considered an important factor. The characteristics of the roots of tetraploid rootstocks may reduce water and nutrient absorption. Motosugi and Yamamoto (2000) reported that the tetraploid rootstocks ‘Riparia Gloire de Montpelier’ (4×) and ‘Couderc 3309’ (4×) showed a much lower flow rate of sap bleeding from a stem stump than did the corresponding diploid rootstocks. Moreover, the stem water potential of ‘Kyoho’ grapevines grafted on tetraploids, measured at midday, was lower than that of vines grafted on the original diploids (Yamaguchi and Motosugi, 2004).

Many studies about the relationship between vine vigor, berry quality, and skin anthocyanin content have been reported. Most of these studies concluded that a reduction of vine vigor could improve berry coloration and other qualities by maintaining a suitable balance between vegetative and reproductive growth (Bell and Henschke, 2005; García-Estévez et al., 2015; Keller et al., 2001; Wolf and Pool, 1988). In the present study, the grapes grafted on the ‘5BB(4×)’ rootstock, whose vines had the least vegetative growth among the rootstocks tested, showed the fastest berry development because they had maintained higher levels of TSS and anthocyanin content during the development period. In the harvested grapes, the anthocyanin content in the ‘5BB(4×)’ berry skin was significantly higher than in the other types of berry skin. Furthermore, TSS of ‘5BB(4×)’ berries in 2014 was significantly higher than that of the other types of berries.

Corso et al. (2016) reported that the berry ripening of
Cabernet Sauvignon (V. vinifera) grafted onto ‘M4’ [(V. vinifera × V. berlandieri) × V. berlandieri × ‘Resseguier n.1’], a medium-vigorous rootstock, was faster than that onto the highly vigorous rootstock ‘1103P’ (V. berlandieri × V. rupestris). Koundouras et al. (2008) and Gambetta et al. (2012) showed that the use of the high-vigor rootstock ‘1103P’ was associated with an extension of the vegetative cycle and a delay in ripening. In our present study, the berry development of ‘Ruby Roman’ grafted on the ‘5BB’ rootstock, a semi-dwarf rootstock, was faster than that on the vigorous rootstock ‘HF’. Moreover, the fruit quality at harvest on the ‘5BB(2×)’ rootstock tended to be better than on the ‘HF(2×)’ rootstock. For example, the cluster weight on four tested years. These results indicate that the relative sites trend. However, there were no notable differences in other words, it cannot be explained by simple competition for nutrients between the vegetative and reproductive organs. In some reports, the effect of rootstock on scion vegetative growth and berry development has been discussed at physiological and molecular levels (Corso et al., 2016; Gambetta et al., 2012). Rootstocks have gained attention as reliable regulators of vine mineral uptake and transport, exerting marked effects on leaf and cluster mineral composition, particularly with respect to potassium (K), magnesium (Mg), and iron (Fe) (Zamboni et al., 2016). Moreover, the role of the rootstock in the control of scion growth and reproductive activity via the modulation of hormone signaling pathways has been identified (Berdeja et al., 2015; Cookson and Ollat, 2013; Corso et al., 2016; Gregory et al., 2013). To elucidate the differences between the ‘5BB’ and ‘HF’ rootstocks in their mechanisms of regulation of scion growth and berry development, it would be interesting to investigate the physiological changes, particularly changes in endogenous hormones, in vegetative organs and berries in a future study.

It is necessary to discuss why the anthocyanin content in the ‘5BB(2×)’ berry skin was lowest in 2014. This might be because the 4 year-old grafted grapevines were too young for ‘5BB(2×)’ to bear fruit. Uehara (1995) had described how grapevines grafted on ‘5BB’ rootstock tended to be succulent in growth at a young age. It also could be explained by the fact that the shoots on ‘5BB(2×)’ showed the most vigorous growth in the first year of our study (2011). The fifth year after grafting (2015), the grapevines may have begun to mature causing the fruit quality to normalize. On the other hand, in our study, the anthocyanin contents in all types of berries harvested in 2015, except for ‘5BB(2×)’, were lower than those in 2014, especially in the two types of ‘HF’ berry. In considering some reasons for these results, one possibility is that the skin disc used in measuring the anthocyanin content was collected from different positions, in 2014, it was collected from the apex of the berry, while in 2015, it was collected from the side. The apex of the berry had a deeper coloration than the side, especially the ‘HF’ berries had less coloration in the side. Other reasons might be that the harvest time was a little earlier in 2015 (August 17) than in 2014 (August 25), or that the weather before veraison (from June 26 to July 10) in 2015 had a continuously lower temperature than in 2014. In fact, the increase of TSS content and the decrease of TA content before veraison were slower in 2015 than in 2014. These reasons could also explain why the TSS content of most of the harvested berries in 2015 was lower than those in 2014, and the TA content in 2015 was higher than in 2014. The anthocyanin content in all of the types of berries showed almost no increase until August 5 in 2014; however, they continuously increased from July 17 in 2015. It might also be because the skin samples were collected from different positions in 2014 and 2015. In 2015, there were significant differences in skin coloration among the four types of berries; berry skin colorations on the ‘5BB’ rootstocks were deeper than those on the ‘HF’ rootstocks, whereas it was deeper on the tetraploid rootstocks than on the diploid rootstocks. Remarkably, the anthocyanin content of the ‘HF(4×)’ berry skin was significantly higher than that of the ‘HF(2×)’ berry skin in 2015, although the vine growth on ‘HF(4×)’ was more vigorous than that on ‘HF(2×)’ in 2014. We could not confirm the relationship between the better coloration of the berries and more vigorous vegetative growth of the ‘HF(4×)’ grapevine, further investigation and study would be necessary in the future. In 2015, TSS content on the tetraploid rootstocks was significantly higher than that on the diploid rootstocks according to a two-way ANOVA. On the other hand, the TA contents in the two types of ‘5BB’ berries were significantly lower than those of the ‘HF’ berries.

In conclusion, in our present study, we observed that the roots of ‘Ruby Roman’ grapevines grafted on the two types of tetraploid rootstock had thicker roots and fewer thin roots than their corresponding diploid rootstocks. Vine growth on the ‘5BB(4×)’ rootstock was inhibited, while berry quality, including sugar content and skin coloration, were simultaneously improved, but these changes were not observed in the grapevines grafted on the ‘HF(4×)’ rootstock. However, because the growth and vigor of the grapevines grafted on the ‘5BB(4×)’ rootstock were weaker, their tolerance to both biotic and abiotic stresses may be decreased, and their long-term fruit production potential is not known. In addition, given that the survival rate of ‘5BB(4×)’
grafted cuttings in the initial stage of grafting and rooting was low, careful consideration and further investigation are appropriate before introduction of the tetraploid rootstock into practical production.

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