Comparison of the growth and leaf mineral concentrations between three grapevine rootstocks and their corresponding tetraploids inoculated with an arbuscular mycorrhizal fungus *Gigaspora margarita*

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Summary

Effects of the arbuscular mycorrhizal (AM) fungus Gigaspora margarita BECKER and HALL on growth and leaf mineral concentrations of the tetraploid grapevine rootstocks Gloire de Montpellier (Gloire, Vitis riparia MICHX.), Rupestris St. George (St. George, V. rupestris SCHEELE), and Couderc 3309 (3309, V. riparia x V. rupestris) were compared with those of their corresponding diploids. The percentage of AM infection in the inoculated tetraploid grapevines of each rootstock was as high (above 90 %) as in the inoculated diploids. Shoot and root growth in the inoculated grapevines of each tetraploid was significantly higher than that in the non-inoculated grapevines. For the original diploid rootstocks, almost the same shoot and root growth was observed regardless of inoculation. Tetraploid and diploid rootstocks with AM fungi-inoculation had significantly higher P concentrations in the leaves than the non-inoculated grapevines, but tetraploid grapevines with AM fungi-inoculation had lower Ca and Mg concentrations. The tetraploid grapevines with thicker roots and more compact root systems were considered to depend more on arbuscular mycorrhizas than the original diploid rootstock cultivars.

K e y w o r d s: grapevine rootstock, tetraploid, arbuscular mycorrhizal (AM) fungus, *Gigaspora margarita*, shoot growth, root growth, P concentration.

Introduction

In Japan, table grape production with large-berry tetraploid grapevine cultivars such as Kyoho and Pione (hybrids of *Vitis vinifera* L. and *V. labruscana* Bailey, tetraploids) has been greatly increased to more than 40 % of the total grape production area in 1999 (MAFF JAPAN 2000). These tetraploid grapevines which are generally grafted onto phylloxera-resistant rootstocks face, however, problems in flower fertility and berry coloring, which are due to their high vigor in the hot and humid climate. Therefore, control of vigor by appropriate rootstocks could improve berry quality. We reported that own-rooted autotetraploid *Vitis labruscana* grapevines, which were derived from spontaneous sports, have shorter shoots and roots but larger root diameter than the original diploid cultivars (Motosugi 2000). Colchicine-induced autotetraploid rootstock cultivars also show less vigorous shoot growth than the original diploid ones (Motosugi *et al.* 2001). Autotetraploid grapevine rootstocks have relatively thicker and shorter roots than the original diploid rootstocks, the root system of tetraploids being very coarse and compact (Motosugi *et al.* 2001). The growth of Kyoho grapevines grafted onto these autotetraploid rootstocks (Motosugi *et al.* 1999). Therefore, these tetraploid rootstock cultivars seem to have a potential to reduce vigor of tetraploid table grape cultivars.

The coarse and compact root system of tetraploid grapevines which is considered to reduce the contact area between root and soil may lower the ability for uptake of water and nutrients from soil. EISSENSTAT (1992) suggested that plant species with thick roots tended to have a higher dependency on mycorrhiza than species with thin roots. Thus, infection with arbuscular mycorrhizal (AM) fungi may be very effective to regulate growth and the ability for water and nutrient uptake in tetraploid rootstocks. There is, however, no information on the arbuscular mycorrhizal infection rate of roots and its effect on growth of tetraploid grapevines.

The AM fungi of the Glomus genera have been used in studies on the relationship between grapevines and AM fungi (MENGE et al. 1983; SCHUBERT et al. 1988, 1990; SCHELLENBAUM et al. 1991; BAVARESCO et al. 1995; KARAGIANNIDIS et al. 1995, 1997; BAVARESCO and FÖGHER 1996; BIRICOLTI et al. 1997; PETGEN et al. 1998; KARAGIANNIDIS and NIKOLAOU 2000; LINDERMAN and DAVIS 2001). However, there are only a few reports on the artificial inoculation with Gigaspora spp. to grapevines (KARAGIANNIDIS and NIKOLAOU 2000; LINDERMAN and DAVIS 2001). Gigaspora spp. produce very large spores (>200 µm) with a high infectivity to plant roots (INVAM 1993). In our studies on Citrus, the inoculation with Gigaspora ramisporophora SPAIN which has been identified recently as Gi. margarita BECKER and HALL promoted growth and improved fruit quality of Satsuma (Citrus unshiu MARC.) grafted onto trifoliate orange (Poncirus trifoliata RAF.) rootstock as well as inoculation with Gl. ambisporum

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SMITH and SCHENCK, *Gl. fasciculatum* (THAXTER) GERDEMANN and TRAPPE and *Gl. mosseae* (NICOLSON and GERDEMANN) GERDMANN and TRAPPE (SHRESTHA *et al.* 1995, 1996). We observed that *Gi. margarita* produced much longer hyphae and showed more stable infectivity of the roots of many crops and fruit trees in fields and orchards in Japan than *Glomus* spp. Therefore, we chose spores of *Gi. margarita* as inoculum for this study and examined whether infection with *Gi. margarita* may enhance growth and improve nutrient uptake of tetraploids of three grapevine rootstocks compared with the corresponding diploid rootstocks.

Material and Methods

The micropropagated rootstocks Gloire de Montpellier (Gloire, Vitis riparia MICHX.), Rupestris St.George (St.George, V. rupestris Scheele), and Couderc 3309 (3309, V. riparia x V. rupestris) and their corresponding autotetraploids induced by colchicine treatment, Gloire(4x), St.George(4x) and 3309(4x), were transplanted into a polyethylene pot filled with vermiculite and acclimated in 1999. After leaf fall, the grapevines were pruned to 4-5 nodes and stored in a refrigerator at 5 °C for several months. Thereafter the grapevines were transplanted individually into plastic pots (diameter: 24 cm) filled with a potting mixture of vermiculite:perlite:zeolite (2:1:1, v/v/v) and transferred to a greenhouse on June 23, 2000. Half of the tetraploid and original rootstocks were inoculated with the AM fungus Gigaspora margarita (Central Glass Co. Ltd., Tokyo, Japan) on July 7 by mixing per pot 3 g soil containing approximately 60 spores with the potting mixture. The other half of the grapevines were left non-inoculated as control. Both inoculated and non-inoculated treatments were replicated 4 times. All grapevines were supplied biweekly with 100 ml per plant of 1/500 strength of a commercial liquid fertilizer, HYPONeX^R (N- P_2O_5 - K_2O_5) 5-10-5; 10 mg N, 8.7 mg P and 8.3 mg K per 100 ml diluted solution) (HYPONeX Japan Co. Ltd., Tokyo, Japan) and watered properly. The grapevines were allowed to grow without any training and trimming of shoots. They were dug from pots on August 19, and the fresh weight of shoot and root was determined. After cutting the rootlets into 2 cm segments, these segments were immediately fixed in FAA (formalin:acetic acid:50 % ethanol, 13:5:200, v/v/v). Ten segments per treatment of each rootstock were stained (PHILLIPS and HAYMAN 1970), and under a light microscope the rate of root infection by AM fungi was estimated according to the method of Ishii and KADOYA (1994). The percentage of AM infection was calculated by the following equation:

> AM infection (%) = (Root length infected / Root length observed) x 100

To determine N, P, K, Ca and Mg concentrations leaves were freeze-dried and ground. The ground tissue was digested with sulfuric acid and hydrogen peroxide. Concentrations of N and P in the digested samples were measured by the indophenol and the vanado-molybdate phosphoric yellow color methods, respectively (JSSSPN 1990). Concentrations of K, Ca and Mg were analyzed by using an atomic absorption spectrometer (model 170-30, Hitachi Ltd., Tokyo, Japan). Data were tested by two-factor (rootstock and AM inoculation) analysis of variance (ANOVA) among each tetraploid and the original diploid rootstock.

Results and Discussion

Due to the long interval (biweekly) of nutrient supply, the amounts of supplied nutrients were relatively low in this study. Therefore, the percentage of AM infection in the inoculated roots of each tetraploid was as high (>90 %) as that in the inoculated roots of the original diploid rootstocks (Tab. 1). No visible AM infection was observed in the noninoculated roots of tetraploids and diploid rootstocks. On inoculated roots of tetraploid grapevines, AM fungi penetrated the roots, elongated hyphae at the outer part of roots and in the intercellular tissue even near the growing tips (Fig. 1 A), in the same manners as at inoculated roots of diploid rootstocks. Numerous spores developed at the root (Fig. 1 B) and many arbuscules were formed in the cortical root cells (Fig. 1 C). These observations demonstrate the symbiosis between the AM fungus Gi. margarita and the roots of the tetraploid and diploid rootstocks. The extent of root colonization of the three tetraploid and diploid rootstocks by the AM fungus was not significantly different, although KARAGIANNIDIS et al. (1997) and LINDERMAN and DAVIS (2001) reported significant differences of root colonization among different rootstocks. In our study, the fungus species was different from their experiments with Glomus spp. and Gi. rosea, which might have resulted in a lack of variance of the AM infection rates between rootstocks.

Table 1

Arbuscular mycorrhizal (AM) formation at colchicine-induced tetraploid and diploid rootstocks 6 weeks after *Gigaspora margarita* inoculation

		AM infection ^a , %				
Rootstock	Treatment	2x ^b	4x ^b			
Gloire	non-inoculated	0.0	0.0			
	inoculated	$88.8 \pm 5.3^{\circ}$	97.6 ± 1.6			
3309	non-inoculated	0.0	0.0			
	inoculated	88.2 ± 3.2	95.7 ± 1.9			
St.George	non-inoculated	0.0	0.0			
C	inoculated	93.1 ± 1.7	91.9 ± 2.3			

^a % of AM infection = (the length of infected roots per total length) x 100.

^b 2x-diploid, 4x-tetraploid.

^c Standard error; n=4.

In non-inoculated grapevines, tetraploid rootstock cultivars showed much less vigor than the original diploid rootstocks (Fig. 2, Tab. 2) as was reported previously (MotosuGi *et al.* 2002). The tetraploid grapevines had coarse root systems compared to the diploid cultivars (Fig. 2). For the diploid rootstocks, no differences in shoot growth were observed regardless of the AM inoculation (Tab. 2). The

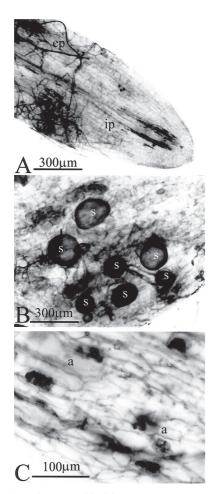


Fig. 1: The arbuscular mycorrhizal fungus *Gigaspora margarita* in roots of tetraploids rootstocks. **A**: Fungal hyphae in a root of 3309 (4x). **ep**: external hyphae, **ip**: intraradical hyphae. **B**: Newly formed spores at the surface of a root of St. George (4x). **s**: spore. **C**: Arbuscules in the cortical cells of a root of St. George (4x). **a**: arbuscule.

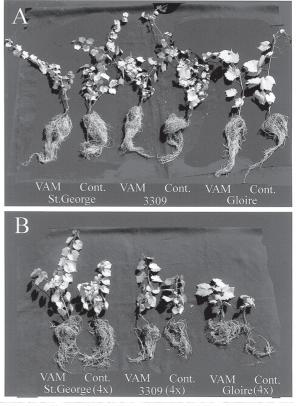


Fig. 2: Effects of the arbuscular mycorrhizal fungus *Gigaspora margarita* on the growth of grapevine rootstock cultivars (**A**) and their tetraploids (**B**). VAM: inoculated, Cont.: control.

inoculated grapevines of each tetraploid rootstock, however, showed significantly enhanced shoot and root growth compared to the non-inoculated grapevines, especially in Gloire(4x) and 3309(4x) (Fig. 2 B, Tab. 2). Therefore, the difference in growth between tetraploids and the corresponding diploids were markedly reduced by AM inoculation

$T\ a\ b\ l\ e\ 2$

Effects of the arbuscular mycorrhizal (AM) fungus *Gigaspora margarita* on growth of colchicine-induced tetraploid and diploid rootstocks

	AM	Shoot fresh weight (g)		Root fresh	weight (g)	Shoot/root ratio	
Rootstock		2x ^a	4x ^a	2x	4x	2x	4x
Gloire	non-inoculated	11.98	2.71	18.63	4.56	0.64	0.62
		(1.14) ^b	(0.53)	(1.57)	(0.93)	(0.02)	(0.12)
	inoculated	11.76	7.49	17.18	11.31	0.72	0.67
		(1.11)	(0.38)	(3.04)	(1.03)	(0.07)	(0.04)
3309	non-inoculated	20.32	5.67	26.82	7.24	0.76	0.85
		(1.85)	(1.47)	(1.86)	(2.34)	(0.05)	(0.08)
	inoculated	19.79	15.10	21.44	12.91	0.93	1.10
		(2.41)	(2.78)	(2.62)	(2.65)	(0.08)	(0.14)
St.George	non-inoculated	16.23	10.54	33.03	16.05	0.49	0.68
		(1.10)	(1.85)	(2.09)	(2.96)	(0.01)	(0.06)
	inoculated	17.59	16.19	29.53	18.12	0.59	0.91
		(2.36)	(2.21)	(3.35)	(2.72)	(0.03)	(0.05)
ANOVA ^c	rootstock	* * *	* * *	* * *	* *	* * *	* *
	AM	NS	* * *	NS	*	* *	*
	interaction	NS	NS	NS	NS	NS	NS

^a 2x-diploid, 4x-tetraploid. ^b Standard error; n=4. ^c Significant at 0.1 % (***), 1 % (**), 5 % (*) or not significant (NS).

(Tab. 2). It is suggested that the growth of the tetraploids with thicker roots, especially in Gloire(4x) and 3309(4x), is more dependent on arbuscular mycorrhizas than that of the diploid rootstocks. The effect of AM inoculation on growth was relatively small in St. George(4x), which had the largest total weight among the three non-inoculated tetraploids. Among the diploid rootstocks, St. George is also known to be the most vigorous rootstock (GALET 1979). Since increase of the growth of tetraploid grapevines by AM infection was generally larger, the water and nutrient uptake of the tetraploid grapevines is considered to have higher mycorrhizal dependency than that of the diploid rootstocks. Shoot/root ratios in tetraploids and diploid rootstocks were generally increased by AM infection (Tab. 2). This suggests that AM fungi-infected roots are more efficient in the uptake of water and nutrients. SCHELLENBAUM et al. (1991) demonstrated that the larger growth of grapevine roots due to mycorrhizal formation was caused by an increase of lateral root number and consequently total root length but did not alter the number of root axes. They also mentioned that the grapevines with mycorrhizal formation developed a more economic root system than those without mycorrhizal formation. For grapevines infected with Gi. margarita, especially tetraploids, the enlargement of the rhizosphere by AM fungi contributes to improve the efficiency of water and nutrient uptake.

The inoculation with *Gi. margarita* significantly increased P concentration in the leaves of tetraploid and diploid rootstocks (Tab. 3). N and K concentrations in the leaves of tetraploid grapevines were much higher than those of diploid rootstocks but were not influenced by AM fungi inoculation (Tab. 3). The inoculation tended to decrease concentrations of Ca and Mg in the leaves of tetraploid but not in diploid rootstocks (Tab. 3). Thus, total P uptake of tetra-

ploid and diploid rootstocks was increased by *Gi. margarita* infection. As the increase of P levels in leaves did not affect growth, other factors such as N might have been limiting. BIRICOLTI *et al.* (1997) reported that AM infection increased P levels but reduced Mg levels and leaf chlorophyll contents in leaves of 5BB rootstock when studying the interaction between AM infection and lime. On the other hand, in 101-14 rootstocks, which are more susceptible to lime-induced chlorophyll content as well as foliar P levels (BAVARESCO *et al.* 1995; BAVARESCO and FÖGHER 1996). Responses of tetraploid rootstocks to AM infection on calcareous soils need further studies.

SANCHES-DIAS and HONRUBIA (1994) suggested that AM infection might increase drought resistance of plants by several mechanisms, including increased water uptake. It is demonstrated that the infection with Gi. ramisporophora or Gi. margarita increased the photosynthetic and transpiration rate of leaves and tree growth of Satsuma (C. unshiu) under water stress grafted onto trifoliate orange rootstock (SHRESTHA et al. 1995, 1996). In the present study, increasing water uptake due to hyphal extraction of soil water may have resulted in the enhanced growth of the tetraploid grapevines inoculated with an AM fungus (Gi. margarita). The inoculation with Gi. ramisporophora also improved fruit quality of C. unshiu grafted onto trifoliate orange rootstock (SHRESTHA et al. 1996). Inoculation with Gi. margarita may also improve growth and fruit quality of grapevines on rootstocks.

In orchard soils in Japan, although the formation of arbuscular mycorrhiza is severely decreased by high amounts of agrochemicals and chemical fertilizers, there are some indigenous AM fungi (ISHII *et al.* 1989). ISHII *et al.* (2000) dem-

Table 3

Effects of the arbuscular mycorrhizal (AM) fungus *Gigaspora margarita* on the N, P, K, Ca and Mg concentrations (% dry weight) in leaves of colchicine-induced tetraploid and diploid rootstocks

Rootstock	AM	Ν		Р		Κ		Ca		Mg	
		2x ^a	4x ^a	2x	4x	2x	4x	2x	4x	2x	4x
Gloire	non-inoculated	1.64	2.79	0.27	0.36	0.96	1.27	0.98	0.86	0.48	0.48
		(0.19) ^b	(0.50)	(0.04)	(0.06)	(0.11)	(0.15)	(0.09)	(0.13)	(0.04)	(0.02)
	inoculated	1.48	2.32	0.39	0.40	0.91	1.26	0.95	0.47	0.45	0.39
		(0.12)	(0.13)	(0.04)	(0.04)	(0.07)	(0.04)	(0.11)	(0.02)	(0.01)	(0.01)
3309	non-inoculated	1.12	3.07	0.22	0.32	0.79	1.41	0.70	0.79	0.43	0.55
		(0.04)	(0.43)	(0.02)	(0.04)	(0.05)	(0.18)	(0.04)	(0.13)	(0.01)	(0.03)
	inoculated	1.60	2.61	0.32	0.44	0.97	1.42	0.80	0.62	0.44	0.49
		(0.15)	(0.47)	(0.02)	(0.07)	(0.07)	(0.20)	(0.04)	(0.13)	(0.02)	(0.01)
St.George	non-inoculated	1.36	1.81	0.27	0.27	0.81	0.95	0.87	0.59	0.52	0.55
		(0.12)	(0.28)	(0.05)	(0.01)	(0.07)	(0.11)	(0.05)	(0.03)	(0.02)	(0.02)
	inoculated	1.23	2.28	0.31	0.36	0.79	1.12	0.75	0.53	0.48	0.53
		(0.10)	(0.31)	(0.02)	(0.02)	(0.02)	(0.05)	(0.07)	(0.07)	(0.03)	(0.02)
ANOVA ^b	rootstock	NS	NS	NS	NS	NS	*	*	NS	*	* * *
	AM	NS	NS	* *	*	NS	NS	NS	*	NS	* *
	interaction	*	NS	NS	NS	NS	NS	NS	NS	NS	NS

^a 2x-diploid, 4x tetraploid. ^b Standard error; n=4.

onstrated that the application of AM stimulatory compounds, *e.g.* extract of orange juice pomace was very effective for the activation of both the inoculated and the indigenous AM fungi. ISHII *et al.* (1996, 2000) also reported that the sod culture by using Bahia grass (*Paspalum notatum* FLÜGGE.) and *Vulpia myuros* (L.) C. C. GMEL. in the *C. unshiu* orchard increased the mycorrhizal formation in the root as compared with bare soil. The application of AM stimulating compounds and/or sod culture is considered to introduce the beneficial effect on growth and berry quality of grapevines grown on soils poor in mycorrhizal fungi. We are currently investigating the effects of the sod culture system with *Vulpia myuros* on mycorrhiza formation and growth of Kyoho grapevines grafted onto the tetraploid and diploid rootstocks.

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