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Original Research Article

The Effects of Crown Gall Resistant Rootstocks on the Growth, Yield and Fruit Quality of Thompson Seedless Grapevine (*Vitis vinifera* L.) cv

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Abstract

The current investigation was conducted during the five successive seasons of 2008 and 2014 on six years, young vines of Thompson Seedless cv. grafted either on NAZ4 or NAZ6 rootstocks, to evaluate their effects on bud burst, fruit quality, and yield grown in a sandy loam soil, spaced at 2×3 meters apart, irrigated by the drip irrigation system, cane-pruned and trellised by the bilateral cordon system in the open field. In addition, the same cultivar was grown on own roots and served as control. Data showed that, grafting on NAZ6 resulted in a significant higher value of yield, cluster weight, some physical properties of berries and gave the highest percent of fruitful buds as well as the minimum vegetative bud percent followed by own-rooted cuttings. Grafting on NAZ4 increased leaf area and cane thickness. Chemical berry characteristic including TSS percentage, low acidity and high TSS/acid ratio were generally noted with Thompson seedless grafting on NAZ6 rootstock. Thompson seedless cultivar recorded higher fruiting bud percentage with ungrafted vines compared with the other rootstocks. Meanwhile, highest bud burst percentage was obtained when Thompson seedless cultivar grafted onto NAZ4 rootstock. Generally, yield, cluster and berry characteristics of own rooted vines of Thompson seedless were best when compared with vines grafted onto NAZ4 rootstocks under this study.

Keywords

Crown gall

Grafting

Resistance

Rootstocks

Vitis vinifera

Introduction

A number of diseases caused by bacterial, fungal, and viral pathogens have resulted in significant reductions in the yields of grape (*Vitis* spp.) (Bavaresco et al., 2003; Montesinos, 2007; Walker et al., 2000). Crown gall disease of grapevines caused by *Rhizobium vitis* (formerly *Agrobacterium vitis*) causes severe economic loss via reduced fruit productivity and mainly occurs during early summer in Iran (Mahmoodzadeh, 2001). Although there have been great increases in our understanding of the infection mechanism of *R. vitis* and ability to control crown gall in grapevines, few strategies are available for the efficient management of this disease in vineyards. *R. rhizogenes* strain K84 was commercially applied to control crown gall in peach trees as a biological control agent (Kawaguchi, 2013); however, it is not effective at preventing grapevine infections caused by *R. vitis* (Chen et al., 2007). There is no effective control method at present. Vines that are free of crown gall can be infected when planted in soil with debris remaining from a vine that was infected with *A. vitis* (Burr et al., 1998). Therefore, the best way to prevent crown gall in the vineyard is to prevent the site from being contaminated with infected plants from the beginning (Goodman et al., 1993). *Vitis vinifera* cultivars are especially susceptible to crown gall and phylloxera (Pongrácz, 1993). One way to avoid problems with these diseases is to graft *V. vinifera* to resistant rootstocks (Mahmoodzadeh et al., 2004). Studies have shown that some rootstocks such as 3309C, 101-14 Mgt, and Riparia Gloire that have been used for phylloxera resistance may also provide resistance to crown gall (Sule and Burr, 1998).

In one study, crown gall susceptible *Vitis vinifera* vines grafted on Gloire rootstocks were infected with *A. vitis,* and still developed symptoms after 3-4years, but they were able to recover compared to non-grafted *V. vinifera* (Yokotsuka et al., 1999). Thompson Seedless as a promising grapevine cultivar that grown successfully under Iran environmental condition and has progressively developed in the last few years, but this cultivar is very sensitive to crown gall disease (Mahmoodzadeh, 2001). Therefore, grafting on some rootstocks is a good solution to overcome such problems. The importance of rootstocks in viticulture is well documented, they are used not only as an effective means of controlling important biological pests such as phylloxera and nematodes, but they can also be used effectively to regulate nutrient exclusion, uptake of water in the vine (Keller, 2001; Walker et al., 2002).

In addition, Reynolds and Wardle (2001) outlined seven major criteria for rootstocks choice in the order of their importance as phylloxera resistance, nematode resistance, crown gall resistance, adaptability to high pH soils, saline soils, low pH soils, wet or poorly drained soils and drought. Many investigations proved that rootstocks affect vine growth, yield, fruit quality through the interactions between the environmental factors and the physiology of scions and rootstock cultivars employed (Gaser, 2006; Ozden et al., 2010; Zhiyuan, 2003). Factors should be considered when selecting a rootstock-scion combination for a specific site (Kocsis and Lehoczky, 2002; Whiting, 2004; Zhiyuan, 2003). The rootstock St. George has been reported to be vigorous, resulting in low bud fruitfulness per unit of growth (Ollat, 2009) and fewer numbers of berries per cluster (Whiting, 2004). Fruit quality and maturity also were reported to be affected by the rootstock (Prior et al., 1993; Mahmoodzadeh et al., 2004; Zerihun and Treeby, 2002). Rootstocks affect vine growth, yield, fruit quality, cluster weight, berry size and soluble solids content (Bavaresco et al., 2003; Nikolaou et al., 2003; Whiting, 2004). In one study, NAZ4 was classified as vigorous stocks, while NAZ6 was classified the low vigorous (Mahmoudzadeh and Dovlatibaneh, 2008). However, in another study, NAZ1, NAZ3, NAZ5 rootstocks and some local grape cultivars in Iran were classified as lower yielding rootstocks with low cluster number and weight, and berry weight (Mahmoodzadeh, 2001). The aim of the present investigation is to evaluate Thompson Seedless grapevine cultivar grafted on the two crown gall disease resistant rootstocks comparing with the own rooted of this cultivar on some plant yield, physical and chemical parameters of growth and fruiting such as bud burst ratio, fruiting bud, cluster and berry characteristics.

Materials and methods

Genetic background of material

Two interspecific hybrids of grapevine were used as resistant rootstocks to crown gall disease (Table 1) (Mahmoodzadeh et al., 2004). One-bud scions taken from Thompson Seedless grapevine as sensitive vines without visible symptoms of crown gall (Table 1) were hand-whip grafted onto 40 cm long cuttings of dormant rootstocks during September 2008 as described by Chadha and Shikhamany (1999). Rooted cuttings of the same vines were used as the control treatment. Cuttings and scions were taken from the Grapevine Collection of Viticulture Research Section of Kahriz Horticultural Research Station, Urmia, Iran.

Table 1. List of the studied grape-vine genotypes

Hybrids as rootstocks*	Scion or self-rooted cultivar
NAZ4: *V. vinifera* cv. 'Jighjigha' × Riparia Gloire NAZ6: *V. vinifera* cv. 'Gharaozum' × Kober 5BB	*V. vinifera* cv. 'Thompson seedless'
*Mahmoodzadeh et al. (2004).	

Trial site, vineyard establishment and design

For callus formation, the scions and the cuttings were maintained in a hot-room (28±2ºC) and humidity (75-85% RH) for four weeks. Grafts and cuttings were potted separately into polyethylene bags with a diameter of 15 cm containing a mixture of loam soil, Perlite and Compost (1:1:2 in volume) and transferred to a greenhouse (20 to 28ºC) for 2 months. The grown grafts and ungrafted cuttings were planted into the nursery for the first growing season. One-year-old bare rooted plants were planted 2×4 m apart in four replications (10 vines per replication) in field plots. Standard pruning, training and other cultural practices were done each year based on methods described by Goodman et al. (1993).

Observations were recorded on the appearance of the vigor of growth and viability of the vine in October of each year. The vines were cane pruned leaving six to eight nodes, depending on cane thickness. The first harvesting took place in March 2010. Experimental data was collected from the year 2010 onwards. Six vines of each stock: scion combination was selected in each replication, and there were four replications for each treatment.

Data collection

Yield and physical characteristics of bunches

Clusters were harvested in each season. Yield per vine (kg) were recorded. Four cluster/vine were pecked to determine average cluster weight (gm), cluster length and width (cm), then 100 berries/cluster were used to determine, average berry weight (g), berry length/ diameter Ratio and Juice volume of 100 berry (cm^3).

Physical characteristics of berries

Average berry weight (g), average berry size (cm^3) and average berry dimensions (length and diameter) (cm) were determined.

Some characteristics of vegetative growth

At growth cessation, the following morphological and chemical determinations were carried out on 4 shoots / the considered vine:

Bud burst percentage was calculated by dividing bursted buds on total number of bud and multiplied by 100, Fruiting buds percentage was calculated by dividing fruiting buds on total number of bursted buds and multiplied by 100, Shoot diameter (cm), shoots length (cm), number of leaves/shoot, leaf area (cm^2) of the apical 5th and 6th leaves using a CI-203- Laser Area-meter made by CID, Inc., Vancouver, USA.

Total leaf area/vine (m^2) was determined by multiplying average number of leaves/shoot by average leaf area then by the number of shoots per vine. Weight of pruning's (Kg) at the dormancy period (winter pruning) was determined.

The number of shoots was counted prior to forward pruning and the cane diameter was measured between the fifth and sixth node at shoot maturity.

Fruit chemical properties

100 berries/sample were used to determine, Total soluble solids were measured using a hand-held temperature-compensated digital refractometer (ERMA, Japan), Total titratable acidity (as grams tartaric acid per 100 milliliters of juice) measured by titrating a known volume of juice with 0.1 NaOH using phenolphthalein as indicator. TSS/acid ratio was calculated.

Statistical analysis

Analyses of variance were performed using SAS statistical software (29). Student's 't' least significant difference (LSD) values were calculated at the 5% significance level to facilitate comparison between treatment means.

Results

The results of the experiment are presented and discussed in the following subsections.

Yield and physical characteristics of bunches

Yield, when averaged for three years of data, varied significantly among the rootstocks, with NAZ6 producing highest yield, NAZ4 producing intermediate yield, and own rooted vines producing the lowest yield (Table 2). Although there was an increase in the yield per vine from 2011 until 2014, the year 2012 saw a severe incidence of winter cold damage in the entire experimental block, resulting in the loss of more than 20% of the yield.

Table 2. Yield, cluster weight, length and width of cluster and some physical characteristics of berries of Thompson seedless as affected by different tested rootstocks during 2011-2014 seasons.

Rootstocks	Yield (kg/vine)	Number of bunches	Cluster weight (gm)	Length of cluster (cm)	width of cluster (cm)	Berry weight (gm)	Juice volume of 100 berry (cm^3)	Berries/ clusters
Own rooted	5.8c	16.8a	208.15b	29.4a	19.6a	1.31c	72.18b	83b
NAZ4	6.6b	14.25b	214.54a	28.17b	17.2b	1.45b	55.12c	75c
NAZ6	8.3a	16.9a	220.16a	29.2a	18.1a	2.1a	85.16a	89a
Significance	*	*	*	**	*	**	*	**
Means having the same letters within a column are not significantly different at 5% levels								

Table 3. Some chemical characteristics of berries of Thompson seedless as affected by different tested rootstocks during 2011-2014 seasons.

Rootstocks	TSS(ºB)	Total acidity (%)	TSS/TA ratio	pH	Vitamin C (mg/100gr)
Own rooted	23.25a	0.42c	55.35a	3.36a	8.5c
NAZ4	19.25c	0.68a	28.30c	3.33b	9.2b
NAZ6	21.00b	0.49b	42.85b	3.31c	11.25a
Significance	*	*	**	*	*
Means having the same letters within a column are not significantly different at 5% levels					

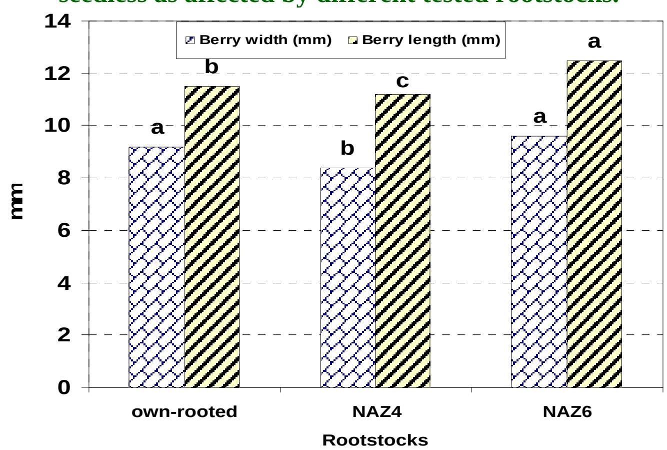
A greater number of bunches were recorded on NAZ6 rootstock during all the years of the study, with the fewest bunches being either on NAZ4 rootstock. Though the number of bunches differed significantly among rootstocks in the individual years of the study, the mean number of bunches did not differ significantly among the rootstocks (Table 2). However, average bunch weight differed significantly among the rootstocks. The greatest bunch weight was recorded on NAZ4 and NAZ6 rootstocks, while the smallest bunch weight was recorded on own rooted vines.

It is obvious from Table 2 that yield/vine, cluster weight, length and width were more pronounced when Thompson Seedless cv. grafted on NAZ6 than compared with Thompson Seedless on NAZ4 or own roots. It is worthwhile, to mention that NAZ6 rootstocks superiority and was given an increase in the yield / vine as well as the weight of a heavy cluster compared with control which marked decrease in the former data. It could be noticed that NAZ6 produced an intermediate effect in those after mentioned parameter in the three seasons under study.

Physical characteristics of berries

As shown in Table 2 and Fig. 1, it is obvious that all berry physical components i.e. berry weight, Juice volume of berry, length and diameter and also berries/cluster ratio were significantly affected by the kind of rootstock or own-rooted. Thompson Seedless vine grafted onto NAZ6 rootstock recorded the highest values followed in a descending order by those grafted onto NAZ4 and own-rooted. On the contrary, Thompson Seedless owns rooted vines gave the highest values in berry weight, berry length and juice volume.

Fig. 1: Physical characteristics of berries of Thompson seedless as affected by different tested rootstocks.



Chemical characteristics of berries

It is interesting to note, that all berry chemical parameters, including total soluble solids, titratable acidity, TSS / acid ratio, pH and Vitamin C ratio were significantly affected by rootstocks (Table 3). Accordingly, Thompson Seedless grafted onto NAZ4 rootstock was found to record the lowest percentages of TSS and TSS / acid ratio, and the lowest percentages of acidity of the berry juice, followed in a descending order by own-rooted which recorded intermediate values, while Thompson Seedless on own - rooted gave the highest values of TSS, TSS/acid ratio, pH and the lowest values of Vitamin C in the three seasons under study.

Some characteristics of vegetative growth

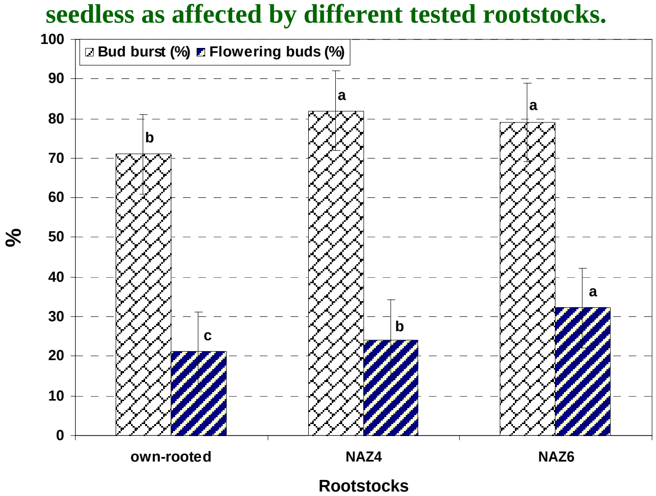
Data presented in (Table 4) show that most of vegetative growth parameters (shoot diameter, shoot length, number of leaves per shoot, leaf area, total leaf area/vine, coefficient of wood ripening and weight of cane pruning, crop load), responded positively to rootstocks. Thompson Seedless grafted onto NAZ4 rootstock recorded the highest values of vegetative growth, followed in a descending order by those grafted onto NAZ6 rootstock and own-rooted vine. The maximum values of shoot length and diameter, total leaf area and pruning weights and the highest number of leaves were given by vines grafted on NAZ4 rootstock as compared to own-rooted vines could be attributed to the total biomass produced in Dog ridge and Salt creek rootstocks which provided the frame work for total leaf area which by its turn was reflected on the amount of old wood retained on the grapevine which may have positively affected the yield and bunch quality that found by Williams and Smith (1991).

Table 4. Some physical characteristics of canes and shoots of Thompson seedless as affected by different tested rootstocks during 2011-2014 seasons.

Rootstocks	Shoot diameter (cm)	Shoot length (cm)	Number of leaves per shoot	Leaf area (cm^2)	Total leaf area/vine (cm^2)	Coefficient of wood ripening	Weight of cane pruning (kg/vine)	Crop load (kg yield/kg cane pruned)
Own rooted	1.03c	166.2b	24.8b	134.28c	18.6c	0.80c	1.12c	4.82c
NAZ4	1.13a	175.2a	26.2a	146.2a	20.7b	0.83b	1.40a	5.11b
NAZ6	1.07b	148.5c	26.5a	139.8b	21.9a	0.85a	1.21b	7.24a
Significance	*	**	**	*	*		**	*
Means having the same letters within a column are not significantly different at 5% levels								

Vegetative growth significant differences in pruning weight were recorded during all the years of the study. Pruning weight was significantly the highest on NAZ4, while the lowest pruning weight was produced by own rooted vines. Pruning weight was intermediate in Thompson Seedless grafted on rootstocks NAZ6 and bud sprouting after forward pruning was quicker on own rooted Thompson Seedless vines, followed by those grafted on NAZ4 rootstock.

Fig. 2: Physiological characteristics of buds of Thompson seedless as affected by different tested rootstocks.



Bud burst and flowering buds percentage

Data in Fig. 2 show bud burst and flowering bud percentage of Thompson as affected by different tested rootstocks during 2010 and 2013 seasons. It was noticed a significant increase in Bud burst percentage of Thompson grafted onto NAZ4 and NAZ6 comparing with own rooted in average three years of study. Also, the own rooted vines recorded the lowest flowering bud percentage comparing with the two other rootstocks. Thompson seedless cultivar recorded higher fruiting bud percentage with NAZ6 vines compared with the other rootstocks.

Discussion

The influence of rootstock on yield and physiological parameters has been reported by many researchers (Bica et al., 2000). The results of these studies suggest that rootstocks differ in root distribution pattern and total root number, which influences the yield and pruning weight and also yield to pruning weight ratio (Satisha et al., 2010). Means of total yield and yield components of Thompson Seedless grafted onto different rootstocks varied significantly among rootstocks during the experimental years of the study, and also when the average yield over the years was taken into consideration. Since the number of cluster was adjusted to 16 per vine, it is supported that any increase in cluster weight should be parallel with the increase in yield by weight, length and width of clusters (Table 2). This result was confirmed by Ferree et al. (1996) who reported an increase yield from grafted Cabernet Franc and white Riesling than from own–rooted vine. Also, Sommer et al. (2001) found that grafted Sultana vines were always more fruitful than own–rooted vines. The obtained results referring to a positive effect of rootstocks on the physical characteristics of berries are in agreementwith those reported by Satisha et al. (2010) who found that bigger and heavier berries, as indicated by higher berry diameter and berry weight, were recorded on vines grafted on to Dog Ridge rootstocks as compared to own- rooted vines. The influence of rootstock on fruit composition has been studied by several workers; Cirami et al. (1984) recorded higher juice pH in Shiraz grafted onto Ramsey, Dog Ridge, Harmony, Schwarzmann and 1613C than in own-rooted vines. In addition, Ruhl et al. (1988) showed that own-rooted 'Riesling', 'Ruby Cabernet' and 'Shiraz' had low to medium juice pH while the own-rooted 'Chardonnay' had higher juice pH; rootstocks 'Harmony', 'Dog Ridge', 'Freedom' and 'Rupestris du Lot' generally caused a high juice pH, whereas '140R', '1202', '5A', 'SO4' and '101-14' had low pH.

Kubota et al. (1993) grafted Fujimori grapes onto seven different rootstocks and found that the highest level of TSS and Vitamin C were observed in berries from vines grafted onto 3306 C. There is a strong relation between sugar content and the content of vitamin C in the berries. Yokotsuka et al. (1999) who reported that, in the period from berry coloration to ripening sugar content of the berry, TSS , the content of Vitamin C and anthocyanines per berry increased as ripening proceeded and that amounts were maximal at 18-20º Brix. The results in this respect are in line with those of Rafaat and El-Gendy (2013) who observed more vegetative growth of Flame Seedless, expressed in high values of biomass on vines grafted on Salt Creek or Freedom rootstock, with the lowest vegetative growth on own-rooted grapes.

Also, Fardossi et al. (1995) found that shoot growth of 'GrunerVeltliner' was slower on '5C' and 'Fercal', but more rapid on '1103P', '725P' and '125AA'; ripening occurred earlier on '1103P', 'G1' and '*Riparia* Sirbu' than on the other rootstocks. In addition, Rizk-Alla et al. (2011) reported that rootstocks Dog ridge, Salt creek and Freedom were effective in increasing the yield and its components, ensuring the best physical properties of bunches, improving the physical and chemical characteristics of berries, achieving the best vegetative growth parameters (i.e. average shoot diameter, average shoot length, average number of leaves/ shoot, average leaf area, total leaf area/vine, coefficient of wood ripening and weight of pruning) and increasing leaf content of total chlorophyll and percentages of total nitrogen, phosphorus and potassium as well as cane content of total carbohydrates in comparison with the non grafted vines.

The economical study indicated that Red Globe grapevines grafted on Dog ridge, Salt creek, Freedom, Harmony, and Paulsen 1103 rootstocks gave the maximum net profit compared with the own-rooted vines. Vegetative growth significant differences in pruning weight were recorded during all the years of the study. Pruning weight was significantly the highest on NAZ4, while the lowest pruning weight was produced by own rooted vines. Pruning weight was intermediate in Thompson Seedless grafted on rootstocks NAZ6 and bud sprouting after forward pruning was quicker on own rooted Thompson Seedless vines, followed by those grafted on NAZ4 rootstock. The results in this respect are in line with those of Rafaat and El-Gendy (2013) who observed more vegetative growth of Flame Seedless, expressed in high values of biomass on vines grafted on Salt Creek or Freedom rootstock, with the lowest vegetative growth on own-rooted grapes. Also, Fardossi et al. (1995) found that shoot growth of 'Gruner Veltliner' was slower on '5C' and 'Fercal', but more rapid on '1103P', '725P' and '125AA'; ripening occurred earlier on '1103P', 'G1' and '*Riparia* Sirbu' than on the other rootstocks.

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Conclusion

This is one of the few studies investigating the effects of resistance to crown gall disease rootstocks on vine vegetative growth status and productivity in a field situation. Therefore, this research provides both basic knowledge about rootstocks and applied information useful to viticulturists. This study also was important because in Iran a majority of the vineyards were infected by crown gall disease farmed or the availability of health vineyard is minimal. The results indicate that the two rootstocks used in this study could be classified into different groups based upon their effects on growth and productivity characteristics of scion measurements.

This classification, based upon one measure of vine vegetative growth status, was similar in several respects to another study in which a different measure of grapevine reproductive status was used to make a classification for crown gall resistance. However, the yield and quality of fruit data indicates that the effect of a rootstock upon the scion's flower bud status was not the sole factor determining the productivity of a vine under crown gall infected farming conditions. Based upon yield data, NAZ4 should not be considered as a rootstock for use in Iran under present of crown gall agent in farm. Yield data obtained from the rootstock NAZ6 in this study has been classified as highly crown gall infection resistant (Chen et al., 2007; Reynolds and Wardle, 2001), would indicate that it could be used in present *Rhizobium vitis* bacterium in farm and perform relatively.

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