



Effect of salicylic acid and potassium sulfate on the primary bud necrosis and fruit set of the following year of Askari grapevine

Jahanshir Ahmadi ¹, Bahram Abedy ^{1*}, Mahmood Shoor ¹, Saeid Eshghi ²,
Reza Amiri Fahlani ³

¹ Department of Horticulture, Faculty of Agriculture, Ferdowsi University of Mashhad, IRAN

² Department of Horticulture, Faculty of Agriculture, Shiraz University, IRAN

³ Department of agronomy and plant breeding, Faculty of Agriculture, Yasouj University, IRAN

*Corresponding author: abedy@um.ac.ir

Abstract

Grape cv. Askari is one of the dominant cultivars of the city of Dena, which is affected by freezing temperatures of the winter every year. In order to study the primary bud necrosis and fruit set of the following year, the present research was carried out in one of the vineyards of Dena County in a two-factor factorial experiment based on a completely randomized block design with three replications and two vines per plot. In this experiment, salicylic acid (0, 150, 300 and 450 mg/l) and potassium sulfate (0, 1 and 2%) were used as spraying in one phase. Bud sampling was performed in three stages, and the primary bud necrosis percentage was recorded in each stage. Salicylic acid at 300 mg/l and interaction of 450 mg/l salicylic acid and 2% potassium sulfate had the highest effect on the reduction of primary bud necrosis of grape at 5% level, and the rest of the treatments did not have any significant effect on the primary bud necrosis percentage. The lowest mortality percentage of primary buds was observed in the second stage of sampling at 5% level. The results of variance analysis showed that the interaction of salicylic acid and potassium sulfate at 5% level had a significant effect on fruit diameter, and the remaining treatments showed no significant effect on the trait. Means comparison of fruit diameter showed that the interaction of salicylic acid at 150 mg/l and 2% potassium sulfate had the highest effect on the diameter of berry, and increasing the percentage of potassium sulfate increased the diameter of berry. Salicylic acid and potassium sulfate treatments had no significant effect on average length, berry weight, pH, TA, TSS and percentage of berry set.

Keywords: bud mortality, diameter, weight, TSS, pH, TA, percentage of berry set

Ahmadi J, Abedy B, Shoor M, Eshghi S, Fahlani RA (2017) Effect of salicylic acid and potassium sulfate on the primary bud necrosis and fruit set of the following year of Askari grapevine. Eurasia J Biosci 11: 16-24.

© 2017 Ahmadi et al.

This is an open-access article distributed under the terms of the Creative Commons Attribution License.

INTRODUCTION

Grape is one of the horticultural crops and is a small fruit that is grown as irrigated and rainfed crop. It can be eaten fresh as table grape or it can be used in processing industries such as the production of raisin and grape juice (Tafazoli et al. 1996).

Grape has complete flowers and requires long and warm growing season for fruit ripening. Grape, belonging to the *Vitaceae* family, is divided into three American, European and Asian groups, of which European one is of higher importance. Iranian grapes are of European type. American grapes have the highest resistant to frost, while Asian grapes are more susceptible. European grapes tolerate up to -15 °C in winter (Tafazoli et al. 1976).

In early spring, all the buds that grow along the petioles are uniform and it is not possible to determine which buds flower. Gradually, in the middle of the growing season, the growth of the branches decreases

and the plant begins the storing, in which a number of buds differentiate for flowering. This phase of flower formation is called induction (Tafazoli et al. 1976).

Grapes are plants with very high vegetative growth, and branches containing cluster grow continuously during the growing season (Tafazoli et al. 1976). The compound bud of the grape contains three independent buds, of which the central bud is called the primary bud, and the two lateral buds are called secondary and tertiary buds. With the death of primary bud, the secondary bud produces branches with less fertility and smaller clusters, thereby reducing the yield. If the primary bud grows in the spring, the secondary and tertiary buds remain dormant, and if the primary bud dies, the secondary bud produces weak branches to

Received: March 2017

Accepted: May 2017

Printed: July 2017

compensate the growth (Rawnsley and Collins 2005). In terms of the appearance, a bud with primary bud necrosis disorder is similar to a healthy bud and it is difficult to distinguish the difference between them by using naked eye (Dry and Coombe 1994).

If the mortality rate of the primary bud is less than 20%, it does not significantly reduce the yield, and if it is more than 20%, it resulted in fruiting dissatisfaction (Rawnsley and Collins 2005).

Necrosis of grape buds may be caused by physiological or developmental disorders. Necrosis of buds has been reported in seedless grape cultivars including Thompson seedless grape, Flame Seedless and Queen of Vineyard (Lavee 1987, Lavee et al. 1981, Morrison and Iodi 1990, Naito et al. 1987, Perez and Kliewer 1990, Perez-Harvey 1991) and seeded cultivars (Dry and Coombe 1994, Naito et al. 1985, Naito et al. 1986).

Reduction of yield with blind nodes (Pastena 1990), decrease of budburst in shadow (May et al. 1976), and in some cases necrosis of buds have been observed.

Flower initiation and many developmental stages in grape occur in the first year, and the development of bud and growth of shoot occur during the second year (Pratt 1974). Symptoms of bud necrosis during the development in the first year are evident during the current season anthesis of inflorescences. In general, buds may be affected until the onset of dormancy (Lavee et al. 1981, Morrison and Iodi 1990, Naito et al. 1987, Perez and Kliewer 1990). Necrosis usually begins from the base of the primary bud, and results in the death of only this bud. Under these conditions, one or more inappropriate secondary buds may grow in the spring. Under more difficult conditions, necrosis may include the secondary bud (usually two), which results in the lack of shoot growth from the node (Lavee et al. 1981, Perez and Kliewer 1990).

Histological experiments showed that bud necrosis of Thompson seedless grape occurred by the formation of irregular and cross-linked compact cells in one or two nodes from the primary bud axis (Morrison and Iodi 1990). Cell breakdown in that area continues through the death and drying of the tissues far from the center.

The cause of grape bud necrosis has remained unclear. High growth rate (Bindra and Chohan 1975, Dry and Coombe 1994, Lavee et al. 1981, Naito et al. 1986) and growing of the entire shoot or a number of buds in the shade (Perez and Kliewer 1990) mainly increased necrosis rate in necrosis-susceptible cultivars.

In terms of the appearance, a necrotic bud looks like a healthy one, which is hard to detect with naked eye (Dry and Coombe 1994). Although it is possible to see the death of primary bud in the field by using a handheld lens, it is necessary to cut the buds to determine the death of the primary bud and to detect the productive buds. Anatomy of the bud involves opening dissection of the bud and use of a microscope to study its internal

structure. The primary bud is the central bud, which seems brown and dry if it is dead (necrotic), while the secondary buds are green. The analysis of the bud anatomy for productivity involves counting the number of cluster primordia in the bud without necrotic symptoms. The necrosis of bud starts from the bud basis and only affects the primary bud. It is not clear why necrosis does not affect the secondary bud, but in severe conditions, the death of the whole buds occurs.

Microscopic anatomy of buds is used to evaluate the bud productivity and to predict the yield potential. Primary bud death can be easily detected by using bud anatomy.

High growth of stem, excessive irrigation, shade (May 1961, Wolf and Warren 1995), high levels of gibberellic acid (Ziv et al. 1981), and reduction of bud carbohydrate (Vasudevan et al. 1998a) are associated with bud necrosis. High growth of stem, such as shoot diameter, the growth rate, and stem internode length are associated with the death of the primary bud. For example, Shiraz cultivar has high growth potential and is prone to the death of the primary bud. It has been shown that branches with a diameter greater than 12 mm had the highest rate of primary bud death (Dry and Coombe 1994).

The correlation between growth rate and the incidence of primary bud necrosis may be accompanied by rapid growth of the shoot in the spring. The peak of fast growth, coupled with increased levels of plant hormones, causes abnormal tissue development. It has been reported that the death rate of the primary bud is directly related to shoot heading, defoliation and thinning of the shoots. The intensity of shoot thinning in Shiraz cultivar increased the spread of primary bud death. As a result, shoot removal increases the growth of the remaining shoots (Dry 1986). However, studies in Chile showed that the moderate level of shoot thinning reduced the incidence of primary bud death in Sultani cultivar (Perez and Kliewer 1990). While in Riesling, the effect of shoot thinning on the incidence of primary bud death varied between the seasons (Wolf and Warren 1995).

Studies have shown that the death of the bud begins after flowering and stops after the onset of the dormancy. If the death of primary bud occurs earlier, the secondary buds show more development than the initial state and generally fill the space occupied by the dead primary bud (Lavee 1981, Morrison and Iodi 1990). It has been shown in a research that the occurrence of bud death at inflorescence development stage was very low (2%), at the time of flowering (14%), at pea size stage (30%) and at leaf drop (43%), and it was higher after the onset of dormancy (Dry 1986).

The death of the primary bud may lead to the development of two branches from the same node (Dry and Coombe 1994). At the same time, the number of cluster decreases and poor fertility may represent high

death rate of the primary bud. Previous reports have shown that the death of the primary bud increased with the onset of the bud dormancy (Lavee et al. 1981, Morrison and Iodi 1990, Vasudevan et al. 1998a).

Formed in the leaf axis, the compound bud of grape is composed of a primary bud and one or two secondary buds. The axis of the primary bud is formed in nodes 6 to 9 before entering into dormancy stage in August or September. Flower primordia are formed in node 4 or 5 and tendril is formed in the next nodes. The main stem develops in the spring of the following season. If the primary bud dies, the secondary buds turn into the secondary branches, but the yield of the secondary branches is less than that of the main branches, because cluster differentiation in the primary buds occurs before the onset of dormancy, while the differentiation of the secondary bud is delayed or depends on the differentiation of the primary bud (Pratt 1974).

Necrosis affects the primary buds and sometimes secondary bud in the compound bud (Dry and Coombe 1994, Lavee 1987, Lavee et al. 1981, Morrison and Iodi 1990, Naito et al. 1986, Wolf and Warren 1995). Bud necrosis is one of the causes of low fertility of grape in vineyard all around the world. The appearance of bud necrosis is different (Lavee et al. 1981, Morrison and Iodi 1990). It has been reported that some buds have developed a necrotic layer in the primary axis (Morrison and Iodi 1990). Some of the buds develop necrosis in the first to fourth layers above the base of the primary axis (Lavee et al. 1981, Morrison and Iodi 1990), while others develop necrosis only at the top of the primary axis basis (Morrison and Iodi 1990). Limited studies on the necrosis of the bud showed that in the late summer, the color of the primary bud varies from light green to brownish green and eventually brown (Prise and Clairev 1990, Wolf and Warren 1995). Furthermore, development of a necrotic area was observed along with the death of the primary bud, although time of necrosis was not reported (Perez and Kliwer 1990).

Anatomical observation of Virginia grape showed that primary bud necrosis begins about 60 days after budburst (15 days after blooming). The first visible symptoms of bud necrosis were observed 20 days after full bloom for a two-week period (Lavee et al. 1981). Primary bud necrosis initiated 3 to 6 weeks after full bloom, but continues until the onset of dormancy. Time of the primary bud death can vary among the cultivars. It has been reported that the primary bud death of Thompson seedless cultivar was three weeks after blooming (Lavee et al. 1981), while according to other experiments in the same cultivar, the primary bud death occurred 6-10 weeks after the blooming stage (Vasudevan and Wolf 1998a).

Primary bud necrosis is a physiological disorder in grape, which causes the primary bud to die. Primary bud necrosis is due to the abortion and subsequent drying of

the primary bud in a developing compound bud (Lavee et al. 1981, Morrison and Iodi 1990, Dry and Coombe 1994, Wolf and Warren 1995). The severity and position of necrosis in the primary bud depends on the developmental stage. In the samples taken from Riesling bud under optical microscope, degenerated regions of the abnormal cells are immediately seen under the axis of the primary bud 60 days after blooming. 90 days after blooming, non-uniform cells become dense and gradually die (Vasudevan et al. 1998a).

Necrosis occurs in the primary axis of some cells (Morrison and Iodi 1990), and in undifferentiated buds, necrosis below the developed terminal meristem results in the death of primary buds (Ziv et al. 1981). Sampling by using an electron microscope showed a similar pattern of tissue degradation, suggesting that cellular degradation was not the result of tissue preparation (Vasudevan et al. 1998a, Vasudevan et al. 1998b). The first visible sign of the primary bud necrosis is caused by degradation and compaction of cells with irregular cell wall (Vasudevan et al. 1998, Morrison and Iodi 1990). The effect of primary bud death on production is important in terms of direct losses of the product and is related to costs management. For example, in 2003, in one of Australia's vineyards, approximately 19,000 t of Shiraz cultivar fruit, equivalent to \$ 35.5 million, was lost due to the death of the primary bud. In some of the vineyards, the prevalence of bud death disorder was more than 60%, which greatly reduced the yield. It is unclear why some vineyards are more affected by this disorder (Rawnsley and Collins 2005). Growth of the secondary bud and the production of thin branches, increase of frost and reduction of the yield are the main problems of Askari grape in Dena County. Therefore, increase in the death of the primary bud leads to pruning management, foliar spray and introduction of new and resistant cultivars to bud death. Due to the death of the bud, vineyard growers inevitably have to choose the branches resulted from the secondary bud and prune it as a fruiting branch, thereby facing reduction in crop yield.

During summer season, the available potassium in the soil of pistachio production areas is reduced due to drought and thermal stress. In general, pistachio trees show some physiological disorders, such as flower bud drop, blank nut, early splitting of fruit, non-split fruit, and fruit deformation, which can be reduced by potassium spraying. It has been reported that potassium foliar application improved fruit weight and percentage of pistachio splitting (Ashworth et al. 1987, Ben-Mimoun 2004).

During the evolution period, plants have gained a wide range of resistant mechanisms against a variety of stresses. Evidence suggests that minerals play an important role in resistance to plant stress (Kant and Kafkafi 2002, Marschner 2012).

Among all the mineral nutrients, potassium plays a very important role in plant growth and metabolism, and it largely leads to the survival of plants that are under different living and non-living stresses. The importance of potassium fertilizer for producing crops and increasing their quality is very well known. As a result, consumption of potassium has increased dramatically in many parts of the world (Pettigrew 2008). Positive and strong correlation was found between potassium fertilizer and seed yield (Dong et al. 2010).

It has been reported that good nutrition of potassium not only increased dry matter of the whole plant and dry leaf area, but also improved water retention in plant tissues under drought stress conditions. Evidence suggests that maintaining membrane's health and stability under drought stress conditions is also necessary for plant drought tolerance (Bajji et al. 2002). Cell membrane stability under drought stress was significantly reduced (Wang and Huang 2004).

Salicylic acid plays an important role as an important messenger molecule in the creation of resistance to local and epidemic diseases in plants in response to the attack of various pathogens (Enyedi et al. 1992). In addition to the increase of plant resistance to disease, salicylic acid can create plant responses to a wide range of oxidative stresses (Shirasu et al. 1997).

Salicylic acid, which is an internal growth regulator, affects various physiological functions and biochemical activities in plants. It is also a natural molecular signal that plays an important role in the creation of a defensive response to various living and non-living stresses (Arfan et al. 2007, Wang et al. 2010).

Plant physiological processes, growth, development, fertility, and response to non-living stresses are affected by salicylic acid (Erfan et al. 2007). The activity of antioxidant enzymes is altered by salicylic acid and plays an important role in protecting plants from oxidative damage by detoxifying of strong oxidizing radicals (Muns and Tester 2008). High activity of antioxidant enzymes improves plant resistance to oxidative damage caused by active oxygen species (Gapinska et al. 2008).

It has been reported that use of salicylic acid affects fertility, growth, photosynthesis, plant's water relation and activity of antioxidant enzymes of plants exposed to different living and non-living stresses. Effectively, salicylic acid reduced toxic effects produced in plants due to the exposure to various non-living stresses (Hayat et al. 2010).

Therefore, it is clear from the above references that salicylic acid and potassium exhibit different physiological roles in plants and potentially reduce the destructive effects produced by different living and non-living stresses. The aim of this study was to investigate the effect of salicylic acid and potassium on physiological and biochemical changes, cold resistance and fruit set in the following year in grape cv. Askari.

MATERIALS AND METHODS

This experiment was carried out to investigate the effect of salicylic acid and potassium sulfate on the changes of primary bud death and quantitative and qualitative characteristics of grape cv. Askari in one of the vineyards of Sisakht County. At the end of August 2014, leaf and soil samples were taken from the testing plants and analyzed separately in the laboratory. Based on the results obtained from analysis of these samples, fertilization was performed during the winter. This experiment was conducted in vineyard (all grapes were 15 years old) with head-training system, drip irrigation and uniform pruning system. The experiment was carried in a factorial design based on randomized complete block design in three replications, and two plants per plot. The first factor was Salicylic Acid (SA) at four levels (0, 150, 300 and 450 mg/l), and the second factor was potassium sulfate at three levels (0, 1 and 2%). Salicylic acid and potassium sulfate were sprayed on plants at a single stage on 9/11/2014. Bud sampling was performed to measure the mortality rate of the primary bud on three dates (11/22/2014, 2/20/2015, and 3/25/2015). After recording the data, the results were analyzed using split plot design in time based on randomized complete block design. The average length, diameter, berry weight, pH, TA, TSS of berry and the percentage of berry set were measured in the following year. After the measurement of the traits, the results were analyzed using a factorial design based on a completely randomized block design. Finally, after data collection, statistical analysis was performed using MSTAT-C software and means were compared by using Duncan's test.

Measuring the percentage of primary bud death of the grape

To determine the mortality percentage of the primary bud of grape at ambient temperature during winter, bud sampling was carried out on three dates 11/22/2014, 2/20/2014, and 4/4/2015. For studying and taking photo of buds, digital microscope (Dinolite) was used. To do so, buds were cut using a scalpel razor and studied under the microscope. For each treatment, at least 6 buds were selected and necrotic buds (**Figs. 1 to 3**) were distinguished from healthy buds (**Fig. 4**). Finally, the percentage of necrotic primary bud and the number of remaining healthy buds were analyzed. Data were analyzed by split plot design in time based on a completely randomized block design.

Characteristics measured in berry

Measurement of length, diameter, weight, pH, TA, TSS and percentage of berry set

Thirty berries were randomly separated for measuring the average length, diameter and weight in each treatment. Data were analyzed in factorial design (salicylic acid at 4 levels and potassium sulfate at 3 levels) based on a completely randomized block design.



Fig. 1. Start of primary bud necrosis in grape



Fig. 2. Progression of primary bud necrosis in grape



Fig. 3. Primary bud necrosis and growth of the secondary bud of grape

Measurement of fruit juice pH

To measure the pH of fruit juice, a number of grape berries were separated and washed. The fruits of each sample were individually extracted by using cheesecloth, and 20 ml of the extract was poured into



Fig. 4. Three healthy buds after cold stress

glass containers. Fruit juice pH was measured using pH meter device. Data were analyzed based on two-factor factorial design (salicylic acid at 4 levels and potassium sulfate at 3 levels) in a completely randomized block design.

Total acidity (TA%)

Total of free organic acids and salts present in the fruit forms the total acidity. 10 cc fruit extract was poured into a dish, and 20 cc distilled water was then added. In the next step, two to three drops of phenolphthalein 0.1 were added to the solution. The solution was titrated with 0.1 sodium hydroxide, and formation of pale red color was the end of titration (phenolphthalein turns colorless in acidic solutions, and red in basis solutions).

Acid content of fruits and vegetables can range from a very low to 50 meq/100 g product. To prepare phenolphthalein solution 0.1, one g of powder was dissolved with 90% ethanol and the solution was brought up to 100 ml. The following equation was used to calculate the amount of acid:

$$A = \frac{S \cdot N \cdot F \cdot E}{C} \times 100$$

Where A is the amount of acid in fruit extract (g/100 ml), S is the amount of NaOH consumed (ml), N is the normality of NaOH (0.1 normal), F is NaOH factor, C is the amount of fruit extract (ml), and E is equivalent of the desired acid (Tartaric Acid = 75 equivalent or 0/075 meq)

Normal (N) = Equivalent per liter

- Factor F or normal capacity is the number of equivalent per liter. Therefore, normal solution has a factor of one.

Data were analyzed based on two-factor factorial design (salicylic acid at 4 levels and potassium sulfate at 3 levels) in a completely randomized block design.

Measurement of total soluble solids (TSS%)

Quantitative measurement of sugars

For this purpose, a manual refractometer was used. Some fruit extract was prepared and a drop of it was poured on the plate of refractometer to measure the amount of TSS. The amount of sugar obtained was

expressed as brix, which was equal to g sugar per 100 g fruit extract.

Data were analyzed based on two-factor factorial design (salicylic acid at 4 levels and potassium sulfate at 3 levels) in a completely randomized block design.

Fruit set percentage

On May 2015, three clusters were labeled on each vine and placed in a plastic bag before being opened. After fertilization and flower drop, the plastic bags were collected and the number of flowers per cluster was counted. The clusters were marked and the number of their berries was counted at harvesting. This practice was performed to evaluate the effect of salicylic acid, potassium sulfate and their interaction on fruit set percentage.

RESULTS

Results of ANOVA analysis and means comparison of primary bud death percentage of grape

The results of analysis of variance of the treatments showed that salicylic acid and sampling dates had a significant effect on the mortality rate of the primary bud of grape at 5%. Based on the results, the rest of the treatments did not have a significant effect on the mortality rate of the primary bud (**Table 1**).

Table 1. Results of analysis of variance related to the effects of SA and k2so4 on the rate of primary bud necrosis

Mean squares		
Source of variation	Df	Primary bud necrosis%
Block	2	34.101 [*]
SA	3	24.317 [*]
K	2	7.076 ^{ns}
SAxK	6	4.674 ^{ns}
Error(a)	22	7.664
Date	2	23.159 [*]
SAxDate	6	2.210 ^{ns}
KxDate	4	5.458 ^{ns}
SAxKxDate	12	3.550 ^{ns}
Error(b)	48	4.804
Cv(%)		128.02

ns: Difference is not significant, *: Difference is significant at five percent, **: Difference is significant at one percent

Means comparison of the treatments showed that increasing salicylic acid levels reduced the mortality rate of the primary bud. With salicylic acid at 300 mg / L, the lowest percentage of death of the first germ was observed. The lowest mortality rate of primary bud was observed when salicylic acid was applied at 300 mg/l. concentrations of 300 and 450 mg/l were placed in class b in terms of the reduction of mortality rate of the primary bud (**Fig. 5**).

The effect of different sampling dates on the mean mortality percentages of the primary bud showed that the highest death rate was seen on the first and third sampling dates, and the lowest mortality rate was observed on the second sampling date (**Fig. 6**).

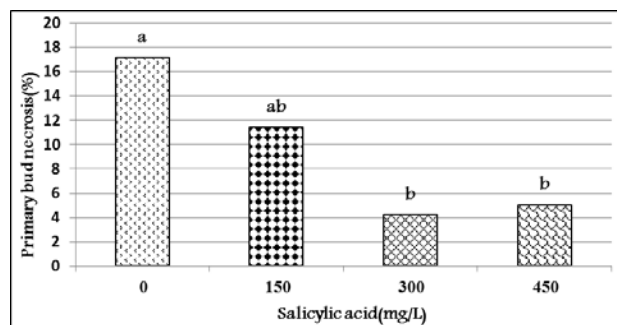


Fig. 5. Effect of salicylic acid on the rate of primary bud necrosis

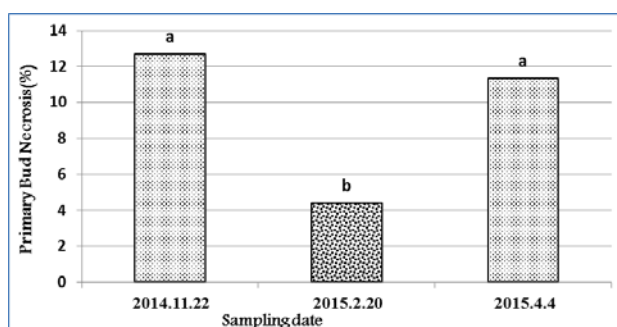


Fig. 6. Effect of sampling date on the rate of primary bud necrosis

Necrosis of tissue or organ occurs among many horticultural products including necrosis of gooseberry buds (Gill 1985), lack of bud formation in almond (Hellali et al. 1987), necrosis of poinsettia bracts (Simon and Smith 1991) and necrosis of grape bud (Christensen and Boggero 1985). Necrosis of grape bud reduces bud burst, yield and profit (Dry and Coombe 1994, Lavee et al. 1981, Perez and Kliewer 1990).

Use of chemical growth inhibitor of succinic acid reduced the incidence of necrosis of the bud (Nieto et al. 1986), while use of gibberellic acid increased the necrosis of the bud (Naito et al. 1986, Ziv et al. 1981). However, gibberellic acid did not have any effect on cultivars that were not susceptible to necrosis of buds (Morrison et al. 1990).

Characteristics measured in fruit

Length, berry weight, fruit juice pH, TA, soluble solids content (TSS) of fruit juice

Analysis of variance and means comparison of quantitative traits of grape juice

Grape clusters of each treatment were harvested separately in the next year after spraying. Diameter, length, weight, acidity, soluble solids and total acidity were measured for each treatment. The results of the analysis of variance are shown in **Table 2**.

Table 2. Results of analysis of variance related to the effects of SA and k2so4 on some traits of grape

Source of Variations	DF	Mean squares of the traits						
		Dimeter	lenght	pH	TA	Tss	Weight	Fruit set%
Block	2	0.000 ^{ns}	0.015 ^{ns}	0.027 ^{ns}	0.012 ^{ns}	7.87 ^{ns}	0.093 ^{ns}	193.282 ^{ns}
SA	3	0.001 ^{ns}	0.015 ^{ns}	0.009 ^{ns}	0.003 ^{ns}	0.747 ^{ns}	0.058 ^{ns}	226.208 ^{ns}
K ₂ SO ₄	2	0.005 ^{ns}	0.013 ^{ns}	0.009 ^{ns}	0.008 ^{ns}	2.276 ^{ns}	0.088 ^{ns}	100.412 ^{ns}
SA×K	6	0.008 [*]	0.016 ^{ns}	0.001 ^{ns}	0.002 ^{ns}	1.181 ^{ns}	0.098 ^{ns}	125.473 ^{ns}
Error	22	0.003	0.011	0.014	0.008	3.757	0.069	98.665
(%)Cv		3.43	5.42	3.42	14.82	10.83	10.74	19.31

ns: non-significant, *: significant at 5% level, and **: significant at 1% level

Fruit diameter

The results of variance analysis showed that the interaction of salicylic acid and potassium sulfate had a significant effect on fruit diameter at 5% level, and the effects of the remaining treatments were not significant. Analysis of means comparison related to fruit diameter showed that the interaction of salicylic acid at 150 mg/l and 2% potassium sulfate had the highest effect on berry diameter, followed by control treatment, and fruit diameter increased as percentage of potassium sulfate increased. The interaction of salicylic acid (450 mg/l) and different concentrations of potassium sulfate showed that increasing potassium sulfate concentration increased berry diameter (Fig. 7). Salicylic acid and potassium sulfate caused an increase in the amount of photosynthesis, carbohydrates and proteins, and reduction in the effects of environmental stresses, thus increasing flowering in different plants.

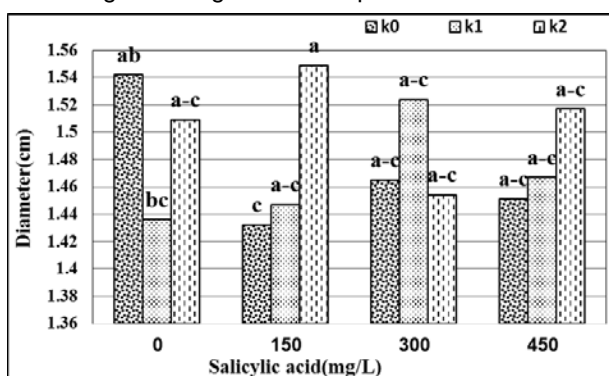


Fig. 7. Effect of sampling date on the rate of primary bud necrosis

It has been reported that potassium foliar application improves the weight of fruit and the percentage of pistachio splitting (Ashworth et al. 1987, Ben-Mimoun 2004). Salicylic acid induces flowering in a number of plants, including lemon (Cleland and Agni 1974).

Analysis of variance related to length, weight, pH, TA, percentage of soluble solids (TSS) and percentage of berry set were showed (Table 2) that different treatments of salicylic acid, potassium sulfate and their interaction did not have significant effect on these traits.

DISCUSSION

Living and non-living stresses cause developmental disorders in grapes and increase the death of the primary bud. Death of the primary bud results in the growth of the secondary bud. Shoots resulting from the secondary buds are weaker than the shoots grown from the primary buds, so the shoots grown from the secondary buds produce lower yield than those from the primary buds. Spraying of salicylic acid reduced the death of the bud, and increase in the concentration of salicylic acid reduced the death of the primary bud. Means comparison showed that the interaction of salicylic acid and potassium sulfate and increase of their concentrations led to a decrease in the percentage of the primary bud death. Necrosis of grape buds may be caused by physiological or developmental disorders, which reduced as a result of using salicylic acid and potassium sulfate. Salicylic acid and potassium sulfate increased the amount of photosynthesis, sugars, protein, proline, antioxidants, and carbohydrate storage of tissue. Through osmotic regulation and protection of cellular structure, salicylic acid and potassium sulfate decrease the mortality rate of bud and necrosis of the tissue as a result of free radicals, which are caused by stress. Furthermore, the interaction of salicylic acid and potassium sulfate increased the diameter of the fruit, which is due to the fact that salicylic acid and potassium sulfate increase photosynthesis and metabolism, regulate plant water relations, increase plant resistance to living and non-living stresses, and increase fertility, production and crop quality.

REFERENCES

- Arfan M, Athar HR, Ashraf M (2007) Does Exogenous Application of Salicylic Acid through the Rooting Medium Modulate Growth and Photosynthetic Capacity in Two Differently Adapted Spring Wheat Cultivars under Salt Stress. *Journal of Plant Physiology* 6(4): 685-694. <https://doi.org/10.1016/j.jplph.2006.05.010>
- Ashworth LJ, Gaona SA, Sube E (1987) Nutritional diseases of pistachio trees: Potassium and phosphorous deficiencies and chloride and boron toxicities. *Physiol Biochem.* 75: 1084-1091.

- Bajji M, Kinet JM, Lutts S (2002) The use of the electrolyte leakage method for assessing cell membrane stability as a water stress tolerance test in durum wheat. *Plant Growth Regulation* 36: 61–70. <https://doi.org/10.1023/A:1014732714549>
- Ben-Mimoun M, Loumi O, Ghrab M, Latiri K, Hellali R (2004) Foliar potassium application on pistachio tree. *Journal of Horticultural Science* 111: 224-228.
- Bindra AS, Chohan JS (1975) Flower-bud killing in Anab-E-Shahi grapes. *Indian Journal Mycology and Plant Pathology* 5: 63-68.
- Bray EA, Bailey-Serres J, Weretilnyk E (2000) Responses to Abiotic Stresses. In: Buchanan, B., Grissem, W., Jones, R., editors. *Biochemistry and Molecular Biology of Plants*. American Society of Plant Physiologists; Rockville, MD, USA: pp. 1158–1203.
- Christensen LP, Boggero JD (1985) A study of mineral nutrition relationships of waterberry in Thompson Seedless. *American Journal of Enology and Viticulture* 36: 57-64.
- Cleland CF, Ajami A (1974) Identification of the flower-inducing factor isolated from aphid honeydew as being salicylic acid. *Plant Physiology* 54: 904–906. <https://doi.org/10.1104/pp.54.6.904>
- Dong H, Kong X, Li W, Tang W, Zhang D (2010) Effects of plant density and nitrogen and potassium fertilization on cotton yield and uptake of major nutrients in two fields with varying fertility. *Field Crops Research*. 119: 106–113. <https://doi.org/10.1016/j.fcr.2010.06.019>
- Dry PR (1986) Primary bud-necrosis of grapevine. Masters thesis University of Adelaide.
- Dry PR, Coombe GG (1994) Primary bud-axis necrosis. I. Natural incidence and correlation with vigour. *Vitis*, 33: 225-230.
- Enyedi AJ, Yalpani N, Sliverman P, Raskin I (1992) Signal molecule in systemic plant resistance to pathogens and pests. *Cell* 70: 879-886. [https://doi.org/10.1016/0092-8674\(92\)90239-9](https://doi.org/10.1016/0092-8674(92)90239-9)
- Gill PA (1985) Lateral bud necrosis in the black currant (*Ribes nigrum*). *Plant Pathology* 34: 297-299. <https://doi.org/10.1111/j.1365-3059.1985.tb01363.x>
- Hayat Q, Hayat S, Irfan M, Ahmad A (2010) Effect of Exogenous Salicylic Acid under Changing Environment: A review of Environmental and Experimental Botany. 68: 14-25.
- Hellali R, Lin J, Kester DE (1978) Morphology of noninfectious bud failure symptoms in vegetative buds of almond (*Prunus amygdalus*). *Journal of the American Society for Horticultural Science* 103: 459-464.
- Kant S, Kafkafi U (2002) Potassium and Abiotic Stresses in Plants. In: Pasricha N.S., Bansal S.K., editors. *Potassium for Sustainable Crop Production*. Potash Institute of India; Gurgaon, India: pp. 233-251.
- Lavee S (1987) Necrosis in grapevine buds (*Vitis vinifera* cv. Queen of Vineyard) III. Endogenous gibberellin levels in leaves and buds. *Vitis* 260: 225-230.
- Lavee S, Melamud H, Ziv M, Bernstein Z (1981) Necrosis in grapevine buds (*Vitis vinifera* cv. Queen of-Vineyard) I. Relation to vegetative vigor. *Vitis* 20: 8-14.
- Marschner P (2012) *Marschner's Mineral Nutrition of Higher Plants*. 3rd ed. Academic Press; London, UK: pp. 178-189.
- May P (1961) The value of an estimate of fruiting potential in the Sultana. *Vitis* 3: 15-26.
- May P, Clingeffer PR, Brien CJ (1976) Sultana (*Vitis vinifera* L.) canes and their exposure to light. *Vitis* 14: 278-288.
- Morrison JC, Iodi M (1990) The development of primary bud necrosis in Thompson Seedless and Flame Seedless grapevines. *Vitis* 29: 133-144.
- Naito R, Yamamura H, Muniesue S (1987) Studies on the necrosis in grapevine buds (III) The time of the occurrence of bud necrosis in 'Kyoho' and the relation between its occurrence and the amounts of nutritional elements in buds. *Bul. Fat. of Agr., Shimane Univ., Japan*, 21: 10-17.
- Naito R, Yamamura H, Yoshino K (1986) Effects of shoot vigor and foliar application of GA and SADH on the occurrence of bud necrosis in 'Kyoho' grape. *Journal of the Japanese Society for Horticultural science* 55: 130-137. <https://doi.org/10.2503/jjshs.55.130>
- Oerke EC (2006) Crop losses to pests. *Journal of Agricultural Science* 144: 31-43. <https://doi.org/10.1017/S0021859605005708>
- Pastena B (1990) Sulle gemme cieche della vite. *Vignevine* 6: 53-59.
- Perez J, Kliwer WM (1990) Effect of shading on bud necrosis and bud fruitfulness of Thompson Seedless grapevines. *American Journal of Enology and Viticulture* 41(2): 168-175.

- Perez-Harvey J (1991) The influence of nitrogen fertilization on bud necrosis and bud fruitfulness of grapevines. Proc. Intl. Symp. Nitrogen in Grapes and Wine, Seattle, Wash., 18-19 June 1991. p. 110-115.
- Pettigrew WT (2008) Potassium influences on yield and quality production for maize, wheat, soybean and cotton. *Physiologia Plantarum* 133: 670-681. <https://doi.org/10.1111/j.1399-3054.2008.01073.x>
- Pratt C (1974) Vegetative anatomy of cultivated grapes-A review. 1974. *American Journal of Enology and Viticulture* 25: 131-150.
- Rawnsley B, Collins C (2005) Improving vineyard productivity through assessment of bud fruitfulness and bud necrosis.
- Shirasu K, Nakajima A, Rajshekar K, Dixon RA, Lamb C (1997) Salicylic acid potentiates an agonist-dependent gain control that amplifies pathogen signal in the activation of defence mechanism. *Plant Cell* 9: 261-270. <https://doi.org/10.1105/tpc.9.2.261>
- Simon BR, Smith MW (1991) The influence of gibberellin biosynthesis-inhibiting growth regulators on bract necrosis in 'Gutbier V.14 Glory' poinsettia. *Scientia Horticulturae* 48: 117-123. [https://doi.org/10.1016/0304-4238\(91\)90158-U](https://doi.org/10.1016/0304-4238(91)90158-U)
- Tafazoli A, Hekmati J, Firozeh P (1375) *Grapes*. Shiraz University Press, 243 pages.
- Vasudevan L, Wolf TK, Welbaum GG, Wisniewski ME (1998a) Reductions in bud carbohydrates are associated with grapevine bud necrosis. *Vitis* 37(4): 189-190.
- Vasudevan L, Wolf TK, Welbaum GG, Wisniewski ME (1998b) Reduction in bud carbohydrates are associated with grapevine bud necrosis. *Vitis* 37: 189-190.
- Wang ZL, Huang BR (2004) Physiological recovery of kentucky bluegrass from simultaneous drought and heat stress. *Crop Science* 44: 1729-1736. <https://doi.org/10.2135/cropsci2004.1729>
- Wolf TK, Warren MK (1995) Shoot growth rate and density affect bud necrosis of 'Riesling' grapevines. *Journal of American Society of Horticultural Science* 120(6): 989-996.
- Ziv M, Melamud H, Bernstein Z, Lavee S (1981) Necrosis in grapevine buds (*Vitis vinifera* cv. Queen of vineyard) II. Effect of gibberellic acid (GA3) application. *Vitis* 20: 105-114.