

EFFECT OF APPLYING GA₃ TREATMENT ON *Vitis vinifera* AGRONOMICAL TRAITS AND EMBRYO GERMINATION RATE

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Abstract. Seedlessness in *Vitis vinifera* is one of the most extensively studied topics. It is well known that GA₃ treatment is among the most widely used plant growth regulators for improving fruit quality and it can also induce seedlessness in grape berries. Here, we tested an effect of GA₃ application on the induction of seedless grape berries and along with on the morphological traits of grape berry. GA₃ application increased berry length of Qora Kishmish significantly at GA₃ 300 mg/L dose to 26% and berry weight to 36% compared to control. Seeded variety Rizamat the cluster and berry weight decreased approximately 10% and 7% at a concentration of 100 mg L⁻¹ and the highest reduction at 300 mg L⁻¹ 45% and 25% compared to the control, respectively. We found that GA₃ application at 300 mg L⁻¹ dose inhibited seed development and induced seedlessness by both Qora Kishmish and seeded Rizamat and Toyfi cultivars. The application of 100 mg/L⁻¹ and 200 mg/L⁻¹ GA₃ completely inhibited embryo formation in the rudiments and significantly reduced growth rate in in vitro condition (11%). Embryo germination in artificial medium decreased up to 9.1%-6.2% at 100 mg/L⁻¹ and 200 mg/L⁻¹ GA₃ by the “Toyfi” cultivar and 4.5%-4.1% at 100 mg/L⁻¹ and 200 mg/L⁻¹ GA₃ by the “Rizamat” cultivar.

Keywords: GA₃, seedlessness, berry, berry morphology, parthenocarpy, stenospermocarpy.

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1. Introduction

Grape (*V. vinifera* L.) cultivar is one of the widely cultivated and economically important crop across the globe (Ramming *et al.*, 2000). The consolidated statistical data of the International Organization of Vine and Wine estimated that in 2023, the global grape (*V. vinifera* L.) area totaled 7 141 570 hectares and total production was 67,3 M. Table grapes accounted for almost 48% (31.9 M), wine grapes for 47,4% (30,8 M) and raisin grapes for 6,9% (4,6 M) of the total global production of 67,3 M (www.oiv.int). The seedlessness trait in table grape is one of the most appreciated characteristics among consumer and also breeders (Karaagac *et al.*, 2012).

Seedlessness in the genus *Vitis* occurs naturally through two mechanisms parthenocarpy, in which seedless fruit develops without fertilization and stenospermocarpy, in which pollination and fertilization, occur but the embryo aborts at an early developmental stage (Stout, 1936; Abdullaev *et al.*, 2025; Pratt, 1971). It has

been reported that the application of gibberellic acid (GA₃) also can induce seedlessness in several fruit crops (Koura *et al.*, 2001).

In addition, a wide spectrum of physiological effects of GA₃ has been reported in different crops, including grape (*Vitis vinifera* L.) cultivar (Bar & Ori, 2015; Bermejo *et al.*, 2016; Cheng *et al.*, 2013; Cheng *et al.*, 2021; Davière & Achard, 2013; Abdullaev *et al.*, 2024, Ubaydullaeva *et al.*, 2023). The growth rate of seeds was tightly regulated by the supply of growth regulators (Coombe & Hale, 1973). The treatment of GA₃ has been demonstrated to induce hormonal imbalance, damage cellular integrity, seed abortion in seeded 'Kyoho', 'Red Globe' and seedless 'Thompson Seedless' grape cultivars (Cheng *et al.*, 2013). The berry size increasing rate of seedless 'Emperatriz' cultivar dependent on the treatment time and concentration of the GA₃ application (Casanova *et al.*, 2009). In the 'Superior Seedless' grape cultivar the cluster and berry weight, the number of berries per cluster, cluster and berry length, berry width effected significantly at 40 mg/L⁻¹ (twice, at the pre and post bloom stages) and 80 mg/L⁻¹ of GA₃ compared to the control 0 mg/L⁻¹ GA₃ (Abu-Zahra 2013). Pre-bloom application of GA₃ at 5, 7.5 and 10 ppm concentrations to standardize for rachis size of Crimson Seedless grape demonstrated the formation of the compact clusters with the highest bunch and berry weight, length and total soluble solids, whereas higher concentrations of GA₃ at 7.5 ppm and 10 ppm coiled rachis and overly loose clusters compared to the control (Satisha *et al.*, 2021). Interestingly, application of GA₃ aborted seeds and inhibited pollen tube growth (Cheng *et al.*, 2013), while moderate concentrations of GA₃ at 50-100 mg·L⁻¹ elongated grape clusters but did not reduce seed number (Gao *et al.*, 2020). In contrast, GA₃ concentration at 100 mg·L⁻¹ decreased seed number and altered fruit set and seedlessness in the berry (Baneh *et al.*, 2017).

However, the effect of GA₃ treatment on Central Asian grape cultivars on the morphological parameters (grape cluster, berry weight, seed number) and on the embryo germination has not been completely elucidated. Hence, we observed the effect of GA₃ treatment on the seedless and seeded grape cultivars morphology, inducing seedlessness and embryo germination rate in in vitro condition.

2. Materials and methods

2.1. Plant materials

To understand the effect of GA₃ treatment on grape berry formation and phenological characteristics of seedless and seeded grape cultivars was studied. In the experiment, Qora Kishmish, Oq Dum were selected as seedless cultivars and for the seeded Rizamat and Toyfi, cultivars (*V. vinifera* L.). Both experimental materials were 8 years old and planted at the special seed increase unit at the Center of Genomics and Bioinformatics grape collection. We excluded fertilization with NPK (nitrogen (N), phosphorus (P) and potassium (K) from experimental grape plants for evaluation of the gibberellin application during development stages.

2.2. Application of gibberellin acid

To test the effect of GA₃ on grape berry development, we applied concentrations of 100, 200 and 300 mg L⁻¹ of GA₃ (Sigma-Aldrich, #G7645, suitable for plant cell culture, ≥90% gibberellin GA₃ basis (of total gibberellins)) according to the method described by Anjum *et al.* (2020). The preparation method of a liquid solution of GA₃

comprises the following steps: we dissolved GA₃ in 2 mL of 96% ethanol, then gradually added 1 L of distilled water to prepare 300 mg/L⁻¹ concentration (other concentrations of GA₃ were prepared accordingly). We stored prepared solutions in refrigerator at 4°C until use.

Before GA₃ treatment, we randomly marked the grape bushes of selected cultivars in three replicates for each GA₃ (100, 200 and 300 mg L⁻¹) concentrations. Six grape clusters were selected in each marked grape bush based on visual observation, ensuring approximately similar sizes. We dipped the marked grape cluster into GA₃ solution 5 day prior to flowering stage to study the effect of GA₃ treatment (Casanova *et al.*, 2009).

2.3. Embryo rescue

To determine embryo germination of studied grape cultivars, we used embryo rescue (Murashige and Skoog medium) method and procedures as pervious reported (Murashige & Skoog, 1962; Abdullaev *et al.*, 2025).

2.4. Statistical analyses

We designed an experiment for statistical analysis as follows: The model of statistical analysis is “One-way ANOVA.” Three replications per GA₃ (100, 200 and 300 mg/L) concentrations were done; each replication consisted of one grape bush and six clusters were marked per bush as experimental units (grape cluster number per concentration of GA₃ = 9 grape bushes × 6 clusters per bush = 54 grape clusters; berry number = 9 grape clusters × 30 berries per cluster = 270 berries per concentration of GA₃). To evaluate the effect of GA₃, berries from selected cultivars were sampled at 35, 45 and 61 days after pollination (DAP) to measure the targeted morphological parameters. R Studio 2024.12.0+467 was used for statistical analyses (R Core Team, 2021).

3. Result and discussion

3.1. Effect of GA₃ application on the grape cluster morphology

In this experiment, the effect of GA₃ treatment on the morphology of two seedless and two seeded varieties was studied. The cluster length of ‘Oq Dum’ exhibited a significantly longer measurement after treatment with 300 mg/L GA₃ (32,4 sm) and 200 mg/L GA₃ (28,8 sm). The enlargement of cluster length following a GA₃ dose of 100 mg/L showed similarity to the control (23,7 sm and 24,5 sm) (Figure 1).

Figure 1 illustrates the effect of GA₃ treatment on cluster length across studied cultivars. The cluster length of seedless ‘Qora Kishmish’ and seeded ‘Toyfi’ cultivars showed similar growth response among all applied GA₃ (100, 200 and 300 mg/L⁻¹) concentrations. The growth response of cluster length showed a significant increase in ‘Rizamat’ cultivar reaching 10.6% at 100 mg/L⁻¹ and 16.3% at 300 mg/L⁻¹ of GA₃ compared with the control. Two used concentrations 200 and 300 mg/L⁻¹ of GA₃ stimulated progressive growth rate of grape clusters of the ‘Oq dum’ cultivar almost 18% and 32% respectively.

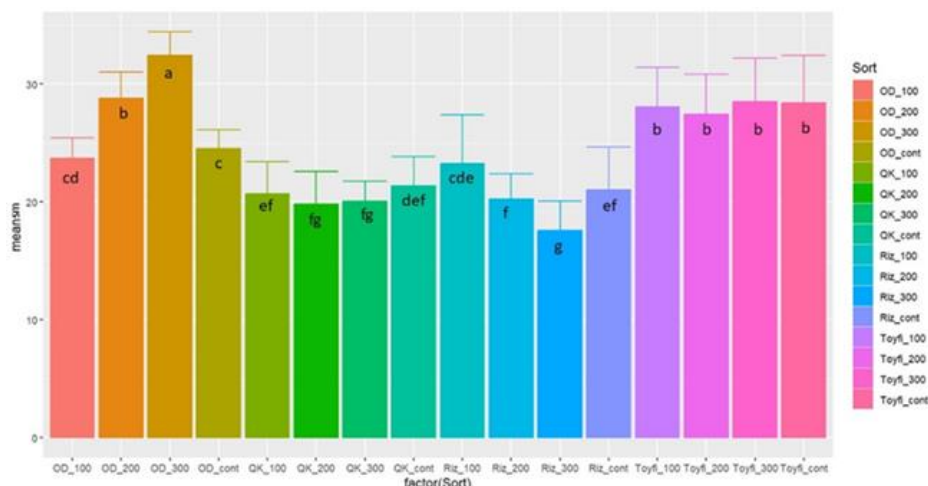


Figure 1. Bar chart illustrates statistical data of the cluster length (sm) of cultivars. The “X” axis ticks capital letters mean the name of cultivars as follows: “OD_100 - Oq Dum cultivar and GA₃ treatment 100 mg/L⁻¹”, “OD_200 - Oq Dum cultivar and GA₃ treatment 200 mg/L⁻¹”, “OD_300 - Oq Dum cultivar and GA₃ treatment 300 mg/L⁻¹”, “QK_100 - Qora Kishmish cultivar and GA₃ treatment mg/L⁻¹”, “QK_200 - Qora Kishmish cultivar and GA₃ treatment 200 mg/L⁻¹”, “QK_300 - Qora Kishmish cultivar and GA₃ treatment 300 mg/L⁻¹”, “Riz_100 - Rizamat cultivar and GA₃ treatment 100 mg/L⁻¹”, Riz_200 - Rizamat cultivar and GA₃ treatment 200 mg/L⁻¹”, “Riz_300 - Rizamat cultivar and GA₃ treatment 300 mg/L⁻¹”. OD, QK, Riz _ cont-control. Cluster length of all cultivars were measured at verasion stage. The “Y” axis demonstrated mean values, the “T” shape bar labs indicate standard error of the mean and the letters indicate statistically significance level

3.2. Berry and seeds growth following GA₃ treatment at day after pollination intervals

With Figure 2, we clearly show the effect of using three (100, 200 and 300 mg/L⁻¹) GA₃ concentrations on the ‘Oq Dum’ cultivar, presenting failed berry development and reduced berry density among clusters. The untreated control clusters remained compact with berries and short pedicels and caused to limit of rachis elongation. Pre-blooming dipping of GA₃ with 100 mg/L⁻¹ concentration stimulated rachis elongation and led to partial loss of the cluster, whereas high concentration (300 mg/L⁻¹) induced excessive rachis elongation and led to lower berry density. In this case, we excluded the ‘Oq Dum’ cultivar for further analysis.

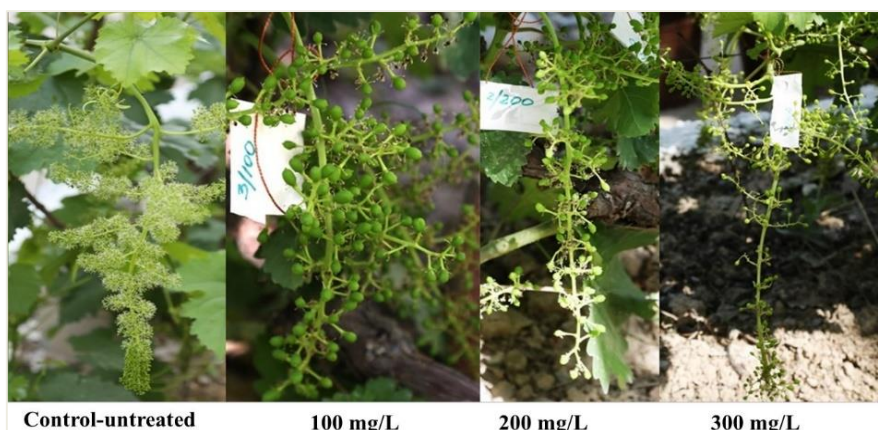


Figure 2. Effect of GA₃ treatment on the cluster of ‘Oq Dum’ cultivar

The seeded ‘Rizamat’ cultivar transited the veraison stage at 45 DAP. A significant acceleration of veraison was observed with GA₃ treatment with 100 mg/L⁻¹ and 200 mg/L⁻¹. The GA₃ treatment induced berry shattering of the ‘Rizamat’ cultivar with 300 mg/L⁻¹ and 200 mg/L⁻¹ concentrations significantly compared to the control (30% and 45%), as seen in Figure 3. According to grape cluster weight, ‘Rizamat’ treated with dipping with 100 mg/L⁻¹ GA₃ recorded the lower values (10%) below control in the study year, respectively. Berry length elongation of the ‘Rizamat’ cultivar showed similarity at 35, 45 and 61 DAP intervals between the control corresponding to each interval and all treatment concentrations of GA₃, with no statistically significant difference. Grape berry showed a decrease in berry weight at 200 mg/L⁻¹ by 4,98% (5.72 g) and an extreme value of weight reduction at 300 mg/L⁻¹ 25.58% (4.48 g) compared to control (6,02 g). Rizamat cultivar cluster was covered with reddish-purple and was more uniform in the control variant compared to the others at 61 DAP (Table 1).

Table 1. Effect of GA₃ treatment on morphological (berry length, width, weight and grape cluster weight) development of seeded and seedless grape cultivars

	35 DAP				45 DAP				61 DAP			
	Control	100 mg/l	200 mg/l	300 mg/l	Control	100 mg/l	200 mg/l	300 mg/l	control	100 mg/l	200 mg/l	300 mg/l
QK	0,40±0,1 ^c	0,42±0,1 ^c	0,45±0,1 ^c	0,55±0,1 ^c	1,00±0,2 ^d	1,06±0,3 ^d	1,12±0,3 ^d	1,37±0,3 ^d	2,52±0,4 ^b	2,41±0,7 ^b	3,0±0,9 ^a	2,5±0,7 ^b
Riz	0,44±0,0 ^c	0,47±0,0 ^c	0,42±0,0 ^c	0,46±0,0 ^c	0,56±0,1 ^c	0,58±0,1 ^c	0,54±0,0 ^c	0,58±0,1 ^c	2,44±0,4 ^b	2,53±0,4 ^b	2,34±0,3 ^b	2,5±0,4 ^b
berry width, sm												
QK	0,26±0,0 ⁱ	0,21±0,0 ⁱ	0,25±0,1 ⁱ	0,17±0,0 ⁱ	0,91±0,3 ^d	1,11±0,3 ^d	1,18±0,3 ^d	1,59±0,3 ^c	1,49±0,5 ^c	1,71±0,3 ^b	1,73±0,3 ^b	2,0±0,3 ^a
Riz	0,26±0,0 ⁱ	0,31±0,0 ^b	0,34±0,0 ^b	0,19±0,0 ⁱ	0,61±0,2 ⁱ	0,73±0,1 ⁱ	0,84±0,3 ^e	0,45±0,1 ^g	1,45±0,4 ^c	1,73±0,4 ^b	1,90±0,4 ^a	1,0±0,4 ^d
berry weight, g												
QK	0,04±0,0 ^j	0,05±0,0 ^j	0,05±0,0 ^j	0,10±0,0 ⁱ	0,66±0,3 ^e	0,82±0,3 ^e	0,86±0,3 ^e	1,68±0,4 ⁱ	3,90±1,3 ^d	4,81±1,3 ^c	4,68±1,3 ^c	6,5±0,7 ^a
Riz	0,42±0,1 ^{gh}	0,34±0,1 ^{gh}	0,19±0,1 ⁱ	0,31±0,1 ^{gh}	1,21±0,3 ^e	0,89±0,5 ^e	0,51±0,3 ^e	0,84±0,3 ^e	6,02±0,8 ^a	5,61±1,3 ^b	5,72±1,4 ^b	4,4±1,5 ^c
cluster weight, g												
QK	48,09±6,9 ^e	63,09±6,9 ^e	81,09±6,9 ^e	102,09±6,9 ^b	172,82±14,4 ⁱ	192,82±14,4 ⁱ	215,82±14,4 ^b	240,82±14,4 ⁱ	322,82±8,0 ^b	356,82±8,0 ^b	392,82±8,0 ^b	429,8±8,0 ^a
Riz	140,8±14,4 ^m	125,8±14,4 ^m	107,8±14,4 ^m	86,8±14,4 ^p	231,5±15,5 ⁱ	211,5±15,5 ^b	272,9±12,9 ^e	163,5±15,5 ⁱ	342,9±12,9 ^e	308,9±12,9 ^d	188,5±15,5 ⁱ	235,9±12,9 ^e

Note: QK - Qora Kishmish, Riz - Rizamat grape cultivars. Values are the means of sections (±SD). Significant differences ($p < 0.05$) between treatments are indicated using different letters according to Tukey's test by R Studio

Table 1 revealed that the berry length of ‘Qora Kishmish’ treated GA₃ with 100 and 300 mg/L⁻¹ recorded intermediate size (2,4 sm and 2,4 sm) without significant differences compared (2,5 sm) to the control across 35, 45 and 61 DAP. Interestingly, 200 mg/L⁻¹ concentration increased 20 % berry length compared to the control whereas the total grape cluster mass overweighed up to 33% treated with 300 mg/L⁻¹. In contrast, ‘Qora Kishmish’ cultivar reached the maximum berry weight at 61 DAP interval treated with 300 mg L⁻¹, approximately 36% (6,5 g) overweighed compared to the control (3.90 g). The specific black color of ‘Qora Kishmish’ completely covered the berry skin at 45 DAP. The value of morphological parameters studied in this experiment were not differently by ‘Toyfi’ cultivar except seedlessness.

Notably, GA₃ treatment with 300 mg/L completely inhibited rudiment formation in all grape berry cultivars (Figure 4). As a result, seeded cultivars were entirely seedless, whereas berries of the untreated control veraisonformed at least one rudiment.

At an early period of rudiment development, we noticed phenotypic change visually in the outer integument layer, demonstrating early wrinkling and inhibiting of growth rate at approximately 20 days after pollination (DAP), especially with GA₃ treatment at 300 mg/L⁻¹ (Figure 5).



Figure 3. Effect of GA₃ treatment on grape cluster of Rizamat cultivar at 65 DAP; *a*- control (untreated), *b*- 100 mg/L⁻¹ GA₃ treatment, *c*- 200 mg/L⁻¹ GA₃ treatment, *d*- 300 mg/L⁻¹ GA₃ treatment



Figure 4. Effect of GA₃ treatment on grape rudiment development in verasion stage. I-Qora Kishmish, II-Rizamat, III-Toyfi, *a*-control, *b*- GA₃ treatment 100 mg L⁻¹, *c*- GA₃ treatment 200 mg L⁻¹, *d* -GA₃ treatment 300 mg L⁻¹ (verasion stage)

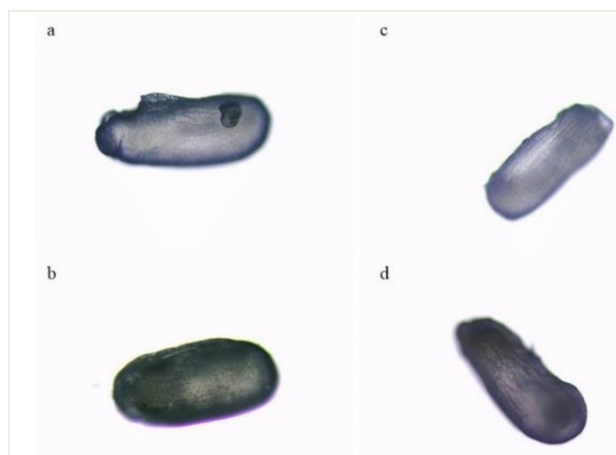


Figure 5. Wrinkled rudiment phenotype following GA₃ treatment Toyfi cultivar,
a - 20 day after pollination (control-untreated), b - 40 day after pollination (control-untreated),
c - 20 day after pollination (GA₃ treatment 300 mg/L⁻¹),
d - 40 day after pollination (GA₃ treatment 300 mg/L⁻¹)

3.3. Embryo development following GA₃ treatment in *in vitro* condition

To test the effect of GA₃ on embryonic development of grape cultivars in *in vitro* conditions, embryo rescue was applied. As shown in Figure 6, from 60 rudiments, embryos were rescued at 65 days after pollination intervals and were cultured on Murashige and Skoog (MS) solid medium (25 ml per Petri dish). Treatment with 300 mg/L⁻¹ of GA₃ completely inhibited embryo development and resulted in total infertility in both seeded cultivars, ‘Rizamat’ and ‘Toyfi’. In contrast, 100 and 200 mg/L⁻¹ of GA₃ treatments did not fully inhibit rudiment growth in either seeded or seedless cultivars; however, embryo differentiation within these rudiments was severely inhibited. Only a few embryos formed under these treatments and all lost their germination potential.

Approximately 27% of the embryos (mean 4.0) rescued from untreated (control) rudiments of ‘Qora Kishmish’ cultivar exhibited normal elongation and subsequently developed into viable plantlets formed shoots and root apices (Figure 7). In contrast to the control, the application of 100 mg/L⁻¹ and 200 mg/L⁻¹ of GA₃ completely inhibited embryo formation and significantly reduced growth rate in *in vitro* conditions. The embryo-inhibitory effect of GA₃ treatment was also observed in two seeded ‘Toyfi’ and ‘Rizamat’ cultivars. Embryo germination in artificial medium decreased up to 9.1%-6.2% with 100 mg/L⁻¹ and 200 mg/L⁻¹ GA₃ by the ‘Toyfi’ cultivar and 4.5%-4.1% at 100 mg/L⁻¹ and 200 mg/L⁻¹ GA₃ by the ‘Rizamat’ cultivar. However, rescued embryos from GA₃-untreated rudiments demonstrated a high rate of germination in artificial medium in both seeded cultivars (Toyfi - 61% and Rizamat - 52%).

Plant species demonstrated diverse responses to GA₃. For instance GA₃ application increased the fruit size of *Prunus salicina* L. up to 62%, weight 94% and acceleration of ripening 3 day prior than control (El-Sharkawy *et al.*, 2014). Several studies report that unpollinated flowers induced parthenocarpic fruits after applying exogenous gibberellins (Gillaspy *et al.*, 1993). However, in *Arabidopsis thaliana*, GA₃ application had a negative effect on fruit growth (Vivian-Smith *et al.*, 2001). In addition, tomato and sweet pepper species formed larger fruits along with inducing parthenocarpic fruit set (Serrani *et al.*, 2007; Tiwari *et al.*, 2012). Induction of parthenocarpic in kiwifruit and bottle gourd was less effective with GA₃ treatment

compared to other plant growth regulators (Lewis *et al.*, 1996). With this experiment, flower of seedless and seeded grapes treated with GA₃ before flower cup dropping to alone at anthesis according to Weaver and others (Weaver & McCune, 1960; Ebadi *et al.*, 1996).

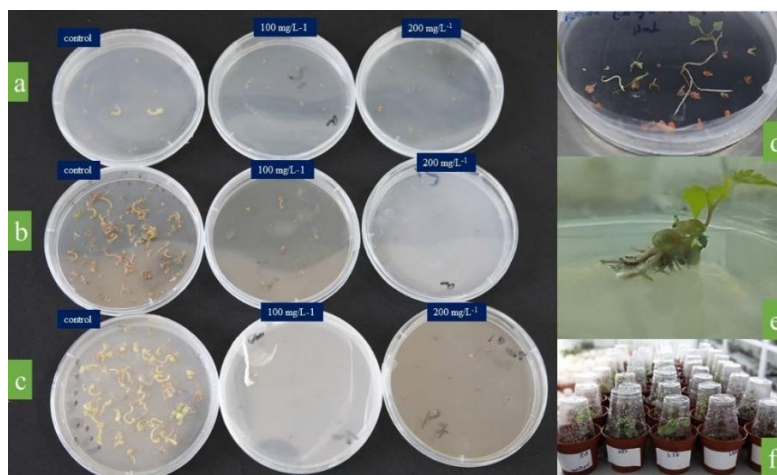


Figure 6. Effect of GA₃ treatment on embryo germination in *in vitro* condition, *a*- Qora Kishmish with control (untreated) and treated GA₃, *b*- Toyfi with control (untreated) and treated GA₃, *c*- Rizamat with control (untreated) and treated GA₃, *d*- rooting of untreated embryo of Rizamat cultivars, *e*- shooting rooting of untreated embryo of Rizamat cultivars, *f*- acclimatization of explants

Berry weight of ‘Qora Kishmish’ cultivar increased the mass up to 36% and average cluster weight to 33% compared to the control (Figure 3). Suehiro *et al.* (2019) reported that GA₃ treatment stimulated the growth in sugar concentration and berry size in grape berries (Casanova *et al.*, 2009). As result a sugar and water content were also accumulated in higher level compared to control. Casanova reported that GA₃ application used for “Emperatriz” seedless cultivars at 80 mg L⁻¹ and 160 mg L⁻¹ dosages led to increase average berry weight by 45% and 80%, compared to control (Casanova *et al.*, 2019). Interestingly, “Emperatriz” seedless showed berry weight increasing only to 20% when GA₃ applied to this cultivar at 290 µM of by other report (Agüero *et al.*, 2000).

When the seeded variety Rizamat was treated with GA₃ at a concentration of 100 mg L⁻¹, the cluster and berry weight decreased by approximately 10% and 7% compared to the control. The highest reduction in grape cluster and berry weight was observed in the experimental variant with a concentration of 300 mg L⁻¹ and the decrease values of both were 45% and 25% less than the control, respectively. It is reported that development of seed and berry formation of Kyoho and Red Globe was inhibited and leading to decrease in berry weight compared to control (Cheng *et al.*, 2015). We found that GA₃ application at 300 mg L⁻¹ dose inhibited seed development and induced seedlessness in both Qora Kishmish and seeded Rizamat cultivars.

Natural seedlessness of *Vitis vinifera* L. cultivars may occur in result of stenospermocarpy or parthenocarpy (Moniruzzaman *et al.*, 2023). Seedlessness of stenospermocarpic *Vitis vinifera* L. cultivars is the most desired trait due to high demand across international consumers. The parthenocarpy *Vitis vinifera* L. cultivar is less profitable for the grape industry due to the lower quality of berries. In the grape

industry, gibberellic acid is widely used to induce seedlessness (Bouquet & Danglot, 1996; Pandolfini, 2009; Royo *et al.*, 2018). The effect of gibberellin acid is a well-characterized and extensively studied subject in plant biology (Coombe & Hale, 1973; B nger-Kibler & Bangerth, 1983; Csukasi *et al.*, 2011). The reduction of seed number and increase of fruit size is one of the traits for improving fruit quality in horticultural practice (Varoquaux *et al.*, 2000). The effect of GA₃ treatment differed significantly in response to the size of inflorescences and bunches; however, yield value and seed number were similar compared to the control in ‘Cabernet Franc’ and ‘Cabernet Sauvignon’ cultivars (Gao *et al.*, 2020). The seed size of *Vitis vinifera* was found to be sensitive to different climate zones (Cabezas *et al.*, 2006; Doligez *et al.*, 2013). We found that exogenous gibberellin acid, due to its elongation effect, can manipulate some parameters of agronomic traits by changing berry size, its mass and compactness of grape cluster, respectively. For instance, seeded ‘Rizamat’ cultivars demonstrated more sensitivity to the 300 mg/L⁻¹ dosage of GA₃, by increasing berry dropp rate and reducing cluster compactness (Figure 5).

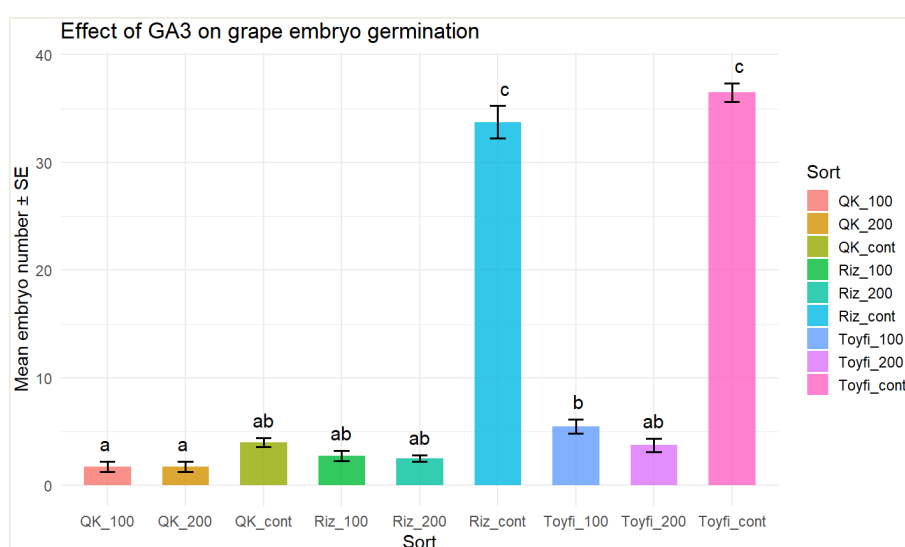


Figure 7. Statistical analyses of GA₃ treatment effect on embryo germination in in vitro condition, cont- control, QK- Qora Kishmish, Riz- Rizamat development following GA₃ treatment (100 -200 mg/L⁻¹). The mean value was taken from four replications, where each contains 15 samples (ANOVA+Tukey HSD, R Studio)

It is reported, that treatment of GA₃ (100 mg/L⁻¹) pre-bloom stage to seeded “Kyoho” and “Red Globe” cultivars induced seedlessness up to 98.6% and 85.2%. However, control plants of that cultivars spontaneously produced up to 3% naturally seedless berries (Cheng *et al.*, 2013). The seedless cultivar of ‘Early Sweet’ born parthenocarpic berries much more after GA₃ treatment at 30 ppm than at 10 ppm, showing sensitivity to the dosages of GA₃ application (Or *et al.*, 2020). It is reported that the absence of seed traces of the ‘Black Finger’ cultivar is indicated by fruit set without fertilization and an increase in the GA₃ level in pistils led to suppressed stenospermocarpy but induced parthenocarpy (Acheampong *et al.*, 2017). Our results are in harmony with those of the previous dated experiment, showing a complete inhibition of seed formation in both seeded (‘Rizamat’ and ‘Toyfi’) and seedless (‘Qora Kishmish’) cultivars, following treatment with 300 mg/L⁻¹ GA₃ (Figure 7).

Application of GA₃ with 100, 200 and 300 mg/L⁻¹ was carried out for improving the fruit quality and fruit set in the ‘Sultanina’ grape cultivar and it was observed that the studied berry size, berry weight, number of berries per cluster, cluster weight, total soluble solids (°Brix) and titratable acidity concentrations showed a significant increase, while the number of berries per cluster decreased compared to the control (Anjum *et al.*, 2020). A following experiment demonstrated that treatment with GA₃ treatment with 320 mg/L⁻¹ influenced growth rate and cluster weight, including several physiological development stages, such as including floral induction, seed germination and fruit and seed formation (Hedden & Kamiya, 1997). In addition, the application GA₃ with 650 mg/L⁻¹ has been showed to growth cluster weight in the ‘Sultanina’ grape cultivar; it was determined that applying 100 mg/L⁻¹ GA₃ produced the most marketable yield with optimal cluster size and berry weight for this cultivar (Korkas *et al.*, 1999). In another study, the effects of GA₃ and Sitofex (CPPU) treatments, either individually or in combination depending on concentration and timing, on shoot and vegetative growth, cluster weight and fruit quality of Thompson seedless grapes were evaluated. According to the results, the effects of the substances applied to the Thompson seedless grape variety on bud movement, vegetative growth, cluster weight and fruit quality were determined. Therefore, it is recommended not to spray Thompson seedless vineyards with high concentrations of Sitofex or GA₃ (3 ppm of CPPU + 40 ppm of GA₃) together, especially during the flowering or berry growth period of the grapes (Elgendy *et al.*, 2012). It could be noticed that the Black Monukka and Red Globe grapevines lost their leaf chlorophyll content, which decreased by increasing the concentration of GA₃ (Duncan, 1995).

Several experimental studies have reported that natural plant hormones and the use of them play a key role during fruit developmental stages by increasing fruit size, reducing seed number and influencing seed size reduction (Pérez *et al.*, 2000; El-Sharkawy *et al.*, 2014; Davière & Achard, 2013; Gao *et al.*, 2020). Recently, an experiment reported the effects of several hormonal changes, including gibberellic acid (GA₃), using embryo rescue technology to assess the correlation between embryo development in seedless grape cultivars. They argued, based on statistical analyzing (embryos rescued from open pollinated for ‘Thompson Seedless’ after 37 days after pollination (DAP), for ‘Flame Seedless’ 45 DAP, for ‘Heshi Seedless’ 60 DAP and ‘Ruby Seedless’ 65 DAP) at DAP intervals, the GA₃ concentration was lower in in vitro ovules with developing embryos than in those without embryos. They found that the GA₃ level decreased significantly if grape berries formed a high number of ovules in the studied seedless grapes and concluded that embryo abortion decreased with lower concentrations of GA₃ (Li *et al.*, 2024). Our experiment is in harmony concentrations of GA₃ with 300 mg/L⁻¹ completely inhibited ovule growth and embryo development and in consequence, embryo abortion in both seedless and seeded cultivars.

4. Conclusion

In this study, we investigated the effect of GA₃ concentrations on grape fruit development in seeded and seedless grape varieties and their role in the loss of embryo viability using the embryo rescue method. Embryos were isolated from rudiments of ‘Rizamat’, ‘Toyfi’ and ‘Qora Kishmish’ cultivars and cultured in vitro condition. Increased GA₃ levels resulted in a decrease in the number of rudiments, which ultimately resulted in their loss. High concentrations of GA₃ completely destroyed

rudiments in both seeded and seedless varieties. Other concentrations of GA₃ had a negative effect on embryo rescue efficacy. Optimal growth rate and development of embryos rescued from rudiments of both cultivars and was observed only in grape clusters not treated with GA₃. This study has enriched our understanding of the interaction between GA₃ concentration and embryo development. We concluded, that the using GA₃ application before flower cup can arise seedlessness for seeded and seedless grape cultivars. The treatment has led to early fruit set, enlargement of berry size because of cell expansion and also reducing seed number of ‘Rizamat’ cultivars, further inducing seedlessness as well.

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Author Contributions

KhAU, SAA: Formal analysis, Data curation, Investigation, Methodology, Visualisation, Resources, Validation, Writing - original draft. AAB: Writing - Data Curation. FIB: Writing - Review, Editing. HNY, MSA, ANY, BOM: Data Curation, Investigation, Resources. ZTB: Conceptualization, Investigation, Methodology, Project administration.

Conflicts of Interest

The authors declare no conflict of interest.

References

- Abdullaev, S.A., Ubaydullaeva, K.A., Eshmurzaev, J.B., Babadjanova, F.I., Abdullaev, A.N., Bolkiev, A.A. & Buriev, Z.T. (2025). Obtaining viable explants from immature embryos of seedless grape cultivars using embryo rescue method. *International Journal of Agriculture and Biology*, 34, 340201. <https://doi.org/10.17957/IJAB/15.2349>
- Abdullaev A.N., Z.T. Buriev., A.M. Asrorov., J.B. Eshmurzaev., A.A. Bolkiev., S.A. Abdullaev., M.M. Darmanov., F.I. Babadjanova., D.E. Usmanov., & Kh.A. Ubaydullaeva. (2024). Evaluation of the Effect of BAP, Kinetin, Meta-Topolin Growth Regulators on Grape Cultivars and Molecular Analysis of SSR Markers. *International Journal of Agriculture and Biology*. 33(01) <https://doi.org/10.17957/IJAB/15.2254>
- Abu-Zahra, T.R. (2013). Effect of plant hormones application methods on fruit quality of Superior seedless grape. *Biosciences Biotechnology Research Asia*, 10(2), 527-531. <http://doi.org/10.13005/bbra/1162>
- Acheampong, A.K., Zheng, C., Halaly, T., Giacomelli, L., Takebayashi, Y., Jikumar, Y., ... & Or, E. (2017). Abnormal endogenous repression of GA signaling in a seedless table grape cultivar with high berry growth response to GA application. *Frontiers in Plant Science*, 8, 850. <https://doi.org/10.3389/fpls.2017.00850>
- Agüero, C., Vigliocco, A., Abdala, G. & Tizio, R. (2000). Effect of gibberellic acid and uniconazol on embryo abortion in the stenospermocarpic grape cultivars Emperatriz and Perlón. *Plant Growth Regulation*, 30(1), 9-16. <https://doi.org/10.1023/A:1006207614388>
- Anjum, N., Feroze, M.A., Rafique, R. & Shah, M.H. (2020). 7. Effect of gibberellic acid on berry yield and quality attributes of grapes cv. sultanina. *Pure and Applied Biology (PAB)*, 9(2), 1319-1324. <https://doi.org/10.19045/bspab.2020.90137>

- Bar, M., Ori, N. (2015). Compound leaf development in model plant species. *Current Opinion in Plant Biology*, 23, 61-69. <https://doi.org/10.1016/j.pbi.2014.10.007>
- Baneh, H. D., Jafari, H., Marandi, R. J., & Abdolahi, R. (2017). Effect of pre-bloom gibberellic acid application on seedlessness and some fruit traits of three Iranian seeded grape cultivars.
- Bermejo, A., Martínez-Alcántara, B., Martínez-Cuenca, M.R., Yuste, R., Mesejo, C., Reig, C., ... & Iglesias, D.J. (2016). Biosynthesis and contents of gibberellins in seeded and seedless sweet orange (*Citrus sinensis* L. Osbeck) cultivars. *Journal of Plant Growth Regulation*, 35(4), 1036-1048. <https://doi.org/10.1007/s00344-016-9602-5>
- Bouquet, A., Danglot, Y. (1996). Inheritance of seedlessness in grapevine (*Vitis vinifera* L.). *Vitis*, 35(1), 35-42. <https://doi.org/10.5073/vitis.1996.35.35-42>
- Bünger-Kibler, S., Bangerth, F. (1982). Relationship between cell number, cell size and fruit size of seeded fruits of tomato (*Lycopersicon esculentum* Mill.) and those induced parthenocarpically by the application of plant growth regulators. *Plant Growth Regulation*, 1(3), 143-154.
- Cabezas, J.A., Cervera, M.T., Ruiz-García, L., Carreño, J. & Martínez-Zapater, J.M. (2006). A genetic analysis of seed and berry weight in grapevine. *Genome*, 49(12), 1572-1585.
- Casanova, L., Casanova, R., Moret, A. & Agustí, M. (2009). The application of gibberellic acid increases berry size of Emperatriz seedless grape. *Spanish Journal of Agricultural Research*, 7(4), 919-927. <https://doi.org/10.5424/sjar/2009074-1105>
- Cheng, C., Jiao, C., Singer, S.D., Gao, M., Xu, X., Zhou, Y., ... & Wang, X. (2015). Gibberellin-induced changes in the transcriptome of grapevine (*Vitis labrusca* × *V. vinifera*) cv. Kyoho flowers. *BMC Genomics*, 16(1), 128.
- Cheng, C., Xu, X., Singer, S. D., Li, J., Zhang, H., Gao, M., ... & Wang, X. (2013). Effect of GA3 treatment on seed development and seed-related gene expression in grape. *PLoS One*, 8(11), e80044.
- Cheng, J., Ma, J., Zheng, X., Lv, H., Zhang, M., Tan, B., ... & Feng, J. (2021). Functional analysis of the gibberellin 2-oxidase gene family in peach. *Frontiers in Plant Science*, 12, 619158. <https://doi.org/10.3389/fpls.2021.619158>
- Clewer, A.G., Scarisbrick, D.H. (2001). *Practical Statistics and Experimental Design for Plant and Crop Science*. New York: John Wiley & Sons.
- Coombe, B. G., Hale, C. R. (1973). The Hormone Content of Ripening Grape Berries and the Effects of Growth Substance Treatments, *Plant Physiology*, Vol.51/4, Pages 629–634
- Csukasi, F., Osorio, S., Gutierrez, J. R., Kitamura, J., Giavalisco, P., Nakajima, M., ... & Medina-Escobar, N. (2011). Gibberellin biosynthesis and signalling during development of the strawberry receptacle. *New Phytologist*, 191(2), 376-390.
- Davière, J.M., Achard, P. (2013). Gibberellin signaling in plants. *Development*, 140(6), 1147-1151. <https://doi.org/10.1242/dev.087650>
- Doligez, A., Bertrand, Y., Farnos, M., Grolier, M., Romieu, C., Esnault, F., ... & This, P. (2013). New stable QTLs for berry weight do not colocalize with QTLs for seed traits in cultivated grapevine (*Vitis vinifera* L.). *BMC Plant Biology*, 13(1), 217.
- Duncan, D.B. (1955). Multiple range and multiple F tests. *Biometrics*, 11(1), 1-42.
- Ebadi, A., May, P., & Coombe, B.G. (1996). Effect of short-term temperature and shading on fruit-set, seed and berry development in model vines of *V. vinifera*, cvs Chardonnay and Shiraz. *Australian Journal of Grape and Wine Research*, 2(1), 1-8.
- Elgendy, R.S.S., Shaker, G.S. & Ahmed, O.A. (2012). Effect of foliar spraying with gibberellic acid and/or sitofex on bud behavior, vegetative growth, yield and cluster quality of Thompson Seedless grapevines. *The Journal of American Science*, 8, 21-34.
- El-Sharkawy, I., Sherif, S., El Kayal, W., Mahboob, A., Abubaker, K., Ravindran, P., ... & Jayasankar, S. (2014). Characterization of gibberellin-signalling elements during plum fruit ontogeny defines the essentiality of gibberellin in fruit development. *Plant Molecular Biology*, 84(4), 399-413. <http://dx.doi.org/10.1007/s11103-013-0139-8>

- Fallahi, E., Heydari, H. & Kilby, M.W. (1995). Maturity, quality, and production of 'Thompson Seedless' grape as affected by frequency of gibberellic acid sprays with and without naphthaleneacetic acid. *Journal of Small Fruit & Viticulture*, 3(1), 49-61.
- Fayziev, J.N., Khakimov, A.A. & Abdullaev, A.N. (2020). The Influence of growth and development regulators on fertility parameters of seedless grape varieties. *International Journal of Scientific and Technology Research*, 9(3), 1959-1961.
- Gao, X.T., Wu, M.H., Sun, D., Li, H.Q., Chen, W.K., Yang, H.Y., ... & He, F. (2020). Effects of gibberellic acid (GA₃) application before anthesis on rachis elongation and berry quality and aroma and flavour compounds in *Vitis vinifera* L. 'Cabernet Franc' and 'Cabernet Sauvignon' grapes. *Journal of the Science of Food and Agriculture*, 100(9), 3729-3740. <https://doi.org/10.1002/jsfa.10412>.
- Gillaspy, G., Ben-David, H. & Gruissem, W. (1993). Fruits: a developmental perspective. *The Plant Cell*, 5(10), 1439.
- Hedden, P., Kamiya, Y. (1997). Gibberellin biosynthesis: Enzymes, genes and their regulation. *Annual Review of Plant Biology*, 48(1), 431-460.
- IPGRI (1997). *Descriptors for Grapevine: Vitis spp.* Rome, International Plant Genetic Resources Institute, 62.
- Karaagac, E., Vargas, A.M., de Andrés, M.T., Carreño, I., Ibáñez, J., Carreño, J., ... & Cabezas, J.A. (2012). Marker assisted selection for seedlessness in table grape breeding. *Tree Genetics & Genomes*, 8(5), 1003-1015. <https://doi.org/10.1007/s11295-012-0480-0>.
- Kim, H.G., Choi, D.G. & Kang, I.K. (2008). Effect of growth regulator treatments on quality and growth in 'Gailiangmeru' grape (*Vitis* spp.). *Acta Horticulturae*, 772, 319-322.
- Korkas, E., Nerantzis, E., Kourtidou-Tymba, P. & Banilas, G. (1999). The effect of gibberellic acid application at different phenological growth stages on yield and quality parameters of 'Sultanina' table grapes (*Vitis vinifera* L.) in Greece. Part I. At development of flower cluster and at fruit set bloomtime. *Vitic. Enol. Sci.*, 54, 44-53.
- Koura, S., Hasegawa, K., Yamamoto, Y. & Yonemoto, Y. (2001, August). Fruit set and fruit growth of seedless cherimoya (*Annona cherimola* Mill.) induced by GA₃ under greenhouse cultivation in Japan. In *IX International Symposium on Plant Bioregulators in Fruit Production* 653, 63-66.
- Leão, P.C.D.S., Carvalho, J.N.D. (2023). Assessment of table grape progenies and correlation between seedlessness and other agronomic traits. *Revista Brasileira de Fruticultura*, 45, e-225.
- Lewis, D.H., Burge, G.K., Hopping, M.E. & Jameson, P.E. (1996). Cytokinins and fruit development in the kiwifruit (*Actinidia deliciosa*). II. Effects of reduced pollination and CPPU application. *Physiologia Plantarum*, 98(1), 187-195.
- Li, G., Li, K., Lu, Y., Fan, X. & Wang, L. (2024). The correlation between embryo rescue and hormonal changes in seedless grapes. *Frontiers in Plant Science*, 15, 1460886. <https://doi.org/10.3389/fpls.2024.1460886>
- Moniruzzaman, M., Darwish, A.G., Ismail, A., El-Kereamy, A., Tsoleva, V. & El-Sharkawy, I. (2023). Seedlessness trait and genome editing - a review. *International Journal of Molecular Sciences*, 24(6), 5660.
- Murashige, T., Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum*, 15(3).
- OIV (1983). *Descriptor List for Grapevine Varieties and Vitis Species*. Paris, Organisation Internationale de la Vigne et du Vin, 232.
- Or, E., Oren, O., Halaly-Basha, T., Koilkonda, P., Shi, Z., Zheng, C. & Acheampong, A.K. (2020). Gibberellin induced shot berry formation in cv. Early Sweet is a direct consequence of high fruit set. *Horticulture Research*, 7.
- Pandolfini, T. (2009). Seedless fruit production by hormonal regulation of fruit set. *Nutrients*, 1(2), 168-177.

- Pérez, F.J., Viani, C. & Retamales, J. (2000). Bioactive gibberellins in seeded and seedless grapes: Identification and changes in content during berry development. *American Journal of Enology and Viticulture*, 51(4), 315-318.
- Pratt, C. (1971). Reproductive anatomy in cultivated grapes - a review. *American Journal of Enology and Viticulture*, 22(2), 92-109. <https://doi.org/10.5344/ajev.1971.22.2.92>
- R Core Team (2021). *R: A language and environment for statistical computing*. Austria, Vienna, R Foundation for Statistical Computing.
- Ramming, D.W., Emershad, R.L. & Tarailo, R. (2000). A stenospermocarpic, seedless *Vitis vinifera* × *Vitis rotundifolia* hybrid developed by embryo rescue. *HortScience*, 35(4), 732-734. <https://doi.org/10.21273/HORTSCI.35.4.732>
- Royo, C., Torres-Pérez, R., Mauri, N., Diestro, N., Cabezas, J.A., Marchal, C., ... & Carbonell-Bejerano, P. (2018). The major origin of seedless grapes is associated with a missense mutation in the MADS-box gene VviAGL11. *Plant physiology*, 177(3), 1234-1253.
- Satisha, J., Sampath, P.K. & Upreti, K.K. (2021). Optimization of GA3 concentration for improved bunch and berry quality in grape cv. Crimson Seedless (*Vitis vinifera* L.). *Journal of Horticultural Sciences*, 16(2), 177-184.
- Serrani, J.C., Fos, M., Atarés, A. & García-Martínez, J.L. (2007). Effect of gibberellin and auxin on parthenocarpic fruit growth induction in the cv Micro-Tom of tomato. *Journal of Plant Growth Regulation*, 26(3), 211-221.
- Stout, A.B. (1936). *Seedlessness in Grapes*. Geneva: New York State Agricultural Experiment Station.
- Suehiro, Y., Mochida, K., Tsuma, M., Yasuda, Y., Itamura, H. & Esumi, T. (2019). Effects of gibberellic acid/cytokinin treatments on berry development and maturation in the yellow-green skinned 'Shine Muscat' grape. *The Horticulture Journal*, 88(2), 202-213. <https://doi.org/10.2503/hortj.UTD-046>
- Tiwari, A., Offringa, R. & Heuvelink, E. (2012). Auxin-induced fruit set in *Capsicum annuum* L. requires downstream gibberellin biosynthesis. *Journal of Plant Growth Regulation*, 31(4), 570-578.
- Usha, K., Kashyap, D. & Singh, B. (2005). Influence of gibberellic acid and N6-benzyladenine on the development of seed and shot berries in the seedless grape (*Vitis vinifera* L.) cv. Perlette. *Australian Journal of Agricultural Research*, 56(5), 497-502.
- Varoquaux, F., Blanvillain, R., Delseny, M. & Gallois, P. (2000). Less is better: New approaches for seedless fruit production. *Trends in Biotechnology*, 18(6), 233-242.
- Vivian-Smith, A., Luo, M., Chaudhury, A. & Koltunow, A. (2001). Fruit development is actively restricted in the absence of fertilization in *Arabidopsis*. *Development*, 128, 2321-2331.
- Weaver, R.J., McCune, S.B. (1960). Further studies with gibberellin on *Vitis vinifera* grapes. *Botanical Gazette*, 121(3), 155-162.
- Weaver, R.J., Van Overbeek, J. & Pool, R.M. (1965). Induction of fruit set in *Vitis vinifera* L. by a kinin. *Nature*, 206(4987), 952-953.