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# Effects of Sodium Nitroprusside and Gibberellic Acid Applications on Direct Germination of Fully Mature Grapevine Seeds and Seedling Growth

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## HIGHLIGHTS

- The in vivo germination protocol of fully mature grapevine seeds just after harvest was determined.
- The grapevine seeds separated from the berries just after harvest, before drying, were germinated in vivo.
- Drying the mature seeds reduced the germination rate.
- NO generator Sodium nitroprusside (SNP) and $GA_3$ increased germination and plant transformation rates and vegetative development parameters of seedlings.
- Genotypic differences in germination rates and vegetative growth characteristics were significant.

**Abstract**

Seeds are widely used in grapevine breeding to obtain new genotypes; however, physical and physiological constraints in seeds reduce the germination rate and slow down breeding programs. The germination rate of mature grapevine seeds and in vitro methodologies that shorten the process of obtaining seedlings have been described. Still, the early germination of fully mature seeds in vivo has not been adequately studied. In this study, the effects of Sodium nitroprusside (SNP) and Gibberellic Acid ($GA_3$) on germination stimulation were examined. At the same time, fresh (F) and dried (D) seeds were used to determine the drying effect of the seeds separated from the fruit flesh. The effects of control (pure water), SNP (100–500 µM), and $GA_3$ (1–5 $gL^{-1}$) 24-hour immersion applications on seed germination, vegetative development, and seedling development were evaluated. While SNP (F-500 µM SNP in cv. Gök Üzüm, 77.94%) and $GA_3$ (F-$GA_3$ 1 $gL^{-1}$ in cv. Royal 70.87%) applications increased germination and plant transformation rates, the effects of applied dose were relatively limited. $GA_3$ treatments promoted germination more in cv. Royal and SNP in cv. Gök Üzüm. $GA_3$ (2.5 $gL^{-1}$) and SNP (500 µM) applications also increased shoot growth and leaf chlorophyll contents. The technique of SNP and GA3 applications can contribute to and accelerate breeding programs carried out with seed-producing vines, as it provides higher germination rates and seed germination and seedling production immediately after the fully mature clusters are harvested.

**Keywords:** Vine; fully matured seed; seedling: germination: stimulants

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## 1. Introduction

Vines are commercially propagated by grafting and cuttings. Seed propagation is used in breeding programs to obtain new hybrids (Ritschel et al. 2014; dos Santos et al. 2019). Dormancy of grapevine seeds results in low germination rates, which disrupts grapevine breeding programs (Ellis et al. 1983; Maeda et al. 1985; Pommer et al. 1988; Conner 2008; Generoso et al. 2019;). Physical dormancy in grapevine seeds is due to the waterproof seed coat (Eriş and Düring 1978; Conner 2008), and it is associated with high endogenous abscisic acid (ABA) levels (Rajasekaran et al. 1982). Cold stratification can lead to a decrease in ABA levels. To eliminate dormancy in grape seeds, stratification is done for 75-90 days in a humid environment at temperatures between 0-5 °C (Ellis et al. 1983; Pommer et al. 1988; Selim et al. 2016; do Brasil and Brasília 2016; Çelik 2014) or with $GA_3$ pre-applications (Kang 1968; Kachru 1969; Manivel and Weaver 1974; Selim et al. 1981) where the benefits of cold stratification can be combined (Kachru 1969; Manivel and Weaver 1974; Pal and Singh 1976; Chohan and Dhillon 1976; Selim et al. 1981; Ergenoglu et al. 1996) have been reported. Recommended $GA_3$ doses are 0.05 - 8 $gL^{-1,}$ and application time varies from 15 seconds to 24 hours (Kang 1968; Manivel and Weaver 1974; Selim et al. 1981; Kara et al. 2020). These methods require significant time, and germination rates often remain below 50%.

Levels of plant growth regulators are effective in stimulating germination or breaking dormancy in seeds, especially GA inactivates ABA in dormant seeds, accelerates the hydrolysis of starch and storage proteins, and stimulates seed germination (Cardemil and Reinero 1982; Hopkins 1995).

Endogen $GA_3$ application to seeds accelerates germination by increasing $\alpha$-amylase activity (Wurzburger et al. 1974), initiates germination by replacing environmental effects such as stratification (Özen and Onay 1999), light and temperature, and directly stimulates embryo growth by providing hydrolysis events in the endosperm (Ünal et al. 2004), ensures elongation by enlarging the cells in the elongation zone (Hopkins 1995). Significant differences have been determined between germination rates and processes depending on factors of applications and grapevine genotypes (da Costa Júnior et al. 2022; Ergenoglu et al. 1996).

Reactive oxygen species (ROS) play an important role in regulating germination under both normal and stress conditions. ROS accumulation is required to break seed dormancy in many plant species (Bailly et al. 2008; Gniazdowska et al. 2010a; Leymarie et al. 2012). $H_2O_2$, a ROS, accumulates more in seeds germinated in saline conditions (Lee et al. 2010; Lin et al. 2012). Hydrogen peroxide mediates the interaction between ABA and $GA_3$ in the stimulation of seed germination in *Arabidopsis* (Leymarie et al. 2012).

Nitric Oxide (NO) acts as a signaling molecule involved in the control and regulation of various plant responses to environmental stresses at almost all stages of development (Delledonne et al. 1998; Durner and Klessig 1999; García-Mata and Lamattina 2001; Beligni et al. 2002; Zhao et al. 2004; Shi et al. 2005; Zhao et al. 2007; Wang et al. 2009), seed germination, root development, cell senescence and stomatal closure (Garcia-Mata et al. 2003) depending on the concentration and the metabolic state in which it is involved, protective or it is also toxically involved in apoptosis (Beligni and Lamattina 2002). The protective effects of NO have been associated with the regulation of ROS levels and toxicity, which are involved in lipid peroxidation (Sharpe et al. 2003; Hung and Kao 2004; Hummel et al. 2006).

NO protects cell subunits of membrane-bound enzymes and substrates by regulating tissue antioxidant capacity and lipid peroxidation (Duan et al. 2007), often providing its regulatory activity through tight coordination with other molecules such as ROS (Wang et al. 2009; Gniazdowska et al. 2010b). Cyanide (CN) is the primary volatile dormancy-breaking compound produced by NO. The donor SNP (Bethke et al. 2006) stimulates germination by enabling ethylene production (Gniazdowska et al. 2007; Ahlfors et al. 2009; García et al. 2011; Soltys et al. 2012) under various conditions such as ozone stress and ion deficiency (Gniazdowska et al. 2010b; Liu et al. 2010). However, there is a lack of information on the effects of NO on the germination of fully matured grape seeds. Although low (10-4–10-8 M) doses of SNP significantly increased germination percentage, root length and shoot control, high doses (10-3–1 M) inhibited seed germination, root and shoot growth, and maximum inhibition was recorded when seeds were soaked in 1 M SNP. 10-5 M SNP provided significant increases in germination rate, root and shoot length (Hayat et al. 2014).

To accelerate breeding programs, in vitro, germination of immature embryos has been used in different species such as grapevine (Val et al. 2010; Generoso et al. 2019; Costa Júnior et al. 2022), pepper (Walter et al. 2018), palm (Mbi et al. 2016). For the same purpose, the production of seedlings from immature embryos has also been studied (Yang et al. 2007; Tian et al. 2008; Tang et al. 2009; Li et al. 2015; Li et al. 2020). However, there is a paucity of studies investigating the early germination of mature grapevine seeds in vivo.

We aimed to develop methodologies that increase the germination rate of fully matured grapevine seeds and shorten the process of obtaining seedlings. For this purpose, we examined the effects of $GA_3$ and SNP (NO donor) applications on the promotion of germination in both F and D seeds of cv. Gök Üzüm and cv. Royal immediately after harvest.

## 2. Materials and Methods

In the experiment, two grape varieties (cv. Gök Üzüm and cv. Royal), two seed types (F and D), (0 (Control), three SNPs (100 μM, 250 μM, and 500 μM), and three $GA_3$ (1 $gL^{-1}$, 2.5 $gL^{-1}$ and 5 $gL^{-1}$) was applied by immersion for 24 hours. In the control, the seeds were soaked in pure water for 24 hours. The seeds were germinated in petri dishes in an environment adjusted to 25°C temperature and 16:8 h (dark: light) photoperiod.

Germinated seeds were grown by planting them in 8*8*10 cm plastic containers in a 3:1 peat: perlite mix media. The study was conducted in a randomized parcel trial design with three replications. Variance analysis was performed on the numerical data obtained in the SPSS 17.0 statistical program and compared with the Student's t-test at the p=0.05 significance level. The effects of pre-germination treatments on seedling development were evaluated with three-way ANOVA.

In the experiment, the seeds of cv. Gök Üzüm (autochthonous variety of Konya Türkiye) and cv. Royal (Karistvala Kolkhuri × Muscat Hamburg) were taken from grapevines in the Selcuk University Faculty of Agriculture Collection and Used. Fully matured clusters of both grape varieties were harvested (August 25, 2021), the clusters were squeezed, the seeds were removed and washed to remove the fruit flesh, and the empty seeds were removed by dipping into water and excluded from the experiment (Olmo 1934). F seeds were forced to germinate by pre-treatments. To determine the effect of drying the seeds after harvest, the washed seeds were laid on drying papers in room temperature without direct sunlight, dried for 72 hours in room conditions, and then used in pre-germination applications as dried (D) seeds. Germination stimulating chemicals $GA_3$ (Cas No. 77-06-5) and SNP (Cas No: 13755-38-9) were obtained from Ciba-Geigy AG.

### *2.1. Effects on seed germination and plant transformation rates*

When the root tips of the seeds germinated in petri dishes containing agricultural perlite reached approximately 2 mm in length, they were considered germinated and transferred to the growth medium. % germination values were determined by the ratio of germinated seeds to the number of seeds treated before germination. Among the plants that developed from germinated seeds planted in growing containers, those that formed their first true leaves were considered to have turned into plants, and their conversion rates to plants were determined by counting them.

### *2.2. Shoot length (cm) and diameter (mm)*

The lengths of the seedlings grown for 70 days were measured with a tape measure, and the shoot lengths (cm), and the diameters were determined by measuring the distance between the 1st and 2nd nodes from two directions with a digital caliper.

### *2.3. Leaf area ($cm^2$), fresh and dry weights (g)*

The leaves in the middle 1/3 of the shoots were taken from each replicate and evaluated as leaf samples. Leaf areas were determined with the Photoshop portable (PS 12.1) package program. Fresh leaf weights were determined by weighing leaf samples. Leaf dry weight was determined by drying in an oven at 70 °C for 72 hours and weighing.

*2.4. Leaf chlorophyll content (SPAD value)*

Chlorophyll contents of 3-4 leaves from the shoot tip of the seedlings were determined with a chlorophyll meter (SPAD-502, Minolta, Japan).

*2.5. Root fresh and dry weight (g)*

All roots of the seedlings grown in containers for 70 days were cut 3 cm away from the root branching point. After washing, the root fresh weights were determined (g), and these roots were kept in the oven (70 °C) for 72 hours and weighed to determine the root dry weights.

## 3. Results

*3.1. Germination and Seedling Rate (%)*

$GA_3$ and SNP were applied to the F and D seeds of cv. Gök Üzüm and cv. Royal immediately after harvest affected the germination rates (Figure 1a). While the lowest germination rate of cv Gök Üzüm was recorded in the control, the highest germination rate was in the D-$GA_3$ 5 $gL^{-1}$ application. The highest germination rate in F seeds was obtained in the F-$GA_3$ 5 $gL^{-1}$ application.

In the D seeds in the cv. Royal, the lowest germination rate was in the control and the highest was in the D-$GA_3$ 5 $gL^{-1}$ application. In the F seeds, the lowest germination rate was in control, while the highest germination rate was again in the F-$GA_3$ 5 $gL^{-1}$ application (Figure 1a).

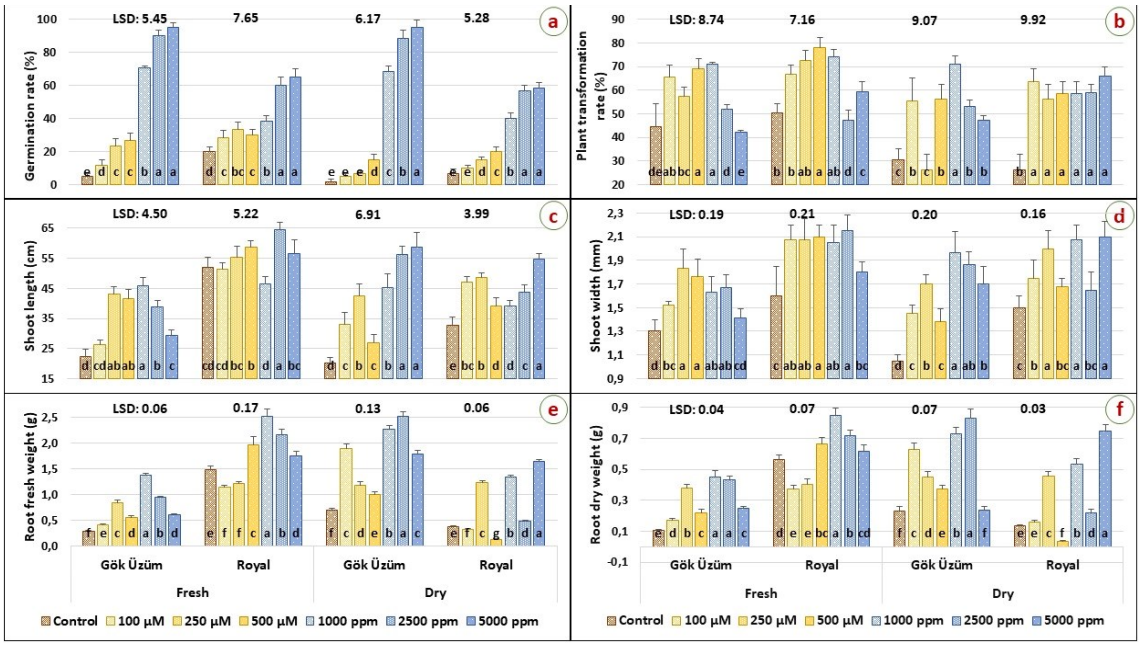


**Figure 1**. Effects of SNP and $GA_3$ treatments on germination rate (a), plant transformation (b), shoot length (c), shoot width (d), root fresh weight (e), and root dry weight (e). Statistically significant ($p<0.05$) differences were indicated by different letters on the bars. Standard error values are given one-sided on the bars.

$GA_3$ and SNP applications affected seedling emergence rates in both grapevine varieties (Figure 1b). Considering only the control, the seedling conversion rate of both cultivars in applications using F seeds was higher in the cv. Royal (50.23%) than in the cv. Gök Üzüm (44.44%). In cv. Gök Üzüm, the effects of SNP applications on the seedling rate were higher in F seeds than in D seeds. The highest seedling rates were determined in F-500 µM SNP (68.93±4.32) and F-$GA_3$ 1 $gL^{-1}$ (70.87±0.96) applications.

In the F seeds of the cv. Royal, all SNP applications increased the seedling conversion rates (Figure 1b), and the maximum value was obtained from the F-500 µM SNP application (77.94±4.34%). In the F-$GA_3$ 1 $gL^{-1}$ application, the seedling conversion rate was 74.01±3.22%. In general, in applications where F seeds are used,

the rate of transformation into plants is higher than that of D seeds. SNP applications increased the rate of transformation into plants more than $GA_3$ applications.

*3.2. Shoot Length (cm) and Width (mm)*

$GA_3$ and SNP applications to F and D seeds of both grape varieties before germination increased shoot growth, although it varied according to the applied dose (Figure 1c). In F seeds, the maximum shoot length was determined in the F-250 μM SNP application (43.25±2.38 cm) in the Royal cultivar, while it was 42.52±4.03 cm in the D-250 μM SNP application in the Gök Üzüm cultivar. This value was 45.83±2.93 cm in the F-$GA_3$ 1 $gL^{-1}$ application, and 45.33±4.48 cm in the D-$GA_3$ 1 $gL^{-1}$ application (Figure 1c).

The height of the seedlings developed from cv. Royal F-seeds were greater in all treatments using control, SNP, and $GA_3$ than in all treatments using D-seeds. In general, the shoot length of cv. Royal seedlings were longer than those of cv. Gök Üzüm seedlings. The maximum shoot length was determined as 58.57±2.40 cm in the F-500 μM SNP application and 64.51±2.59 cm in the F-$GA_3$ 2.5 $gL^{-1}$ application, while it was 48.75±1.25 cm in the D-250 μM SNP application and 54.67±2.02 cm in the D-$GA_3$ 5 $gL^{-1}$ application.

$GA_3$ and SNP applications to the seeds of both grape varieties affected the shoot width (Figure 1d). Control and F-SNP applications increased shoot width. In D-seeds that were used, a thicker shoot width was obtained in the control and SNP treatments. In other words, shoot width remained weaker in D-$GA_3$ applications. All doses of both SNP and $GA_3$ applications supported shoot thickening. The maximum shoot width was 2.15±0.13 mm in the F-$GA_3$ 2.5 $gL^{-1}$ application, and 2.08±0.13 mm in the D-$GA_3$ 5 $gL^{-1}$ application in Royal.

*3.3. Root Fresh and Dry Weight (g)*

$GA_3$ applications increased the root fresh weight of seedlings developed from F-seeds and D-seeds of both grape varieties more than SNP. This increasing effect was more evident in those treated with D-$GA_3$, but no significant change could be noticed with the increase in dose.

In the seedlings developed from cv. in Royal F-seeds, the highest fresh root weight (2.52±0.14 g) was obtained from the F-$GA_3$ 1 $gL^{-1}$ application, while in the seedlings developed from the D-seeds, the highest value (1.65±0.04 g) was obtained from D-$GA_3$ 5 $gL^{-1}$ application (Figure 1e).

While pre-germination applications to F and D seeds increased root dry weights, the highest values were in F-$GA_3$ 1 $gL^{-1}$, D-$GA_3$ 2.5 $gL^{-1,}$ and F-250 μM SNP applications (Figure 1f).

In the cv. Royal F-seeds applications, F-100 μM SNP and F-250 μM SNP, gave lower root dry weight than the control (0.56±0.03 g), while the highest value was obtained from the F-$GA_3$ 1 $gL^{-1}$ (0.85±0.05 g) application. In the D seeds applications, the lowest root weight (0.04±0.00 g), including the control (0.13±0.01 g), was obtained from the D-500 μM SNP application, while the highest value (0.75±0.04 g) was recorded in the D-$GA_3$ 5 $gL^{-1}$ application.

*3.4. Leaf Fresh and Dry Weight*

The leaf dry weights of the seedlings developed from the cv. Gök Üzüm F-seeds were higher in the control and SNP-applied ones. D-$GA_3$ applications increased fresh leaf weights in seedlings. While the fresh leaf weight was 0.45±0.06 g in the control of F-seeds, it was recorded as 0.89±0.04 g in the F-500 μM SNP application. The fresh leaf weight was 0.18±0.02 g in the D-seeds control and 0.84±0.08 g in the D-$GA_3$ 2.5 $gL^{-1}$ application (Figure 2a).

The fresh leaf weight of the cv. Royal seedlings of F-seeds were determined as 0.54±0.04 g in the control, 0.74±0.01 g in the F-500 μM SNP application, and 0.94±0.06 g in the F-$GA_3$ 2.5 $gL^{-1}$ application, while it was 0.48±0.06 g in the D-seeds control. D-500 μM SNP and D-$GA_3$ in 2.5 $gL^{-1}$ applications were recorded as 0.60±0.08 g and 0.69±0.03 g, respectively (Figure 2a).

The effects of SNP and $GA_3$ applications on F and D seeds of both grape cultivars on leaf dry weight were significant and similar to fresh leaf weights (Figure 2b). In the cv. Gök Üzüm, the lowest (0.050±0.008 g) leaf dry weight was determined in the F-$GA_3$ 5 $gL^{-1}$ application, which was also below the control. While the lowest leaf dry weight of this variety was determined as 0.026±0.004 g in the D-seeds control, it was 0.132±0.021 g and 0.168±0.011 g in D-SNP 500 μM and D-$GA_3$ 2.5 $gL^{-1}$ applications, respectively (Figure 2b).

The dry leaf weight of the cv. Royal seedlings developed from F-seeds was 0.087±0.005 g (F-250 µM SNP) and 0.194±0.011 g (F-$GA_3$ 2.5 $gL^{-1}$), and in D-seeds, it was 0.098±0.015 g (control) and 0.147±0.006. g (D-$GA_3$ 2.5 $gL^{-1}$) and no significant change could be determined between applied doses (Figure 2b).

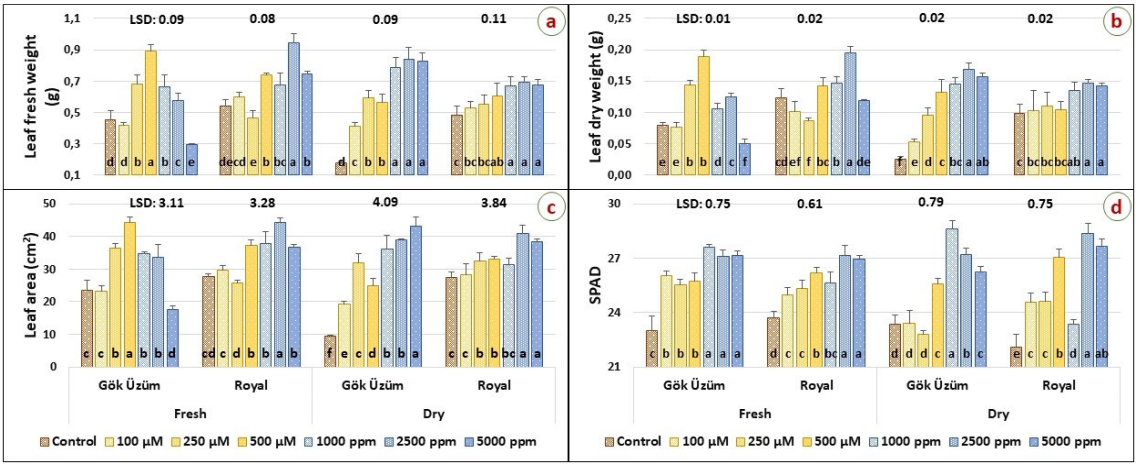


Figure 2. Effects of SNP and $GA_3$ treatments on leaf fresh weight (a), leaf dry weight (b), leaf area (c), and leaf chlorophyll content (SPAD value) (d). Statistically significant ($p<0.05$) differences were indicated by different letters on the bars. Standard error values are given one-sided on the bars.

*3.5. Leaf Area*

In the cv. Gök Üzüm F and D seeds, 250 µM and 500 µM SNP, $GA_3$ 1 $gL^{-1,}$ and $GA_3$ 2.5 $gL^{-1}$ applications increased the leaf area (Figure 2c). In the cv. For Royal F and D seeds, the highest leaf area was obtained from the D-$GA_3$ 2.5 $gL^{-1}$ application. Only in the F 250 µM SNP application, the average leaf area remains below control (Figure 2c).

*3.6. Leaf Chlorophyll Content (SPAD Value)*

SNP and $GA_3$ applications increased leaf chlorophyll content in the cv. Gök Üzüm F-seeds seedlings (Figure 2d), and in the D-seeds seedlings, the highest leaf chlorophyll content was obtained by D-$GA_3$ 1 $gL^{-1}$ application. In both cultivars F and D-seeds applications, $GA_3$ increased leaf chlorophyll content more than SNP, but the effects of dosages were not clear.

*3.7. Effects of Pre-applications on Germination and Seedling Development*

The effects of pre-germination treatments on germination and seedling development were examined by genotype (two grape cultivars), use of seeds in fresh (F) and dried (D), and pre-germination treatments (0 (Control), three SNPs (100 µM, 250 µM, and 500 µM), and three $GA_3$ (1 $gL^{-1}$, 2.5 $gL^{-1,}$ and 5 $gL^{-1}$) data were evaluated by three-way analysis of variance (Table 1). In the germination rate, genotype*drying*application was insignificant, other parameters and the relationships between them were important. In the seedling conversion rate, genotype*drying was unimportant, other parameters and the relationships between them were important. All parameters examined and the relationships between them regarding shoot length and root dry weight were important.

In assessing effects on shoot width, genotype*treatment was unimportant. Other parameters evaluated in this context and the relationships between them were important. In terms of root and leaf fresh weight, drying was unimportant, other parameters and the relationships between them were important. In leaf dry weight evaluation, drying and genotype*drying were insignificant, other parameters and the relationships between them were important. In leaf area evaluation, genotype*drying was unimportant, other parameters and their relationships were important. In the evaluation of leaf chlorophyll content effects, genotype was unimportant, while other parameters and their relationships were important. When the applications were evaluated as a whole, SNP and $GA_3$ applications applied to F and D seeds before germination increased the germination rate and plant transformation rates in the seeds of both grape cultivars and generally encouraged seedling development.

**Table 1**. Three-way Anova for relationships between treatments

| | Germination rate | Plant transformation rate | Shoot length | Shoot width | Root fresh weight | Root dry weight | Leaf fresh weight | Leaf dry weight | Leaf area | SPAD |
|---|---|---|---|---|---|---|---|---|---|---|
| Genotype (G) | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.192 |
| Drying (D) | 0.000 | 0.000 | 0.000 | 0.006 | 0.053 | 0.000 | 0.074 | 0.111 | 0.010 | 0.000 |
| Application (A) | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| G*D | 0.015 | 0.950 | 0.000 | 0.008 | 0.000 | 0.000 | 0.000 | 0.079 | 0.776 | 0.044 |
| G*A | 0.000 | 0.000 | 0.000 | 0.120 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| D*A | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| G*D*A | 0.053 | 0.003 | 0.000 | 0.003 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |

## 4. Discussion

Since physical and physiological dormancy in grapevine seeds (Rajasekaran et al. 1982; Ellis et al. 1983; Maeda et al. 1985; Pommer et al. 1988; Conner 2008; Val et al. 2010; Generoso et al. 2019) reduces or delays seed germination (Generoso et al. 2019), $GA_3$, soaking and beak cutting (Wang et al. 2022), SNP and for 90 days of stratified seeds $GA_3$ application (Kara et al. 2020), for remove seeds from berries five weeks after flowering $GA_3$ and Kinetin applications (Pandey and Singh 1988), for removed immature zygotic embryos 80 days after flowering keeping them in 2.5 $gL^{-1}$ $GA_3$ + 55 °C water for 15 minutes and then keeping them at room temperature for 24 hours (Zhang et al. 2023) were recommended.

In a previous study, cv. Romi seeds that were intended to germinate immediately after harvest did not germinate. When the seeds were stratified at 5 - 18 °C for 30 - 120 days, as the stratification time increased and at the same time the dose of $GA_3$ was increased from 0.05 $gL^{-1}$ to 5 $gL^{-1}$ the germination rate increased. By soaking in pure water for 24 hours, the germination rate reached 4.2% recorded (Selim et al. 1981).

Ergenoglu et al. (1996), reported that stratification at 5 °C for 21 days increased the germination rate and shortened the germination time and that $GA_3$ applications caused excessive elongation and poor development in seedlings. In previous studies, stratification was accepted necessary to break dormancy in seeds (Ellis et al. 1983; Pommer et al. 1988), and the proposed methodologies were time-consuming and relatively low-yield (Ellis et al. 1983; Pommer et al. 1988; Conner 2008) and depends on the genotype (da Costa Júnior et al. 2022).

$GA_3$ applied to seeds generally increases germination. It promotes growth by increasing the plastids in the cell walls, converts carbohydrates into sugar, and reduces the pressure on the cell wall. Thus, it ensures water intake into the cell and cell elongation (Arteca 1996; Hopkins 1995). Moreover, $GA_3$ applications also promote the production of some hydrolase enzymes such as $\alpha$-amylase (Arif et al. 2008). Moreno et al. (2011) indicated that $GA_3$ promotes net carbon fixation and transport, promoting correct carbon allocation to roots and fruits. Dormancy was broken in *in vitro* $GA_3$ applications (Val et al. 2010; Generoso et al. 2019; da Costa Júnior et al. 2022), but in our study, *in vivo* grapevine seeds germination was achieved at high rates (in cv. Gök Üzüm F control 5.00±1.67, in cv. Royal 20.00±2.89) with $GA_3$ and (in cv. Gök Üzüm $GA_3$ 5 $gL^{-1}$ 95.00±2.89, in cv. Royal $GA_3$ 5 $gL^{-1}$ 65.00±5.00) SNP (in cv. Gök Üzüm SNP 500 μM 26.67±4.41, in cv. Royal SNP 250 μM 33.33±4.41) pre-applications to fresh seeds.

Grape seeds have a physical and physiological rest requirement (Cadot et al. 2006; Val et al. 2010; Çelik 2014; Generoso et al. 2019; Kara et al. 2020; Wang et al. 2022). Since physiological dormancy is not found in the embryo, but in the tissues surrounding it (da Costa Júnior et al. 2022), it was possible to break dormancy with SNP and $GA_3$ applications immediately after harvest, before completely drying the seeds.

We broke physical and physiological dormancy in *in vivo* culture with SNP and $GA_3$ applications to seeds with mature zygotic embryos in two grape cultivars immediately after harvest. We obtained a high rate of germination and seedlings from matured grape seeds in the harvest season. Both factors are known to naturally inhibit germination. The decrease in germination rate even after 72 hours of drying proved by this.

In the control of F and D seeds, 24-hour of soaking in water contributed to overcoming both physical and physiological barriers.

There is a great need for new varieties of cultivated plant species that can be produced in a short time. These new genotypes, the products of breeding programs, may be more resistant to biotic and abiotic factors, and productive and high-quality varieties. Methods that will accelerate this process will contribute to the acceleration of plant breeding.

Our results may be a good indication that matured seeds can be used for seed germination regardless of the cultivar or species of the *Vitis* genus. However, further studies with other species, hybrids, and varieties should be conducted for verification.

When our results were evaluated together, applications of NO donor SNP and $GA_3$ to grapevine F and D seeds immediately after harvesting increased germination and plant transformation rates. The effects of SNP and $GA_3$ dosage on germination and transformation into plants remained at relatively limited levels. $GA_3$ treatments promoted germination more compared to SNP. $GA_3$ and SNP applications positively affected shoot length and leaf chlorophyll content.

According to these results, $GA_3$ and SNP can be used to reduce losses in the process of converting seeds obtained through hybridization into plants, especially in grapevine breeding. With the protocol created in our study, a stable and repeatable method was presented that could prevent wasting time during the rest period for seed germination, which is especially necessary for grapevine breeding and other biotechnological studies.

## 5. Conclusions

In this study, the methodology for in vivo germination of fully matured grapevine seeds immediately after harvest was described. Although $GA_3$ promoted the germination of F and D seeds by approximately 40% even at the lowest application, this effect was more limited in the conversion rates to plants (55-65%).

Germination rates were higher in both grape varieties in treatments using F seeds. In D-$GA_3$ treatments, the germination rate was over 65% in the cv. Royal and over 95% in the cv. Gök Üzüm. In mature seeds, drying after harvest reduced the germination rate.

The germination-stimulating effect of SNP treatments was significant, and this activity was higher in D seeds of both grape cultivars.

The stimulatory effect of SNP applications to F seeds was more evident in plant conversion rates, shoot length and diameter, leaf fresh weight, and leaf area.

This methodology increased the number of germinating seeds. By shortening the time required for germination and seedling production, the time required to obtain new grapevine varieties can be reduced and grapevine breeding programs can be accelerated.

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