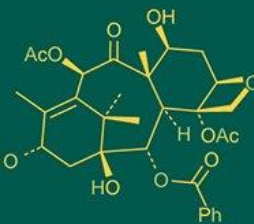
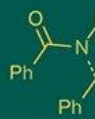


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Breaking seed dormancy in the coloured grape variety 'Red Globe'

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Abstract

The present study was conducted to evaluate the effectiveness of different pre-sowing seed treatments for improving seed germination of grape variety 'Red Globe'. Six treatments comprising combinations of water soaking, de-beaking, hydrogen peroxide (H_2O_2), gibberellic acid (GA_3), α -amylase, and cold stratification were evaluated under a completely randomized design with three replications of 100 seeds each. Significant differences were observed among the treatments. No germination was recorded in seeds subjected to water soaking alone (T_1) or de-beaking followed by water soaking (T_2). De-beaking followed by GA_3 (1000 ppm) treatment (T_3) resulted in 5.33% germination, while GA_3 (1000 ppm) combined with three weeks of chilling at 4 °C (T_4) improved germination to 8.67%. Sequential treatment with 0.5 M H_2O_2 , GA_3 (1000 ppm), and chilling at 4 °C for 3 weeks (T_5) further increased germination to 14.33%. The highest germination (84.00%) was achieved with sequential soaking of seeds in water, H_2O_2 (1M), α -amylase (500 ppm), and GA_3 (100 ppm) and chilling at 4 °C for 4 weeks (T_6). The study demonstrated that the integrated application of oxidative, enzymatic, and hormonal treatments effectively overcame seed dormancy and significantly enhanced germination in "Red Globe" grape seeds.

Keywords: *Vitis vinifera*, seed dormancy, Red Globe, hydrogen peroxide, gibberellic acid, α -amylase, seed germination

Introduction

Grape (*Vitis vinifera* L.) is one of the most economically important fruit crops cultivated worldwide for fresh consumption, wine making, raisin production, and juice. 'Red Globe' is premium coloured and seeded grape variety with high commercial value due to its bold berry size and good eating quality. Natural bold berry size makes it a suitable parent for various breeding programs targeting genetic improvement of fruit quality traits. But 'seed dormancy' present in 'Red Globe' put the challenges for the breeder in producing hybrid progenies. 'Red Globe' is one of the important parent in ongoing coloured grape improvement program conducted by the author. Hence the effort were made to break the seed dormancy particularly with a view to raising grape hybrid seedlings.

Seeds that do not germinate under favorable conditions are considered dormant. Seed dormancy has evolved to ensure that germination is delayed until the environmental conditions for seedling growth and establishment are optimum. Seed dormancy in grape is primarily attributed to the presence of endogenous germination inhibitors, underdeveloped embryo physiology, and restricted mobilization of food reserves. Common dormancy-breaking approaches include mechanical scarification, acid scarification, oxidative treatments, application of growth regulators such as gibberellic acid (GA_3), enzymatic digestion of storage tissues, and cold stratification. Conventional methods such as water soaking and mechanical scarification have shown limited success in overcoming dormancy. Therefore, various physical, chemical, and hormonal treatments have been investigated to improve germination. Kachru *et al.*, 1972 [2]; used stratification, growth substances and subjecting the seeds to running water to induced seed germination in grape variety 'Black Muscat' and found that water soluble inhibitors responsible for masking the effect of growth substances and thereby disturbing the metabolism of germination in the seeds.

Cold stratification is considered one of the most effective methods for reducing physiological dormancy by altering endogenous hormonal balance, particularly decreasing abscisic acid (ABA) while enhancing gibberellin activity.

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Similarly, exogenous application of gibberellic acid (GA₃) stimulates embryo growth and activates hydrolytic enzymes responsible for reserve mobilization. Nafea *et al.*, 2023 [3], observed that seed germination percent of grape variety 'Rashmeri' and seedling growth has positive correlation with the GA₃ concentration and stratification (at 5±1 °C) period. Hydrogen peroxide (H₂O₂) has recently gained attention as an important signaling molecule involved in dormancy release, oxidative metabolism, and activation of antioxidant defense systems during germination. Perko *et al.*, 2019; tried hydrogen peroxide application for enhancing seed germination of grape variety 'Zelenec'. They found hydrogen peroxide has significant impact with higher concentration (30% H₂O₂). Enzymes such as amylase, protease, and lipase are liable for solubilizing spare food material in the form of starch, protein and lipid correspondingly in seed and deliver energy and other fundamentals food material to germinating embryo. The starch is catalyzed by enzyme amylase that provides the food materials for growth and development of embryo (Renu Joshi, 2018) [6]. Combining these treatments may produce synergistic effects leading to enhanced and uniform germination. Kesen *et al.*, 2025 [4], reported that seed germination ability and seedling development of grape seeds show significant differences depending on cultivar and germination conditions. The cultivar, treatment type, and their interaction had significant effects on germination. The present investigation was undertaken to evaluate different pre-sowing seed treatments and identify an effective protocol for improving seed germination in shy germinating grape variety "Red Globe".

Materials and Methods

The experiment was conducted during year 2023-2024. Healthy and freshly harvested matured berries of 'Red Globe' were used to extract the seeds. The seeds were thoroughly washed with running water to remove adhering pulp, air-dried under shade, and used for the experiment. Six treatments involving mechanical scarification, oxidative and enzymatic scarification, gibberellic acid (GA₃) application, and cold stratification were tested. The treatments were as follows: normal seeds soaked in water for 24 hours (T₁); de-beaked seeds soaked in water for 24 hours (T₂); de-beaked seeds soaked in GA₃ at 1000 ppm for 24 hours (T₃); seeds soaked in GA₃ at 1000 ppm for 24 hours, followed by chilling at 4 °C for 3 weeks (T₄); seeds soaked in 0.5 M H₂O₂ for 24 hours, then in GA₃ at 1000 ppm for 24 hours, followed by chilling at 4 °C for 3 weeks (T₅); and sequential soaking of seeds in water for 24 hours, H₂O₂ at 1M for 24 hours, α-amylase at 500 ppm for 48 hours, and GA₃ at 100 ppm for 24 hours followed by chilling at 4 °C for 4 weeks (T₆). Seeds were sown immediately in the germination media comprising vermicompost + perlite + cocopeat + vermiculite (1:1:1:1). Germination was recorded as radicle emergence. Seed Germination percentage was calculated as (number germinated/number seeds treated) × 100. Seeds exhibiting normal radicle emergence were considered germinated. The experiment was laid out in a Completely Randomized Design (CRD) comprising six seed treatments with three replications and statistical analysis was carried out in MS-Excel. Each replication consisted of 100 seeds. For each treatment the number of seeds treated and the number germinated were recorded.

Results

The effect of different pre-sowing seed treatments on the germination of 'Red Globe' seeds was evaluated under six treatment combinations, each comprising three replications of 100 seeds (Table 1). Significant variation in seed germination was observed among the treatments, indicating that the germination response was markedly influenced by the dormancy-breaking treatments applied. Analysis of Variance (ANOVA) revealed a highly significant treatment effect ($F = 653.198$; $p < 0.001$), indicating that the observed differences in germination were due to the treatments rather than random variation.

No germination was recorded in treatments involving soaking of intact seeds in water for 24 hours (T₁) or de-beaking followed by soaking in water for 24 hours (T₂). These findings indicate that hydration alone or de-beaking without additional physiological stimulation was ineffective in overcoming the inherent dormancy of 'Red Globe' grape seeds. The application of gibberellic acid (GA₃) at 1000 ppm following de-beaking (T₃) resulted in a marginal improvement in germination, with an average of 5.33%. Incorporation of cold stratification (3 weeks at 4 °C) after GA₃ treatment (T₄) further enhanced germination to 8.67%, suggesting that chilling treatment partially alleviated seed dormancy by promoting physiological changes required for embryo growth. Similar observation also reported by Nafea *et al.*, (2023) [3] while working on seed germination of grape variety 'Rashmeri'. A further increase in germination was observed under 'T₅', where seeds were sequentially treated with 0.5 M hydrogen peroxide for 24 hours, GA₃ (1000 ppm) for 24 hours, and subsequently subjected to three weeks of chilling at 4 °C. This treatment recorded an average germination of 14.33%, indicating that hydrogen peroxide, when combined with GA₃ and cold stratification, exerted a positive effect on dormancy release.

The highest germination percentage was obtained with T₆, in which seeds were sequentially soaked in water for 24 hours, hydrogen peroxide (1 M) for 24 hours, α-amylase (500 ppm) for 48 hours, and GA₃ (100 ppm) for 24 hours followed by chilling at 4 °C for 4 weeks. This treatment recorded an average germination of 84.00%. Enhancement in seed germination in seven different grape varieties of grape through scarification with hydrogen peroxide and gibberellic acid followed by chilling treatment at 1-5 °C was also observed by Ellis *et al.*, 1983 [1]. The germination achieved under T₆ was approximately six times greater than the next best treatment (T₅) and represented a substantial improvement over all other treatments evaluated. Overall, the treatments may be ranked in the following order based on average germination: T₆ (84.00%) > T₅ (14.33%) > T₄ (8.67%) > T₃ (5.33%) > T₁ = T₂ (0.00%). These results clearly demonstrate that the sequential application of hydrogen peroxide, α-amylase, GA₃ and chilling was considerably more effective in overcoming seed dormancy than the conventional treatments involving water soaking, de-beaking, GA₃, or chilling alone.

Discussion

In the present investigation absence of germination in T₁ and T₂ confirmed that water imbibition alone or mechanical de-beaking is insufficient to break dormancy in 'Red Globe'. Which indicates that 'Red Globe' seeds are characterized by deep physiological dormancy, which cannot be effectively overcome without appropriate biochemical or hormonal

interventions. The limited germination observed in treatment 'T₃' suggests that exogenous gibberellic acid partially stimulates embryo growth by promoting the synthesis of hydrolytic enzymes involved in reserve mobilization. The improvement in germination following cold stratification combined with GA₃ (T₄) indicates the importance of chilling in modifying endogenous hormonal balance. Cold stratification has long been recognized as an essential requirement for breaking physiological dormancy in grape seeds and facilitating normal embryo development (Ellis *et al.*, 1983 and Wang *et al.*, 2022) [1, 7]. The addition of hydrogen peroxide in 'T₅' further enhanced germination, supporting its role as an important signaling molecule involved in dormancy release. However, despite this improvement, germination remained relatively low, indicating that hydrogen peroxide and GA₃ alone were insufficient for complete dormancy release. Unwarranted plumule elongation in seedling was observed when gibberellic acid (GA₃) applied @ 1000 ppm hence in treatment 'T₆' the GA₃ dose was reduced to 100 ppm.



Fig 1: Enhanced seed germination in grape variety 'Red Globe' with treatment 'T₆'.

The remarkable germination achieved under treatment 'T₆' demonstrates the synergistic interaction among hydrogen peroxide, α -amylase, and GA₃. Hydrogen peroxide likely enhanced seed permeability and initiated oxidative signaling required for dormancy alleviation. Subsequent treatment with α -amylase facilitated rapid hydrolysis of stored starch reserves into soluble sugars, thereby ensuring an adequate energy supply for embryo growth. The final application of GA₃ further stimulated the synthesis of endogenous hydrolytic enzymes and promoted radicle emergence. The consistently high germination across replications indicates that the treatment was both effective and reproducible.

Conclusion

The superiority of 'T₆' suggests that successful germination of 'Red Globe' grape seeds requires simultaneous enhancement of oxidative metabolism, enzymatic reserve mobilization, hormonal regulation and stratification with chilling temperature rather than reliance on a single dormancy-breaking approach. These findings are consistent with previous reports demonstrating that integrated chemical and enzymatic treatments are more effective than individual applications for improving germination in physiologically dormant seeds. In conclusion, the sequential treatment involving water soaking, hydrogen peroxide (1 M), α -amylase (500 ppm), and GA₃ (100 ppm) followed by

stratification with chilling temperature (4 °C) proved to be the most effective protocol for breaking seed dormancy and achieving maximum germination (84.00%) in "Red Globe" grape.

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