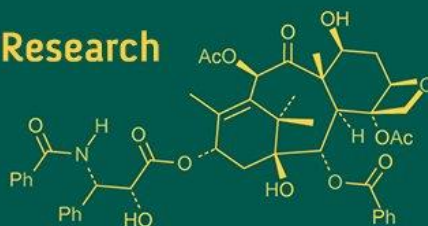
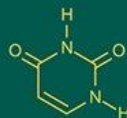


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Optimizing in-ovulo embryo rescue in the grape cultivar 'Fantasy Seedless'

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Abstract

The development of seedless grape cultivars is a major objective of many grape breeding programme. But the stenospermocarpic nature of most seedless grape cultivars impose limitation on conventional breeding approach. Embryo rescue overcomes this limitation by enabling the recovery of viable hybrid seedlings from crosses involving seedless female parents. The present study aimed to optimize an in-ovulo embryo rescue protocol for the seedless grape cultivar 'Fantasy Seedless' to facilitate its use as a female parent in breeding programmes with 'Red Globe'. Two culture media were evaluated for their effectiveness for in-ovulo germination and seedling recovery. Nitsch medium supplemented with indole-3-butyric acid (IBA) @ 0.5 mg L⁻¹, 6-benzyladenine (6-BA) @ 1.5 mg L⁻¹, and 200 mg L⁻¹ casein hydrolysate (T₁) significantly outperformed half-strength MS medium containing the same growth regulators (i.e., IBA @ 0.5 mg L⁻¹ + 6BA @ 1.0 mg L⁻¹) and 100 mg L⁻¹ casein hydrolysate (T₂). T₁ recorded 42.60% ovule germination compared with 10.60% under T₂ and produced 151 rescued seedlings with an overall embryo rescue efficiency of 30.20%, whereas T₂ yielded only 34 seedlings with 6.80% efficiency. The superior performance of T₁ demonstrates that Nitsch medium provides a more suitable environment for the growth and development of immature embryos.

Keywords: *Vitis vinifera*, embryo rescue, fantasy seedless, in-ovulo germination, seedlessness

Introduction

Grape (*Vitis vinifera* L.) is one of the most important fruit crops; cultivated worldwide for fresh consumption, wine making, raisin making and juice purpose. Most of the grape breeding programme has primary objective to develop seedless cultivars. However, conventional breeding of seedless grapes is difficult due to stenospermocarpic nature of most of the seedless grape genotypes (Stout, 1936) ^[10] wherein embryos often abort before maturity. Embryo rescue has been used for producing progenies from seedless x seeded and seedless x seedless crosses by rescuing weak or abortive embryos. Embryo rescue help to overcome the limitations imposed by embryo abortion associated with stenospermocarp and thus encourage the utilization of seedless parent as female in various breeding initiatives. Hannig (1904) ^[4] for first time successfully cultured plant embryos under aseptic conditions. Two approaches i.e., Ex-ovulo (excising immature embryos then cultured on medium under sterile conditions) and In-ovulo embryo culture are commonly used in embryo rescue experiments. In ovulo embryo rescue has been widely used since its first application in grapes (Emershad and Ramming, 1982) ^[2]. In-ovulo involves culturing of whole ovules growing them *in vitro* until plantlets are formed.

Fantasy Seedless is premium coloured grape variety with high commercial value and widely known for its better fruit quality. It is being used as a female parent in the ongoing breeding programme conducted by the author. The development of an efficient embryo rescue protocol enables the successful utilization of Fantasy Seedless as a female parent in grape breeding programmes, to induce seedlessness in its hybrid progenies. Furthermore, the embryo rescue protocol provides an effective strategy for Seedless x Seeded hybridization using 'Fantasy Seedless' as a female parent. Such crosses help to combine seedlessness of the maternal parent with the desirable fruit quality traits of the seeded pollen parent. This approach significantly increases the probability of recovering seedless hybrid progenies while broadening the genetic base available for cultivar improvement.

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Materials and Methods

The genotype, time of ovule sampling, medium for ovule culture, and plant growth regulators are the key factors that decide the success of embryo rescue (Li *et al.*, 2015) [5]. Among these, the most critical are genotype, the developmental stage of ovules, and the composition of the growth medium (Sharma *et al.* 1996) [9]. The early growth of seedless grapes is like that of normal seeded grapes, but choosing the right moment to take out and culture the ovules is crucial for successful embryo rescue (ER). The optimal time for ovule collection varies depending on the genotypes and the climatic conditions under which it is grown as it significantly affects grapevine phenology and the embryo development. Generally, the ideal ovule sampling for ER is when ovules are in the latest developmental stage while not aborting. In many seedless grape genotypes, it falls often around *veraison* stage (the onset of berry ripening (Yamashita *et al.* 1998 and Notsuka *et al.* 2001) [11, 8]. In the present study, ovule sampling was performed at 35–40 days after flowering (DAF) which generally coincides with the '*veraison*' stage, and represents an optimal developmental stage for embryo rescue. Total 1000 ovules from the cross of 'Fantasy Seedless x Red Globe' were isolated from bunches sampled during 35–40 days after flowering; and total 500 ovules were inoculated on each medium. 'T-test' statistics was employed for the analysis of experimental results. Statistical analysis was carried out in MS-Excel. Percentage Change or Relative Difference for the ovule germination and embryo rescued plant recovery was calculated using formula: $\frac{\{\text{Treatment B}\} - \{\text{Treatment A}\}}{\{\text{Treatment A}\}} \times 100 (\%)$.

Sample preparation before inoculation: A seedless x seeded cross between Fantasy Seedless and Red Globe was carried out through emasculation and controlled pollination. The spray of 6-Benzyl Adenine (6-BA) @ 30 ppm was given during parrot green stage of panicles followed by the second spray of 6-BA @ 30 ppm after a week to support ovule development (Bharathy *et al.*, 2005) [1]. Then grape berries from Fantasy Seedless were harvested at 35–40 days after flowering (DAF), corresponding to the optimal growth stage prior to embryo abortion. The harvested berries were initially washed with 0.1% Tween 20 followed by three to four rinses with running tap water to remove surface contaminants. Under aseptic conditions, the berries were surface sterilized by immersing them in 0.1% mercuric chloride (HgCl₂) solution for 10 minutes, followed by three to four rinses with sterile distilled water to eliminate residual sterilant. The berries were subsequently dipped in 70% ethanol to ensure complete surface sterilization. Sterile ovules were then carefully excised from the berries and inoculated onto embryo rescue (ER) medium. In-ovulo germination approach was used in this study. Prior to inoculation, the laminar airflow cabinet was sterilized by wiping the working surface with ethanol and exposing it to ultraviolet (UV) light for 20 minutes. The cultured ovules were incubated at 25±1 °C under dark for a month and then moved to cool-white fluorescent lamps providing 16-h photoperiod to promote embryo development and germination.

In vitro embryo rescue (ER) techniques generally consist of three stages i.e., embryo development within cultured ovules, embryo germination on medium, and plantlet formation. For embryo germination and plantlet

development requires appropriate culture media, similarly proper hardening of the ER plantlet under controlled condition before its field transplanting is also an important aspect in ER Plant development.

Medium for embryo germination: Nitsch medium, MS medium and ER medium are the most widely used basal media for embryo rescue (Bharathy *et al.* 2005; Gray *et al.* 1990, Liu *et al.* 2008; Yamashita *et al.* 1998) [1, 3, 6, 11]. In the current study two nutrient media, namely Nitsch medium (T₁) and half-strength Murashige and Skoog (MS) medium (T₂) were evaluated for their suitability in supporting ovule culture during embryo rescue. Both media were supplemented with 60 g L⁻¹ sucrose as the primary carbon source and 292 mg L⁻¹ glutamine (2 mmol L⁻¹) to provide an additional source of organic nitrogen for the growth and development of immature embryos. To promote embryo growth and differentiation, 0.5 mg L⁻¹ indole-3-butyric acid (IBA) was added to both media, while 6-benzyladenine (6-BA) was incorporated at 1.0 mg L⁻¹ in the half-strength MS medium and 1.5 mg L⁻¹ in the Nitsch medium. Casein hydrolysate, was included at 100 mg L⁻¹ in the half-strength MS medium and 200 mg L⁻¹ in the Nitsch medium to support embryo development. Amongst several types of natural adjuvants; casein hydrolysate is widely used in case of grape tissue culture as a rich source of amino acids (Sharma *et al.* 1996; Liu *et al.* 2008) [9, 6]. Calcium chloride (CaCl₂), has an important role in cell division, membrane stability, and embryo growth. It was supplemented at 166 mg L⁻¹ in the half-strength MS medium and 332 mg L⁻¹ in the Nitsch medium. To minimize microbial contamination during prolonged *in vitro* culture, 25 mg L⁻¹ miconazole nitrate was incorporated into both media. In addition, 1.5 g L⁻¹ activated charcoal was added to adsorb inhibitory compounds and improve the culture environment, while 8 g L⁻¹ agar was used as the gelling agent to solidify the medium. The pH of both media was adjusted to 6.0 before sterilization by autoclaving. The prepared media were then used for ovule inoculation and subsequent embryo development under controlled *in vitro* culture conditions.

Medium for plantlet development: Woody Plant medium is generally used as a popular medium along with MS medium for plantlet development *in vitro* (Lloyd and McCown, 1980). For plantlet development Woody Plant (WP) medium was prepared as the basal medium; which was supplemented with 6-benzyladenine (6-BA) @ (0.226 mg L⁻¹ and indole-3-butyric acid (IBA) @ 0.10 mg L⁻¹ to promote shoot development. Casein hydrolysate (500 mg L⁻¹) was added as a source of organic nitrogen and amino acids to support plantlet growth, while 30g L⁻¹ sucrose served as the carbon source. The medium also contained 3.0g L⁻¹ activated charcoal to adsorb inhibitory compounds released during culture and improve the growth environment. Agar @ 8.0g L⁻¹ was used as the gelling agent. To minimize fungal contamination, miconazole nitrate (25 mg L⁻¹) was added. MES buffer (0.976 g L⁻¹) was incorporated to maintain a stable pH during culture. The pH of the medium was adjusted to 6.0 before sterilization by autoclaving and subsequent use for embryo germination and plantlet development.

Plantlet hardening: Germinated seedlings were hardened when they reached the height of the culture bottle screw cap.

Seedlings were transplanted into an autoclaved potting mixture of cocopeat, vermiculite, soil, and perlite (2:1:1:0.5) after gently washing the roots with sterile double-distilled water to remove the adhering culture medium. Prior to transplanting, the roots were dipped in Hoagland nutrient solution for 30 seconds. To ensure gradual acclimatization, the seedlings were covered with a perforated plastic bag for 3-4 days. Then exposure to ambient conditions was increased progressively by opening and subsequently removing the plastic cover over several days. Once the seedlings were able to remain exposed for 6 hours per day, the plastic cover was removed permanently, allowing the plants to grow under normal greenhouse conditions.

Results

The effect of two different culture media compositions on embryo rescue efficiency was evaluated. The efficiency of embryo rescue was significantly influenced by the treatment applied, as evidenced by differences in ovule germination and seedling recovery (Table 1). Number of ovules germinated on medium and total number of seedlings rescued from germinated ovules were found significantly different at both 5% and 1% level of significance.

The Nitsch medium (T_1) supplemented with IBA (0.5 mg L^{-1}), 6-benzyladenine (6-BA) (1.5 mg L^{-1}), and casein hydrolysate @ 200 mg/L recorded substantially higher in-ovulo germination (42.60%) compared to the half-strength MS medium (T_2) supplemented with 6-BA 1.0 mg L^{-1} , IBA (0.5 mg L^{-1}), and casein hydrolysate @ 100 mg/L (10.60%).

These results indicate that T_1 was more effective in promoting ovule viability and subsequent embryo development under *in vitro* conditions. Among the germinated ovules, T_1 produced total 151 rescued seedlings, corresponding to an embryo rescue plant development rate of 70.93%, whereas T_2 yielded only 34 seedlings with a comparatively lower development rate of 62.49%. Although the conversion efficiency of germinated ovules into seedlings was relatively similar between treatments, the markedly higher number of germinated ovules under T_1 resulted in significantly greater absolute seedling production.



Fig 1: Embryo rescued plants of Fantasy Seedless x Red Globe developed *in-vitro*



Fig 2: Hardening of embryo rescued plants before field transplanting

Discussion

The overall embryo rescue seedling recovery, calculated as the percentage of rescued seedlings from the total inoculated ovules, further demonstrated the superiority of T_1 . An overall recovery of 30.20% was achieved under T_1 , which was more than fourfold higher than the 6.80% obtained under T_2 . This substantial improvement highlights the effectiveness of T_1 in enhancing both ovule germination and successful embryo establishment. If we compared the *percentage change* for ovule germination and the seedlings rescued from germinated ovules on medium; then medium T_1 reported 75.12% and 77.48% *relative difference* respectively over T_2 medium for these variables.

The improved response may be attributed to the balanced nutrient composition of Nitsch medium, which supports better the physiological requirements of immature embryos during *in vitro* culture. In contrast, the poor response observed under T_2 suggests that the treatment conditions were less conducive to embryo development or failed to adequately support post-germination growth. These findings

emphasize that optimizing the embryo rescue protocol is essential for maximizing the recovery of viable hybrid seedlings.

Conclusion

Overall, the results demonstrate that Nitsch medium (T_1) supplemented with IBA (0.5 mg L^{-1}), 6-benzyladenine (6-BA) (1.5 mg L^{-1}), and casein hydrolysate @ 200 mg/L is a significantly more efficient treatment for embryo rescue, providing higher ovule germination, greater seedling recovery, and superior overall embryo rescue efficiency compared with the half-strength MS medium (T_2) supplemented with 6-BA 1.0 mg L^{-1} , IBA (0.5 mg L^{-1}), and casein hydrolysate @ 100 mg/L . The results indicated that the T_1 medium provides a promising platform for embryo rescue in 'Fantasy Seedless'. Further optimization of the culture conditions to enhance ovule germination and embryo rescue plant recovery could further improve its efficiency and applicability in seedless grape breeding.

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References

1. Bharathy PV, Karibasappa GS, Patil SG, Agrawal DC. In ovulo rescue of hybrid embryos in Flame Seedless grapes: influence of pre-bloom sprays of benzyladenine. *Scientia Horticulturae*. 2005;106:353-359.
2. Emershad RL, Ramming DW. In-ovulo embryo culture of *Vitis vinifera* L. cv. Thompson Seedless. *HortScience*. 1982;17:576.
3. Gray DJ, Mortensen JA, Benton CM. Ovule culture to obtain progeny from hybrid seedless bunch grapes. *Journal of the American Society for Horticultural Science*. 1990;115:1019-1024.
4. Hannig E. Zur Physiologie pflanzlicher Embryonen I. Ueber die Cultur von Cruciferen-Embryonen ausserhalb des Embryosacks. *Botanische Zeitung*. 1904;62:45-80.
5. Li J, Wang X, Wang X, Wang Y. Embryo rescue technique and its applications for seedless breeding in grape. *Plant Cell, Tissue and Organ Culture*. 2015;120:861-880. doi:10.1007/s11240-014-0656-4.
6. Liu SM, Sykes SR, Clingeleffer PR. Effect of culture medium, genotype, and year of cross on embryo development and recovery from *in vitro* cultured ovules in breeding stenospermocarpic seedless grape varieties. *Australian Journal of Agricultural Research*. 2008;59:175-182.
7. Lloyd G, McCown B. Commercially feasible micropropagation of mountain laurel, *Kalmia latifolia*, by use of shoot-tip culture. *Proceedings of the International Plant Propagators' Society*. 1980;30:421-427.
8. Notsuka K, Tsuru T, Shiraishi M. Seedless-seedless grape hybridization via in-ovulo embryo culture. *Journal of the Japanese Society for Horticultural Science*. 2001;70:7-15.
9. Sharma DR, Kaur R, Kumar K. Embryo rescue in plants: a review. *Euphytica*. 1996;89:325-337.
10. Stout AB. Seedlessness in grapes. *New York State Agricultural Experiment Station Technical Bulletin*. 1936;238:1-68.
11. Yamashita H, Shigehapa I, Haniuda T. Production of triploid grapes by in ovulo embryo culture. *Vitis*. 1998;37:113-117.