

Effect of alternative carbohydrates on micropropagation in plant species with special reference to *Vanilla planifolia* Jacks.

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ABSTRACT

One of the challenges posed by cultivating certain agricultural species, such as *Vanilla planifolia* Jacks., is obtaining high-quality plantlets to meet the high global demand for vanillin. However, despite the progress achieved, this crop is severely damaged by fungal infections such as *Fusarium oxysporum* f. sp. *vanillae* (*Fov*). Through *in vitro* selection, the RH₃₅₀ genotype, which shows a certain level of resistance to this pathogen, is currently available. Therefore, in this study, it was proposed to review the role of different concentrations and sources of carbohydrates in the micropropagation of various plant species. According to the literature, sucrose is the most commonly used carbohydrate, probably because it contributes to greater shoot proliferation and shoot growth. Considering that vegetative propagation techniques require a precise propagation protocol to obtain plants faithful to the promising genotype of interest, it was proposed to develop the present literature review to evaluate the advances obtained in this area between 2000 and 2025. The impact of different carbon concentration and sources is the primary focus of the micropropagation process of the valuable genotype RH₃₅₀ which was obtained through *in vitro* selection, against *Fov* filtrates for future field evaluation by vanilla producers.

Keywords: carbon sources, orchid, plant tissue culture, *Fusarium oxysporum*, *in vitro*.

INTRODUCTION

In vitro plant culture has proven to be an essential tool, especially useful in clonal propagation and germplasm conservation (Rodríguez-Deméneghi, 2023). However, as plant cells and tissues develop in a controlled artificial environment, they present impairments in the optimal functioning of photosynthesis (Xiao *et al.*, 2011). This situation limits the ability of plants to generate their own assimilable carbon (George *et al.*, 2008), which adversely affects the micropropagation process itself (Fotopoulos and Sotiropoulos, 2004).

To compensate for this deficiency, carbohydrate sources are typically added to the culture medium. In addition to sucrose, which is the most commonly used disaccharide due to its low cost and availability, other carbohydrate sources that are translocated and metabolized in plants are used for this purpose (Hema and Murthy, 2007). These include hexose monosaccharides (glucose, fructose, galactose, and mannose), pentoses (arabinose, ribose, xylose), disaccharides (maltose, lactose, cellobiose, trehalose), and some trisaccharide

(raffinose) (George *et al.*, 2008), as well as some sugar alcohols, also known as polyols, among which are sorbitol, glycerol, as well as mannitol, etc. In addition to their energy function, these exogenous carbon sources are important signaling molecules that interfere with osmotic regulation, morphogenetic responses, biochemical synthesis, and cell differentiation (Lemoine *et al.*, 2013; Ikeuchi *et al.*, 2013), regeneration, and developmental control (Bhojwani and Razdan, 1986). They also participate in central metabolic pathways such as the Krebs cycle and glycolysis. Because each species may have different needs for these carbohydrates, it is imperative to assess their impact on each species and at each stage of cultivation (Thomson and Thorpe, 1987).

These requirements have not yet been defined and optimized in micropropagation systems due to the lack of understanding surrounding certain aspects of carbon utilization and metabolism in cell and tissue cultures. Despite their importance in cell growth, maintenance, and differentiation *in vitro* (Romano *et al.*, 1995). In recent years, particular emphasis has been placed on evaluating the effects of different carbon sources and concentrations, both alone and in combination with other nutrients and growth regulators. This is done in order to maximize seedling quality and propagation rates and to optimize micropropagation work. However, despite the voluminous information available on the subject, there is still no precise analysis of the impact of different carbohydrate sources on micropropagation work, especially in the propagation of such valuable species as *Vanilla planifolia* Jacks. Thus, the goal of the current literature review was to evaluate the advancements in this field from 2000 to 2025. This could support efforts to enhance the micropropagation protocol of the RH₃₅₀ genotype, which was acquired through *in vitro* selection against the main fungal pathogen that poses a serious threat to this crop, *Fusarium oxysporum* f. *vanillae* (Fov).

Carbon sources commonly used in vitro culture

The most popular carbon source for plant micropropagation is sucrose, particularly for orchid species, which have little to no *in vitro* photosynthetic capacity. Several studies have demonstrated that the source and concentration of carbohydrates in the culture have a significant impact on the growth and spread of shoots, which is a crucial component of successful plant regeneration *in vitro*. The use of a concentration of 30 gL⁻¹ sucrose has proven to be the most effective concentration for stimulating the growth of some plant species. According to various authors, this sucrose dose caused a high rate of shoot multiplication in *Zingiber officinale* Roscoe, *Dendrobium nobile* Lindl., and *Cymbopogon flexuosus* Nees ex Steud. W. Watson (Nayak *et al.*, 1997; Rout *et al.*, 2001; Ravi-Kumar *et al.*, 2011).

Similarly, *Cattleya cernua* Lindl. Van den Berg benefited from the application of such a concentration of sucrose, whereas *Cattleya tigrina* A. Rich. benefited from the effective stimulation of protocorms conversion into seedlings (Hisayuki *et al.*, 2021; Silva, 2022). The addition of 30 gL⁻¹ sucrose to *Metroxylon sagu* Rottb. can result in improvements in height growth, leaf number, and shoot fresh weight (Sumaryono *et al.*, 2012). It was found that this carbon source helps to encourage shoot elongation in *Quercus suber* L., demonstrating its effectiveness as the primary carbon source in micropropagation (Romano *et al.*, 1995). Similarly, the use of 30 gL⁻¹ sucrose in *Solanum tuberosum* L. has been shown to promote a high proliferation rate and significant starch accumulation (Ahmad *et al.*, 2021).

However, it has been observed that the optimal sucrose concentration can vary significantly between orchid species and even between different stages of development *in vitro*. Several studies have indicated that different species respond better to lower sucrose concentrations (Martins *et al.*, 2015; Koene *et al.*, 2019). The advantages of using lower concentrations of sucrose for better shoot growth outcomes have been underlined in this context. When Nayak *et al.* (1997) added a concentration of 20 gL⁻¹ sucrose to *D. nobile* they were able to increase shoot length.

The optimal results for *Phalaenopsis* mass propagation were found when a concentration of 15 gL⁻¹ sucrose was combined with a four-hour immersion frequency in temporary immersion systems (like RITA® bioreactors). However, other orchids might react better to media like MS that contain 20–30 gL⁻¹ sucrose (Murashige and Skoog, 1962).

Numerous studies, including those on *Cattleya maxima* Lindl. suggest that sucrose concentration may not significantly affect germination *in vitro* because high germination percentages have been obtained even in the absence of sucrose or with low concentrations of sucrose. Higher sucrose concentrations between 40 and 60 gL⁻¹ are effective in other studies (Sasamori *et al.*, 2021). However, it has been proposed that utilizing a higher sucrose concentration (90 gL⁻¹) is not beneficial for plant growth (George *et al.*, 2008).

Accordingly, it has been recommended that high sucrose concentrations in the growing medium may also harm plants by impeding their ability to absorb nutrients and water (Koene *et al.*, 2019). At higher sucrose concentrations in the culture media, Yildiz *et al.* (2007) observed that three sugar beet lines exhibited a marked decrease in shoot regeneration in addition to an increase in phenolic concentration and necrosis.

These outcomes are also in line with those of Kadota *et al.* (2001), who discovered that high sucrose concentrations were harmful to pears (*Pyrus communis* L.). This could be because a higher carbohydrate concentration causes a decrease in osmotic potential. During micropropagation in temporary immersion systems, high sucrose concentrations are known to induce osmotic stress in orchid tissues, which can occasionally inhibit orchid growth or even raise the risk of certain physiological disorders such as vitrification. Because sugars provide carbon to bacteria and fungi, high concentrations of carbohydrates can also change the structure of the photosynthetic apparatus, prevent the synthesis of chlorophyll and lowering the photosynthetic capacity of the tissues (Texeira Da Silva, 2004). This is particularly low *in vitro* (Rolland *et al.*, 2006). One aspect of interest that has been observed is the fact that sucrose is not always the best carbohydrate (Blanc *et al.*, 1999).

In terms of morphogenic development, it was discovered that using a concentration of 19.82 gL⁻¹ of glucose and fructose produced shoots with superior growth compared to those obtained with sucrose in *Phalaenopsis gigantea* J.J.Sm. and *Cattleya tigrine* A. Rich (Silva *et al.*, 2022). Since fructose and glucose both exhibit a shoot multiplication rate that is at least as high as sucrose, they may be helpful substitutes in *in vitro* culture protocols and have been utilized successfully in the genus *Dendrobium* (Khusairy *et al.*, 2022; Hernández, 2024).

Other recent studies have focused on trehalose, a non-reducing disaccharide involved in critical metabolic pathways and the synthesis of advanced glycation products. In orchids, there is evidence that the ability to effectively utilize trehalose is linked to their mycorrhizal symbiotic relationship with fungi in nature, making it a particularly good carbon source for orchid protocorms and plantlets (Ponert *et al.*, 2021). When compared to sucrose, the application of 30 gL⁻¹ trehalose greatly increased the formation of shoots and roots in species such as *Phalaenopsis* and *Dendrobium officinale* Kimura & Migo (Lee *et al.*, 2015; Nhut *et al.*, 2022).

It has been observed in certain studies, such as those conducted on *Fagus* species, that both sucrose and glucose are appropriate for increasing shoot length; however, in certain clones of this species, glucose demonstrated an exceptional level of effectiveness (Cuenca and Vieitez, 2000). Similarly, it has been shown that early stages of *V. planifolia* development, specifically protocorm formation and seed germination, are significantly impacted by the addition of 20 gL⁻¹ of glucose to the culture medium (Gayatri and Kavyashree, 2005).

Other studies have highlighted that not only glucose but also fructose is involved in the induction of axillary buds of several species of the *Prunus* genus when concentrations of 16 gL⁻¹ are used (Sumaryono *et al.*, 2012; Cheong and An, 2015). These researchers discovered that while the overall vigor of the seedlings produced was marginally lower than that seen with this concentration of sucrose, the use of this concentration of glucose and fructose resulted in sufficient shoot elongation. This also led to an increase in fresh weight. According

to Al-Khayri (2011), *Phoenix dactylifera* shoot length increased moderately when 20 gL⁻¹ fructose was added to the culture medium. A wide range of carbohydrates, including glucose, fructose, and oligosaccharides, are appropriate for the growth and development of *Phalaenopsis* shoots. This is according to research by Hew and Mah (1989) on the asymbiotic mechanism for the quick uptake of the monosaccharide fructose by differentiated and undifferentiated tissues of *Dendrobium* (Arditti, 1990). However, when the effects of sucrose, glucose, and maltose on *Rubus chamaemorus* L. were compared, it was found that both glucose and maltose outperformed sucrose in terms of the efficiency of shoot multiplication (Nayak *et al.*, 1997). Recent studies on *Doritaenopsis* orchids suggest that maltose may be more effective than sucrose in promoting protocorm-like bodies (PLBs) and subsequent seedling development, while sorbitol may affect seedling regeneration (Rahman *et al.*, 2005).

Maltose, a byproduct of the hydrolytic breakdown of starch, is another source of carbohydrates that has drawn special attention from orchids. It is involved in the metabolism of carbohydrates in these species, particularly in CAM plants (Crassulacean Acid Metabolism). One of the reasons maltose performs better than sucrose is thought to be that it has the lowest rate of extracellular hydrolysis, which inhibits the release of phenolic compounds into the culture medium (Kumar *et al.*, 2015).

The use of maltose produced more vigorous shoots in *D. nobile* than those obtained with similar concentrations of sucrose, which was particularly noteworthy (Nayak *et al.*, 1997). Maltose has been less successful in promoting shoot growth and proliferation in other contexts, such as the smaller-sized shoots grown on apple rootstocks, where it is the least effective carbon source (Bahmani *et al.*, 2009). Carrion-Pereira *et al.* (2019) discovered that increasing plant regeneration occurs when sucrose is substituted for maltose in *Urochloa brizantha* Hochst. ex. A. Rich. *in vitro* culture media.

In *Stevia rebaudiana* Bertoni, fructose was found to be effective in maintaining a constant number of shoots, followed by sucrose, maltose, and glucose (Preeth *et al.*, 2011). However, for the *in vitro* propagation of the Marsol chestnut hybrid cultivar, Akyüz (2025) found that the best results were obtained when 30 gL⁻¹ of sucrose was added to the culture medium. Glucose produced results that were better than maltose but not as good as sucrose.

Acclimatization of seedlings to the *ex vitro* environment is a significant challenge in *in vitro* culture. Optimizing the supply of carbohydrates in the later stages of *in vitro* culture can influence the ability of seedlings to develop a strong photosynthetic capacity and improve their survival after transplanting. As a result, it has been noted that an adequate supply of carbohydrates at this stage improves the starch and sucrose reserves in the leaves of the micropropagated plants. These act as organs for energy storage and provide the acclimatization stage for the development of new leaves that are appropriate for the *ex-vitro* environment. Thus, improving acclimatization performance (Fuentes *et al.*, 2005). Similarly, it has been noted that adding 40 gL⁻¹ of sucrose to *Cattleya loddigesii* Lindl. plants during their acclimatization phase are ineffective.

This could be because the high concentration of sugar causes excessive osmotic stress, which lowers metabolic activity *in vitro* (Jo *et al.*, 2009) and subsequently impacts *ex vitro* establishment. Nonetheless, it has been found that the photosynthetic apparatus operates most effectively when the amount of sucrose in the culture medium is decreased. Secondary oxidative stress and slower vine plant growth during acclimatization can result from a higher concentration of sucrose in the culture medium, which alters the synthesis of pigments, particularly carotenoids (Sundryeva *et al.*, 2020).

Impact of various carbon concentrations and sources on vanilla

V. planifolia is an orchid of immense economic importance, recognized worldwide for its aromatic pods, from which vanillin is extracted, a flavoring agent highly valued in the food,

beverage, cosmetics, and pharmaceutical industries. Natural vanilla extract is ranked as the second most expensive spice globally (Cardone *et al.*, 2020).

Despite its high value, traditional vanilla propagation, primarily through stem cuttings, is a labor-intensive, time-consuming, and inefficient process. This results in low multiplication rates and insufficient availability of quality planting material. This limitation directly contributes to the high cost and scarcity of natural vanilla on the market. Global demand for vanillin is projected to exceed 59,000 tons by 2025; however, natural vanilla barely meets 1% of this annual demand. This disparity creates considerable economic pressure to find more efficient production methods. In addition to propagation limitations, conventionally cultivated vanilla is susceptible to the effects of climate change, pests, and diseases.

The reliance on asexual propagation also affects the levels of genetic variation, which reduces the species' ability to adapt and resist different biotic and abiotic factors. Searching for propagation alternatives is a current priority.

Micropropagation represents a vital solution to overcome the challenges of conventional vanilla propagation, allowing for the mass production of disease-free seedlings and facilitating the genetic improvement of this highly valued orchid. However, research on how various elements, such as carbon source and concentration, can enhance mass micropropagation techniques for this valuable species is still ongoing.

To date, 30 gL⁻¹ of sucrose has been added to the culture medium commonly used for this crop's shoot multiplication (Oliveira *et al.*, 2013; Banerjee and Roy, 2023). Other authors, however, have confirmed that utilizing a lower sucrose concentration (20 gL⁻¹) can result in improvements in shoot and root proliferation and length (Samir, 2005; Oliveira, 2013).

More recent studies carried out on *V. planifolia* (Córdova-Nieto *et al.*, 2025, non-published data) have also shown significant differences between the carbohydrate sources and concentrations evaluated (sucrose, glucose, galactose, maltose, and fructose) in terms of shoot length. The use of 15 and 30 gL⁻¹ of maltose and 30 gL⁻¹ of glucose, as well as a similar concentration of fructose, were those that showed the most promising results.

Based on these authors' analysis of the effect on photosynthetic pigment content, the optimal concentrations of maltose for chlorophyll A content were 15 and 30 gL⁻¹, while the lowest concentration (15 gL⁻¹) was found for chlorophyll B content.

A concentration of 15 gL⁻¹ of sucrose and maltose yielded the highest concentration of carotenoids. Figure 1 shows the beneficial effect of some carbohydrate sources and concentrations that were most suitable for this species.

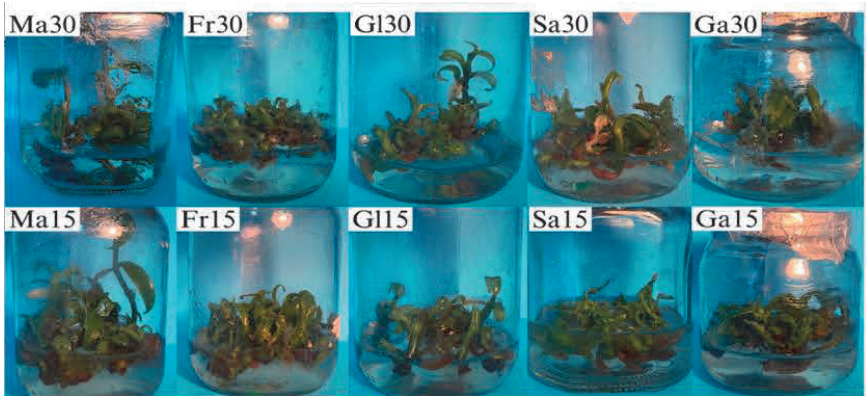


Figure 1. Effect of two concentrations (15 and 30 gL⁻¹) and five carbohydrate sources (*maltose*, *Ma*; *fructose*, *Fr*; *glucose*, *Gl*; *sucrose*, *Sa*; *galactose*, *Ga*) on the characteristics of *in vitro* *V. planifolia* RH₃₅₀ seedlings.

The findings indicated that maltose outperformed fructose in terms of shoot growth and production, while fructose outperformed sucrose and other carbohydrates in terms of shoot proliferation. When compared to the other carbohydrates assessed, galactose performed the worst in terms of shoot growth and proliferation. Finally, studies conducted on *V. planifolia* RH₃₅₀ and other species of the same genus confirm that carbohydrates are essential for establishing an effective micropropagation system. Finding their source and dosage will not only support environmentally conscious, technologically sophisticated, and sustainable production. It may also open up new avenues for study in the fields of plant physiology, biotechnology, and metabolomics when applied to species like *V. planifolia*. These species have significant ecological and commercial value.

CONCLUSIONS

The findings of the review emphasize the significance of carbohydrates for the *in vitro* culture of plants, particularly orchid species, whose photosynthetic efficiency is generally lower *in vitro* than *in vivo*. Therefore, selecting and having an adequate supply of a suitable carbon source is one of the most important factors affecting the success of micropropagation attempts. Even though 30 gL⁻¹ of sucrose has been shown to produce the best results in a variety of plant species, it hasn't always been the best concentration or carbon source to produce higher-quality shoots and faster rates of proliferation. It is crucial to carefully consider the species and cultivation stage because the choice of carbon source can have a direct impact on the outcomes of *in vitro* propagation processes.

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