SPECIALIZED METABOLITES AND USE OF CULTIVATION TECHNIQUES IN THE IN VITRO PRODUCTION OF ROSA SPP

METABÓLITOS ESPECIALIZADOS E USO DE TÉCNICAS DE CULTIVO NA PRODUÇÃO IN VITRO DE ROSA SPP

METABOLITOS ESPECIALIZADOS Y USO DE TÉCNICAS DE CULTIVO EN LA PRODUCCIÓN IN VITRO DE ROSA SPP

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ABSTRACT

Rose (Rosa spp.) is a species native to Asia and is considered one of the most popular flowers in the world, as well as being the most widely cultivated flower. Roses belong to the family Rosaceae and the genus Rosa, with more than 100 species, and thousands of varieties, hybrids and cultivars. World flower production occupies more than 190,000 hectares, representing a figure of 16,000 million dollars. Currently, the in vitro culture technique is used to commercially propagate a multitude of ornamental species and other economically important plants, with higher multiplication rates in a shorter time using traditional techniques and free of pests and diseases. In vitro cultivation of roses, research has been carried out on direct organogenesis, indirect organogenesis, callus induction, cell culture, among others. The concentration of secondary metabolites accumulates as a function of geographic distribution and seasonal variation. Therefore, the objectives of the present article are to mention the different types of in vitro culture in roses and the in vitro production of secondary metabolites biosynthesized by roses.

Keywords: Essential Oil. Flower Essence. Gas Chromatography–Mass Spectrometry. In Vitro Production.

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RESUMO
A rosa (Rosa spp.) é uma espécie nativa da Ásia e é considerada uma das flores mais populares do mundo, além de ser a flor mais amplamente cultivada. As rosas pertencem à família Rosaceae e ao gênero Rosa, com mais de 100 espécies e milhares de variedades, híbridos e cultivares. A produção mundial de flores ocupa mais de 190.000 hectares, representando um valor aproximado de 16 bilhões de dólares. Atualmente, a técnica de cultivo in vitro é utilizada para a propagação comercial de uma grande variedade de espécies ornamentais e outras plantas de importância econômica, apresentando maiores taxas de multiplicação em menor tempo quando comparada às técnicas tradicionais, além de possibilitar a obtenção de plantas livres de pragas e doenças. No cultivo in vitro de rosas, pesquisas têm sido realizadas sobre organogênese direta, organogênese indireta, indução de calos, cultura celular, entre outras abordagens. A concentração de metabólitos secundários acumula-se em função da distribuição geográfica e da variação sazonal. Portanto, os objetivos do presente artigo são apresentar os diferentes tipos de cultivo in vitro em rosas e a produção in vitro de metabólitos secundários biossintetizados por essas plantas.

Palavra-chave: Óleo Essencial. Essência Floral. Cromatografia Gasosa–Espectrometria de Massas. Produção In Vitro.

RESUMEN
La rosa (Rosa spp.) es una especie nativa de Asia y es considerada una de las flores más populares del mundo, además de ser la flor más ampliamente cultivada. Las rosas pertenecen a la familia Rosaceae y al género Rosa, con más de 100 especies y miles de variedades, híbridos y cultivares. La producción mundial de flores ocupa más de 190.000 hectáreas, representando un valor aproximado de 16.000 millones de dólares. Actualmente, la técnica de cultivo in vitro se utiliza para la propagación comercial de una gran variedad de especies ornamentales y otras plantas de importancia económica, con mayores tasas de multiplicación en menor tiempo en comparación con las técnicas tradicionales y con la ventaja de obtener plantas libres de plagas y enfermedades. En el cultivo in vitro de rosas, se han realizado investigaciones sobre organogénesis directa, organogénesis indirecta, inducción de callos, cultivo celular, entre otros. La concentración de metabolitos secundarios se acumula en función de la distribución geográfica y la variación estacional. Por lo tanto, los objetivos del presente artículo son mencionar los diferentes tipos de cultivo in vitro en rosas y la producción in vitro de metabolitos secundarios biosintetizados por estas plantas.

Palabras clave: Aceite Esencial. Esencia Floral. Cromatografía de Gases–Espectrometría de Masas. Producción In Vitro.

1 INTRODUCTION

The rose (Rosa spp.) is an exotic plant of great ornamental interest. Rose is a species native to Asia and is considered one of the most popular flowers in the world, as well as being the most widely cultivated flower. Rose is one of the most sought-after ornamental plants as a cut flower (Qi et al., 2018), it is one of the most sold species within the international flower trade (Henuka et al., 2015). Roses belong to the family Rosaceae and the genus Rosa, with more than 100 species, and thousands of varieties, hybrids and cultivars. The plant is classified as woody, with a growth habit of 1 to 2 m in height. The species has simple leaves divided into 5 or 7 lobes, and solitary flowers of various colors (Prata, 2009). World flower production occupies more than 190,000 hectares, representing a figure of 16,000 million dollars. Therefore, it is worth mentioning that in 2004, the main Mexican flower exported was the rose, which showed a value of 10 million US dollars (SDR, 2006). At present, as a result of commercial crossing and selection, it is estimated that there are between 30,000 and 35,000 cultivated varieties of roses, usually referred to as Rosa hybrida (Gudin, 2003). Nowadays it is one of the best-known species, cultivated and sought after as a cut flower, due to its unsurpassed beauty, the wide variety of colors, tones and combinations it presents, its soft fragrance and the diversity of forms (Yong, 2004). English women in the Victorian era served sophisticated dishes with crystallized rose petals (Rosa x grandiflora). Today, the rose is offered in salads, jellies and cakes. According to Prata (2009), Brazilian gastronomic culture has not stimulated the use of flowers in food, finding these "foods" in exotic culinary recipes and at a high cost, unlike countries in Europe, where gastronomy commonly uses flowers for food products. All flower companies today are faced with the great need to have a better-quality product without affecting the environment, to be more competitive by increasing production, minimizing costs and maximizing profits. For this reason, new management practices such as in vitro cultivation biotechnology are being sought, which offer an alternative, in a responsible manner, to improve agricultural production (De La Cadena-Vera, 2005). In rose, studies have been conducted at the proteomic level (Dafny-Yelin et al., 2005; Lu et al., 2019); genomic (Xu and Liu, 2018; Detcharoen et al., 2023); transcriptomic (Liu et al., 2022; Li et al., 2018); molecular biology (Bendahmane et al., 2013; Shi et al., 2022), pest (Diakaki et al., 2019), phytopathology (Muñoz et al., 2019; Salgado-Salazar et al 2018), microbiology (Zhang et al., 2011), among other studies. Currently, the in vitro culture technique is used to commercially propagate a multitude of ornamental species and other economically important plants, with higher multiplication rates in a shorter time using traditional techniques and free of pests and diseases. The species of roses that are worked in vitro culture are: Rosa hybrida, Rosa canina, Rosa indica, Rosa damascena, Rosa multiflora, Rosa chinensis, Rosa centifolia, Rosa pérsica and cultivars. In vitro cultivation of roses, explants such as lateral buds, shoot tips, among others, are used for shoot multiplication and rooting (Carelli and Echeverrigaray, 2002). The growth regulators are used to proliferate brotes in vitro in roses: naftalen-acetic acid (NAA), giberellic acid, between others (Carelli and Echeverrigaray, 2002), which are used to rooting in vitro in roses. In vitro cultivation of roses, research has been carried out on direct organogenesis using regeneration media. Explants have also been used for indirect organogenesis in in vitro rose culture using PGRs. Therefore, the objectives of the present article are to mention the different types of in vitro culture in roses and the in vitro production of secondary metabolites biosynthesized by roses.

1.1 IN VITRO CULTURE CONDITIONS

The most widely used method for micropropagation of roses is based on bud and shoot multiplication, which is the in vitro equivalent of in vivo propagation from cuttings. It is also possible to produce plants by the formation of adventitious shoots and somatic embryos; however, apprehensions of genetic instability and increased complexity have discouraged the widespread use of these methods in rose micropropagation (Castilla, 2005).

1.2 PHYSICAL FACTORS

The three characteristics of light that influence vitro growth are wavelength, flux density, and duration of light exposure or photo period (George et al., 2008). Horn (1992) mentions that a flux density of 1 to 3 klux (1000 or 3000 Lux, 16-46 μmol m-2 s-1) is sufficient for shoot proliferation in rose.

On the other hand, a photoperiod of 16 h of light showed better growth and multiplication of rose shoots (Bressan et al., 1982). Temperature is a decisive variable during the stages of rose micropropagation. Leyhe & Horn (1994) recorded optimum shoot and root formation at 21 °C for different cultivars of R. hybrida. On the other hand, Rahman et al. (1992) stated that a temperature of 28 °C improved in vitro rooting of rose. However, many researchers have used a temperature of around 25 °C for shoot multiplication and rooting (Pati et al., 2006). In greenhouse-grown roses, it has been found that the effects of elevated temperatures are observed of high temperatures are observed mainly on new shoots, when they are exposed to them from early stages of exposed to them from early stages of their development. It was even found that the proportion. The proportion of different anthocyanins are found in the plant changes drastically when they are grown at high temperatures drastically when cultivated at high temperatures (Dela et al., 2003).

1.3 HUMIDITY

In general, it is accepted that the relative humidity in the in vitro culture vessel is approximately 98 to 100%. Consequently, in vitro plants have more transpiration and anatomical abnormalities under ex vitro conditions which can result in a high mortality rate during acclimatization (Altman & Loberant, 2003). Therefore, different methods have been tested to reduce the relative humidity of the air inside the container, for example, a study conducted with grapevine shoots testing different diameters of holes in the container closure showed that shoots grown in ventilated containers were taller than shoots grown in unventilated containers and had a higher chlorophyll content (Gribaudo et al., 2003).

1.4 IN VITRO CULTURES CHARACTERISTICS

The species of roses that work in vitro culture are Rosa hybrida, Rosa canina, Rosa indica, Rosa damascena, Rosa multiflora, Rosa chinensis, Rosa centifolia, Rosa persica and cultivars. Pati et al. (2005) used for initial sterilization of rose explants, 70% ethanol for 20 to 30 seconds, followed by a 0.1% solution of $HgCl_2$ for 5 to 7 minutes and washing with distilled water. Then a solution of sodium hypochlorite at 5.25% active ingredient and 0.1% Triton X for 5 to 10 minutes, followed by washing with distilled water. In addition, a solution can be prepared for internal disinfection based on different antibiotics (tetracycline, amoxicillin, gentamicin or ampicillin), at different concentrations and duration. Pati et al. (2005) reported that 3% sucrose concentration in the culture medium increases the length and number of roots of rose plants. increases the length and number of roots of rose plants. In in vitro cultivation of roses, the explants used are axillary meristems, apical meristems, lateral buds and shoot tips for shoot multiplication and rooting (Carelli and Echeverrigaray, 2002). Growth regulators used for in vitro shoot proliferation in roses are benzyl-aminopurine (BAP), thidhiazuron (TDZ), naphthalenacetic acid (NAA), indole acetic acid (IAA), indole butyric acid (IBA) and gibberellic acid 3 (GA3) (Carelli and Echeverrigaray, 2002). The growth regulators used for in vitro rooting in roses are naphthalene acetic acid (NAA), indole acetic acid (IAA), indole butyric acid (IBA), 2.4-dichlorophenoxyacetic acid (2,4-D) and gibberellic acid (GA3). The explants used for direct organogenesis in vitro culture of roses are leaves, petiole, root and leaflets.

The regeneration media used with PGR´s are MS with NAA, BAP and IBA. The explants used for indirect organogenesis in in vitro culture of roses are Shoot primordials, segments, callus, excised embryos, leaf segment, leaf, root, stem segments. The PGR´s used are 2,4-D, NAA, GA3, Kn, BAP, TDZ and IBA. Media used are MS, ½ MS, KB. Indirect organogenesis responses in vitro cultures of roses are Callus, pot primordia and adventitious shoots.

Roses can be propagated sexually and asexually; the former involves the fusion of haploid gametes (fertilization) and occurs on a large scale, and the latter can be done by grafting, budding or rooted cuttings (Yong, 2004), however, these techniques may have some limitations, as they are costly, time and labor intensive, slow to produce, and the spread of pests and diseases may occur (Kumud et al., 2015). According to Hasegawa (1980), the incorporation of BAP into the medium, at a concentration between 0.1 and 10 mg/L, is essential for the initiation of rooting. between 0.1 and 10 mg/L, is essential for the initiation of rooting.

The difficulties presented by both propagations are solved through plant tissue culture to massively propagate genetically identical and improved seedlings, free of pests and diseases in a reduced space and in less time (Sharry et al., 2015). Under in vitro conditions, vitropathogens can be harmful, since their proliferation increases when there is an optimal concentration of nutrients in the culture medium; in some cases, contamination is not always visible in the initial stages of culture due to the high content of salts or sucrose and variation in pH, but their multiplication rate can increase as subcultures are carried out (Reed et al., 1998).

The losses of the plant material in vitro can be 10-35 % by the presence of fungi and yeast, and 20 - 55 % by bacteria. Sun et al. (2013), managed to obtain healthy seedlings of Rosa chinensis "Xiangbin", from stem nodules, sown in MS basal medium supplemented with 1.0 mg/L 6-BA and 0.2 mg/L IBA; and, 80 % rooting in 50 % MS medium with 0.3 mg/L NAA; Diniz et al, (2014), obtained 5 shoots/explant, on MS medium with 1.0 mg/L BAP, and 100 % of the seedlings rooted using 100 % MS salts, without the addition of phytohormones. Table 1 shows the in vitro culture conditions according to the growth regulators.

Table 1

Roses in vitro culture growth regulator types and concentrations

Species/cultivars	Objetive	Growth regulators (mg/L) concentrations	Reference
Rosa hybrida cv. Baronesse	Shoot proliferation	3.0 BAP + 0.5 NAA	Carelli and Echeverrigaray, 2002
Rosa damascena var. Jwala	Rooting	0.1 NAA + 0.2 IBA	Kumar et al., 2001
Rosa damascena	Petiole direct organogenesis	Induction: ½ MS + TDZ (1.5) + NAA (0.05) Médium regeneration: MS + BAP (0.5) + NAA (0.01)	Pati et al., 2004

Rosa hybrida cv. Carefree Beauty	Leaf segment, Shoot primordium	2,4-D (2.2-22.1) or NAA (2.0-20.11) callus on MS media	Hsia and Korban, 1996
Rosa hybrida cv. Baronesse	Meristems axillary	Shoot multiplication	Carelli and Echeverrigaray, 2002
Rosa sp.	Seedling and callogenetic response from axillary buds and leaves.	Murashige and Skoog (MS) 100%, benzylaminopurine (BAP) 0.4 mg/L, kinetin 0.3 mg/L, naphthalene-acetic acid (NAA) 0.3 mg/L, and 6 g sigma Agar; adjusting the pH to 5.8	Villa and Arbeláez (2019)
Rosa Al-Taif (Rosa damascena f. trigintipetala (Diek) R. Keller)	*In vitro* rooting of micro shoots	MS medium supplemented with 0.2 mg/L NAA, 80 g/L sucrose, 0.5 g/L activated charcoal and 50 µmol-m-2-s-1 PPFD were the optimal conditions for 100% adventitious root induction.	Al-Ali et al., 2024
‘Kashan’ and ‘Hervy Azerbaijan’ Damask Rose (*Rosa damascena* Mill.)	*In Vitro* Shoot Multiplication and Rooting	At the doses of 2 mg-L-1 BAP and 0.4 mg-L-1 GA3 for the genotypes 'Kashan' and 'Hervy Azerbaijan', the highest regeneration coefficient (4.29 and 4.28) and leaf number (23.33-24.33) were obtained in the explants.	Kaviani et al., 2024
Rosa hybrida Breeding Line 15R-12-2	Callus Derived from Petals	Thirty components, including esters and alcohols, were detected in the petal callus. The 2-ethylhexan-1-ol, showed a relative content of 59.01% when extracted with hexane as solvent.	Lee et al., 2023
Rosa germoplasm	Cryo-Technologies	Se compararon dos métodos de criopreservación rápida (-196 °C), la vitrificación por gotitas y la encapsulación-deshidratación para rosa (Rosa × hybrida L., cultivares 'Ioana', 'Mariana', 'Vulcan'). The highest regeneration frequencies were obtained for cv. Vulcan' in the two cryopreservation procedures, 72% in droplet nitrification and 65% after encapsulation-dehydration. The rate of shoot multiplication and height (morphogenetic response) to storage in liquid nitrogen was direct multiple shoot formation for all genotypes.	Halmagyi et al., 2022
Morphological Characters, Phytochemical Profile and Biological Activities	Novel Garden Roses Edible Cultivars.	The cultivars investigated have a favorable aroma for human consumption, high concentration of phenols and vitamin C, high	Simin et al., 2023

		antioxidant content and moderate neuroprotective activity. Metabolites such as 3-O-glycosyl quercetin and quinic acid were quantified.	

Source: Elaborated for the Authors.

Embryogenic friable tissues (EFT) from rose (R. hybrida cv. Royalty) filament cultures were obtained and cocultured with Agrobacterium tumefaciens or A. rhizogenes, carrying a vector with the neomycin phosphotransferase II (npt II), glucuronidase (GUS) or firefly luciferase (LUC) genes. The putative transformed colonies were selected on kanamycin. Fifty to 60 lines of transformed embryogenic callus were obtained from each gram of TFE inoculated with Agrobacterium. The transformed embryogenic callus was transferred to maturation medium to form somatic embryos, which then produced plants (Firoozabady et al., 1994).

In R. damascena and R. borboniana, two of the most commercially important species due to their aroma and essential oil content, micropropagation protocols have been established using nodal segments and protoplast culture has been studied as viable alternatives for rapid propagation and obtaining new varieties (Pati et al., 2001).

Secondary metabolites production in roses in vitro culture

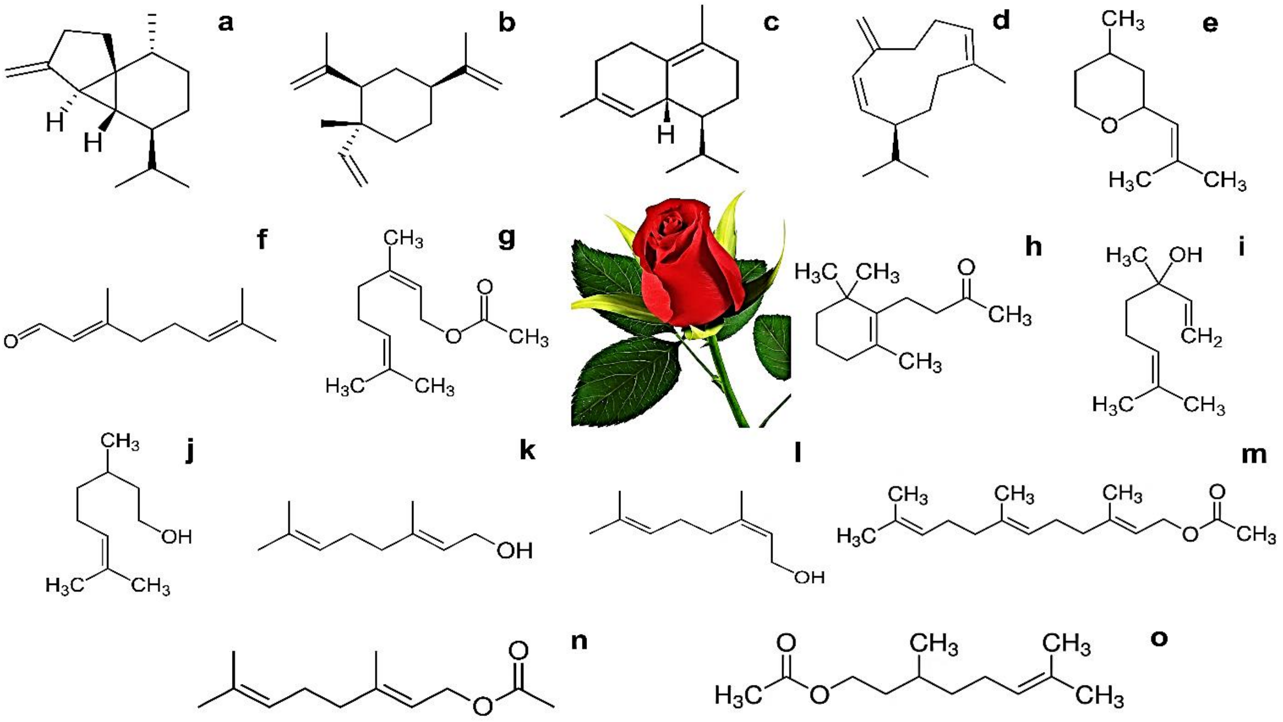
Some bioactive compounds derived from secondary metabolism mostly reported in Rosa gallica are Flavonoids: antioxidant, antimicrobial and anti-inflammatory activity (Ochir et al., 2013). Essential oils: generally found in petals with analgesic and anti-inflammatory properties (Koczka et al., 2018). Other phenolic compounds have been reported by Song et al. (2020). The flowers of R. chinensis present several medicinal, ornamental and gastronomic uses (Pressi et al., 2019). Kumar et al., (2018), validated an analytical HPLC method (in terms of linearity, accuracy and repeatability) to determine ten polyphenols of importance in fresh flowers of R. bourboniana and R. brunoni, which were: gallic acid, catechin, epicatechin, rutin, m-coumaric acid, quercitrin, myricetin, quercetin, apigenin and kaempferol. Separation time for polyphenols was 16 min, by RP-HPLC (Phenomenex, Luna C18 column, 5 μm, 250 mm × 4.6 mm) with linear gradient elution of water and acetonitrile (0.02% trifluroacetic acid) with a flow rate of 1 mL/min at λ 280 nm. Standard calibration curves are linear over a range of 0.39-500 μg/mL. On the other hand, Tuncay and Demirezer (2021), mention that an analytical method validated by the European Pharmacopoeia does not yet exist for the industry, therefore, these authors developed a rapid and reliable RP-HPLC-DAD method to identify and quantify flavonoids from the methanolic extract of R. damascena petals from Turkey (Isparta), Pakistan and different parts of Iran. The method of further identification and separation of flavonoids was achieved on a reversed-phase C18 column maintained at 25 oC by gradient elution with a flow rate of 1.2 mL/min, injection volume of 10 mL and detection at 330 nm. Six different flavonol glycosides were identified: 1) kaempferol 3-O-glucoside, 2) kaempferol 3-O-galactoside, 3) kaempferol 3-O-rhamnoside, 4) quercetin 3-O-glucoside, 5) quercetin 3-O-galactoside, and 6) quercetin 3-O-rhamnoside. It was determined that kaempferol 3-O-glucoside was used as a marker for quantification and validation analyses. The highest amount of kaempferol 3-O-glucoside was found in Isparta rose, with 0.7047%. Saeed et al., (2017), extracted and identified the constituents of R. damascena essential oil as well as the effect of season and climatic conditions of selected regions in Pakistan. The metabolites of the essential oil were obtained through steam entrainment distillation method, while the analysis was carried out by GC coupled with MS. O-cymene, d-limonene, (R)-(+)-citronellal, n-heneicosane, eugenol methyl ether, p-menth-1-en-8-ol and eucalyptol were extracted and quantified. GC-MS analysis referred several important volatile components, such as (R)-(+)-citronellal, o-cymene, d-limonene, eucalyptol, p-menth-1-en-8-ol, eugenol methyl ether and n-heneicosane, with an extraction yield from 3.8% to 91.9%. Also, they mention that the spring season showed a high yield of essential oil compared to summer and winter.

2 SECONDARY METABOLITES OF ROSE FLOWER ESSENCE

Figure 1 shows the terpene-like chemical skeletons of the floral aroma of roses. Figure 2 shows the phenylpropanoid skeletons of the floral aroma of roses.

Figure 1

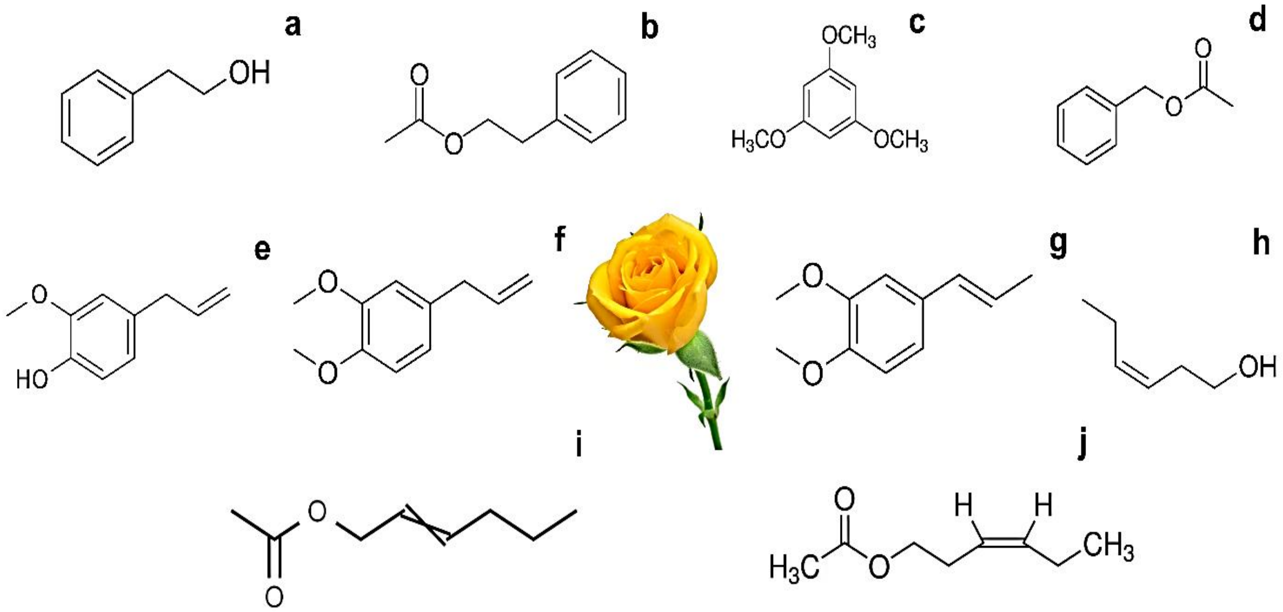
Terpene-like secondary metabolites biosynthesized in rose flower essence. a) β-Cubebene (sesquiterpene, C15H24, 204.35 g/mol); b) (−)-β-Elemene (sesquiterpene, C15H24, 204.35 g/mol); c) d cadinene (sesquiterpene, C15H24, 204.35 g/mol); d) germacrene D (sesquiterpene, C15H24, 204.35 g/mol); e) rose oxide (monoterpene, C10H18O, 154.25 g/mol); f) citral (monoterpene aldehyde, C10H16O, 152.24 g/mol); g) Neryl acetate (terpenoid, C12H20O2, 196.29 g/mol); h) Dihydro-β-ionone (C13H22O, 194.31 g/mol); i) Linalool (monoterpenoid, C10H18O; 154.253 g/mol); j) Citronellol (monotepernoid, C10H20O, 156.27 g/mol); k) geraniol (monoterpenoid, C10H18O, 154.25 g/mol); l) Nerol monoterpenoid (C10H18O, 154.25 g/mol); m) farnesyl acetate (C17H28O2, 264.4 g/mol); n) geranyl acetate (C12H20O2, 196.29 g/mol) and o) citronellyl acetate (C12H22O2, 198.30 g/mol



Source: Elaborated for the Authors.

Figure 2

Phenylpropanoids/benzenoids: a) 2-phenylethanol (C8H10O, 122.16 g/mol); b) 2-phenylethyl acetate (C10H12O2, 164.20 g/mol); c) 1,3,5-trimethoxybenzene (168.19, C6H3(OCH3)3); d) dimethoxytoluene (CH3C6H3(OCH3)2, 152.19); e) benzyl acetate (CH3C(O)OCH2C6H5, 150.18 g/mol); f) eugenol (C10H12O2, 164.20 g/mol); g) methyl eugenol (C11H14O2, 178.23 g/mol); fatty-acid derivaties: h) cis-3-hexenyl-1-alcohol (C6H12O, 100.15 g/mol); i) 2-hexenyl acetate (C8H14O2, 142.19 g/mol); j) cis-3-hexenyl acetate (CH3CO2CH2CH2CH=CHC2H5, 142.20 g/mol)



Source: Elaborated for the Authors.

Table 2 shows the genes related to rose scent essence conditions.

Table 2

Genes related to the scent related to the perfume of roses

Vía	*Gen*	Spp.	Reference
Terpenoids	*RhGDS*	*R. hybrid* "Fragrant Cloud"	Hendel-Rahmanim, et al., 2007
	RhCCD1	*R. damascene*	Huang et al., 2019
	RhCCD4	*R. damascene*	Huang, et al., 2009
	RrLIS	*R. rugosa* Thunb. "Tangzi"	Feng et al., 2014
	RhAAT	R. hybrid "Fragrant Cloud"	Guterman Wt al., 2006, Shalit et al., 2003
	RrAAT	*R. rugosa* "Tangzi"	Feng et al., 2014
	RrDXS	*R. rugosa* "Tangzi"	Feng et al., 2014
	RrDXR	*R. rugosa* "Tangzi"	Feng et al., 2014
	RcGDS	*R. chinensis* "Old blush"	Dubois et al., 2012
	RhNUDX1	*R.chinensis* "Old blush"	Magnard et al., 2015

Phenylpropanoids/ benzenoids	RhPAAS	*R. hybrida* "Frangant Cloud"	Farhi et al 2010
	Rose-PAR	*R. x damascena* Mill	Chen et al 2011
	RyAAAT3	*R. hybrida* "Yves Piaget"	Hirata et al 2012
	RyPPDC	*R. hybrida* "Yves Piaget"	Hirata et al., 2016
	Rc EGS1	*R. chinensis* Jacq. Var. Spontanea	Wu et al., 2004
	RcOOMT1	*R. chinensis* "Old blush"	Wang et al., 2012
	RcOOMT2	*R. hybrida* "Fragrant Cloud" and "Golden Gate" *R. chinensis* Jacq. Var. Spontanea, *R. hybrida* "Fragrant Cloud" and "Golden Gate"	Wu et al., 2003 Lavid et al., 2012
	RcOMT1	*R. chinensis* var. Spontanea	Wu et al., 2003
	RcOMT2	*R. chinensis* var. Spontanea	Wu et al., 2003
	AADC	R. "Hoh-Jun"	Sakai, et al., 2007
	RhMYB1	*R. hybrida* "Jinyindao"	Yan et al., 2011

Source: Elaborated for the Authors.

3 CONCLUSIONS

The most used techniques for the micropropagation of roses from plant organs and tissues are bud, bud and callus culture, as opposed to embryo and protoplast culture, which are rarely used due to their high degree of difficulty. In vitro propagation of roses has proved to be a great alternative for mass propagation of genetically identical plants, free of pests and diseases. Modern roses are the most widely propagated species; however, in vitro cultivation of miniature roses, as well as the scaling up of cell production in bioreactors, remains to be investigated, which represents a current challenge. Currently, many reproductive protocols exist for in vitro propagation of roses. However, the new challenges for the micropropagation industry are economic efficiency, automation, complete control and optimization of the microenvironment.

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