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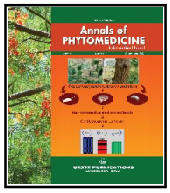

Review Article : Open Access

A review on the *in vitro* regeneration of the timeless panacean medicinal and aromatic herb: *Rosmarinus officinalis* (L.)

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Abstract

Rosmarinus officinalis (L.) (*Salvia rosmarinus* Spenn.) an antediluvian aromatic herbal delight of the Mediterranean threshold roves its niche into the orb of the versatile cuisine, pharmaceuticals and cosmetics on the grounds of its ethnobotanical properties. A perpetual propagation becomes the prerequisite fashioning the protocols of *in vitro* regeneration of *R. officinalis*. With the literary and swot of the herb, on the rise in the recent past, insculps of the use of leaves as explants noted for the muster of volatile bioactive essence befits the ramification, followed by the potent use of sodium hypochlorite (NaOCl), hydrogen peroxidase (H_2O_2) and lemon juice as antecedents of sterilization as in double sterilization hoisting the per cent of explant endurance. The efficacy of N(2-chloro-4-pyridyl)-N'-phenylurea (CPPU) and benzyl amino purine (6-benzyl amino purine) in shoot induction and NAA at half MS suit on as the best rooting media stands imbued.

1. Introduction

Rosmarinus officinalis (L.) (*Salvia rosmarinus Spenn.*) as rosemary belongs to the lamiaceae family, is a fragrant perennial herb cradled from the rocks and coasts of the Mediterranean terrain. Botanically delineated as an erect, bushy evergreen shrub with a plant reach of 1.5 to 2 m height and 1m plant spread with spiny leaves of glossiness nature, infnorescences of spiciform type with white or blue flowers (Manoharachary and Nagaraju, 2016).

Latinate *Rosmarinus*, "Dew of the Sea" espied for its pristinely minty, sage-like, peppery, balsamic taste with a bitter, woody flavor. The ambit of aroma underpins its multifarious nature. Ergo Rosmarinus is cherished for its promiscuous niche. Owing to its range of aromas, it allures an eloquent place of laud in ubiquitous cuisine. Among the myriad cultivars, Tuscan Blue Rosemary thrives on the top of a chef's prize due to its lemon-pine aroma. Spice Island Rosemary for its savory clove and nutmeg while rosemary Sissinghurst Blue Rosemary has evident smoky flavor, the best choice for barbeque. An herb that blends its way in both spice and sweet casserole.

Its history dates back to 500 B.C. as a part of the Ancient Greek and Roman civilizations where the crop was esteemed for its potentiality of memory power, and thus sprigs of rosemary enthralled as hair adornments for scholars and academic aspirants and of the victorian times as floral tiaras for brides of England symbolizing love, fidelity, and remembrance. A redolent facet in the cosmeceutical preparations of Egyptians to tolerate the baroque temperatures of the desert up to 45ºC. The occurrence of carnosic acid and carnosol in this crop is the most widely adaptable anti-bacterial agent (Jordán *et al.*, 2012). The media used for culturing the plantlets under *in vitro* conditions is one of the most critical factors which contribute to the success of *in vitro* propagation. The supplementation of hormones for the regulation of growth and development is crucial. Hence, this review focuses on the successful growth regulators employed for micropropagation of this crop.

1.1 Essential oil components

Thus, hallmarks for a plausible sojourn into the demesne realm of cosmetics sequel to the essential oils and extracts of the herb and withal to aromatherapy (Sri Bhuvaneswari *et al.*, 2021; Duraisami *et al.*, 2021). Owing to its ethnobiological properties, Rosemary has been the quintessence herb of versatile treat. *R. officinalis* 'Collingwood Ingram' and Rosemary, Blue Spires (*R. officinalis* 'Blue Spires') were tended especially for the redolence (Begum *et al.*, 2013), a keynote in aromatherapy. The leathery leaves of the herb thrive as an economically extravagant botanical part known for its muster of the volatile oil in the labiate trichomes ergo the oil production is higher in summer relative to the winter season as in agreement with the reports of Tawik *et al.* (1998).The colossal constituents of rosemary oil have camphor (5-31%), 1,8-cineol (15-55%), pinene (9-26%), borneol (1.5-5.0%), camphene (2.5-12.0%), pinene (2.0-9.0%), limonene(1.5-5.0%), verbenone (2.2-11.1%), caryophyllene (1.8-5.1%) and myrcene (0.9-4.5%).

Bioactive molecules at work in rosemary lists down as monoterpenes (alpha-pinene, beta-pinene, camphene, mycrene, alpha-phellandrene, limonene, alpha- and y-terpinene, paracymene)

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Sesquiterpenes (beta-caryophyllene) diterpenes (carnosic acid, carnosol, rosmarol, epirosmanol, isorosmanol, and rosmaridifenol), triterpenes (oleanolic acid, ursolic acid, betulin, α-amyrin, and β-amyrin) and phenolic acids (caffeic acid, chlorogenic acid, and rosmarinic acid) and flavonoids (Begum *et al.,* 2013; González - Minero *et al.,* 2020). Therein escalates its essentiality in the biome of perfumery, medicine, and food preservatives owing to its classic touchstone in phytochemicals (Figure 1).

2. *In vitro* regeneration of *R. officinalis*/*S. rosmarinus*

In vitro perpetuation of medicinal and aromatic plants (MAPs) is an ample technique for the conservation of rare and endangered plant species, and to produce a higher number of high-value plant materials for commercial cultivation (Grigoriadou and Krigas *et al.,* 2019). In accord with the protocol of *in vitro* regeneration by Murashige, the selection of the mother plant and explant preparation is a pre-requisite, followed by media preparation for shoot induction and multiplication and rooting trialed for a thriving survival through acclimatization. Casting an eagle's view onto the accessible research literature a blend of the corollary is put forth.

2.1 Explants

Misra and Chaturvedi (1983) reported that the single nodal stem segments were better explants than shoot tips for the establishment of field-grown plants. Axillary shoots were obtained in a span of 25-30 days from the 10% of green nodal segments cultured in WM media. About 5000 plants could be produced from a survived single nodal plant in one year. Mascarello *et al.* (2017) addressed the classical protocol of micropropagation and investigated the various reasons for promoting the callus induction and subsequent establishment from the leaf explants excised from plants raised in the open air while the apical and internodal explants reaped from plants raised either in open air or greenhouse placarded higher percentage of mortality. Husain and Jawad (2019) revealed the vivid effect of the plant part in plantlet initiation marking the apical meristem (62.2%) over the lateral buds (42.5%).

2.2 Sterilization

Masoody *et al.* (2015) done a trials using ethanol mercuric chloride and sodium hypochlorite, wherein the use of 0.75 % sodium hypochlorite for 15 min was the most effective by getting sterile explants after two weeks of culture. Mercuric chloride at a concentration of 0.06 mg/l had a significant effect on leaves sterilization for 6 min which gave the highest number of healthy leaves, while a concentration of 50% alcohol, gave the highest number of healthy leaves for the sterilization time of 3 min. Masoody and Florin (2015) dry ran the sterilization course of the apical buds using 70% ethanol for 30 sec and NaOCl and required the procurement of the highest average number of healthy buds (3.24) with a sterilization span of 20 min. A step further explants were double sterilized using NaOCl, followed by H_2O_2 combined with lemon juice (concentration 5/100 V/V) positively affected the increasing percentage of sterilized explants as against siver nitrate ($AgNO_3$) as sterilizing agent substantiated by Sakr *et al.* (2018), the cultivars of rosemary.

2.3 Media

Misra and Chaturvedi (1983) reported that 6-benzyl amino purine showed higher effectiveness when compared with Kinetin while used for shoot induction in shoot tips excised from aseptically grown plants. Maximum numbers of shoots were formed per explants at 0.2 mg/l 6-benzyl amino purine in 30 days. Root induction was 80% successful at the rate of 0.25 mg/l IPA. Leelavathi and Narendra (2013) observed that MSBM and 6-benzyl amino purine (8.88 μM) with IAA (2.85 μM) was the most suitable medium for initiation shoots and multiple shoot formation from *in vitro* apical bud of *R. officinalis*. On hardening and field transferring of these axenic plants, a survival frequency of 80% was garnered. Masoody and Florin (2015) evaluate the effect of growth regulators on *in vitro* callus formation from the apical buds. According to this study, the convergence across BA and NAA at the concentrations of 2.0 and 1.5 mg/l, correspondingly, produced the maximum callus volume (10.2 mm^3) in the MS medium and supplementation. The best improvements were produced at a concentration range of 2.0 mg/l BA and 2.0 mg/l NAA.

Masoody and Florin (2015) delineated the effect of the treatment of the container-grown stock-young leaf explants with BA and NAA on the callus culture. With a concentration of 1.5 mg/l BA and 1.5 mg/l NAA, the color of the cultured callus changed from white into brown, whereas 2.0 and 3.0 mg/l BA changed the appearance of the callus from light brown into brown. The maximum volume of callus (14.4 mm^3) was obtained at 1.5 mg/l BA and 1.5 mg/l NAA, whereas the minimum volume (0.9 mm^3) was obtained at 0.5 mg/l BA. The size of the callus significantly decreased when NAA concentrations were increased to 2.0 mg/l or higher, proving that greater concentrations prevent callus development. Whereby deeming the concentration of 1.5 mg/l BA and 1.5 mg/l NAA at its best for the procurement of pure white callus culture at a volume of (14.4 mm^3) and providing onto the impact of the growth regulator concentration. After 45 days of culture, a larger amount of somatic embryos rosemary plant in MS environment was shown to have a beneficial impact at a dosage of 2 mg.

Vasile *et al.,* (2015) aimed to study the multiplication ability of *R. officinalis* apical tissue in environments with low phytohormone additions and thereabout proceeded to recommend an *in vitro* culture of the rosemary apex, on a low hormonal balance medium (0. 2mg/l BA+ 0.3 mg/l AIA), for 45 days, in order to achieve an advantageous multiplication on mediums with low doses of phytohormones. While high doses of auxin (8-10 mg/l 2,4D) and cytokinin was recommended to regenerate the callus. Mascarello *et al.* (2017) experimenting the cultural growth of *R. officinalis* on the media without the growth regulators vs media with IAA at 0.5 and 1 mg/l, respectively. Despite greater rooting percentages were achieved in the influence of IAA (75 and 78.6) at 0.5 and 1mg/l, correspondingly, while, rooting occurred at a rate of 50% on medium without growth factors.

Striving towards windfall in the constellation of protocol Sakr *et al.*(2018) jotted CPPU and TDZ as the most effective PGRs for shoot stimulation and embryo formation at a lower concentration than 6-benzyl Amino Purine at the same concentration. These contradicted the reports of Leelavathi and Kuppan (2013) MSBM + 6-benzyl amino purine (8.88 μM) + IAA (5.70 μM) as the optimal medium for the induction and growth of whitish-green compact callus from the apical bud explants as well as the statements of Leelavathi *et al.* (2013) as that shoot emergence with 1-2 leaves were observed in all the concentrations of growth regulators with

varying percentage responding to MSBM + 6-benzyl Amino Purine (8.88 µÌ) + IAA (2.85 µÌ) and MSBM + Kn (13.92 ìµ) + IAA (2.85 µÌ). The disparity between the sequels could be changing the use of a higher concentration of 6-benzyl amino purine alone or the appropriate combination with auxins for its triumph. In accord with Masoody and Florin Stanica (2015), the combination of BA and NAA significantly influenced the callus's dry and fresh weight. Likely Sakr *et al.* (2018) adverted to root initiation in MS free for some cultivars of rosemary while MS medium containing NAA at half strength of MS Media was deemed to be the best with the highest number of rooted shoots against IAA of full MS Media whose roots seemed much thinner and shorter.

2.4 Tissue culture protocol

A standard protocol for faster clonal multiplication by using *in vitro* axillary buds of *R. officinalis* on MS media, supplemented with 6-benzyl amino purine (8.88 µM) + IAA (2.85 µM) for the induction of multiple shoots to a large number was found by Leelavathi *et al.* (2013). Rooting cropped up on inoculation with the rooting medium MSBM fortified with 6-benzyl amino purine (8.88µÌ) + NAA (2.68 µÌ) + IBA (4.92 µÌ) after 28 days of culture. Embarked onto the final step of micropropagation, the acclimatized plants avowed a 75% survival frequency once transferred to soil. Mascarello *et al.* (2017) addressed the classical protocol of micropropagation and investigated the factors promoting the callus induction for the further establishment of cell culture, wherein he they divulged the befitting use of leaves taken from stock plants of *R. officinalis* grown in the open air over the apical and internodal explants which were subjected to the high percentage of mortality. The former explants (leaves) came to pass in which the induction of callus was promising. Following two months of culture, callus growth was seen. Subcultures in the same environment for four weeks enhanced the biomass. When friable calli of *R. officinalis* were transferred into the liquid medium with the same hormonal composition in order to expand biomass production *in vitro*, the 50% rooting appeared in media without the growth regulators, whereas a higher rooting percentage was obtained in media containing IAA (75 and 78.6) at 0.5 and 1 mg/l, respectively. Under protected circumstances, 1 mg/l IAA produced the greatest proportion (64%) for rooted plantlets.

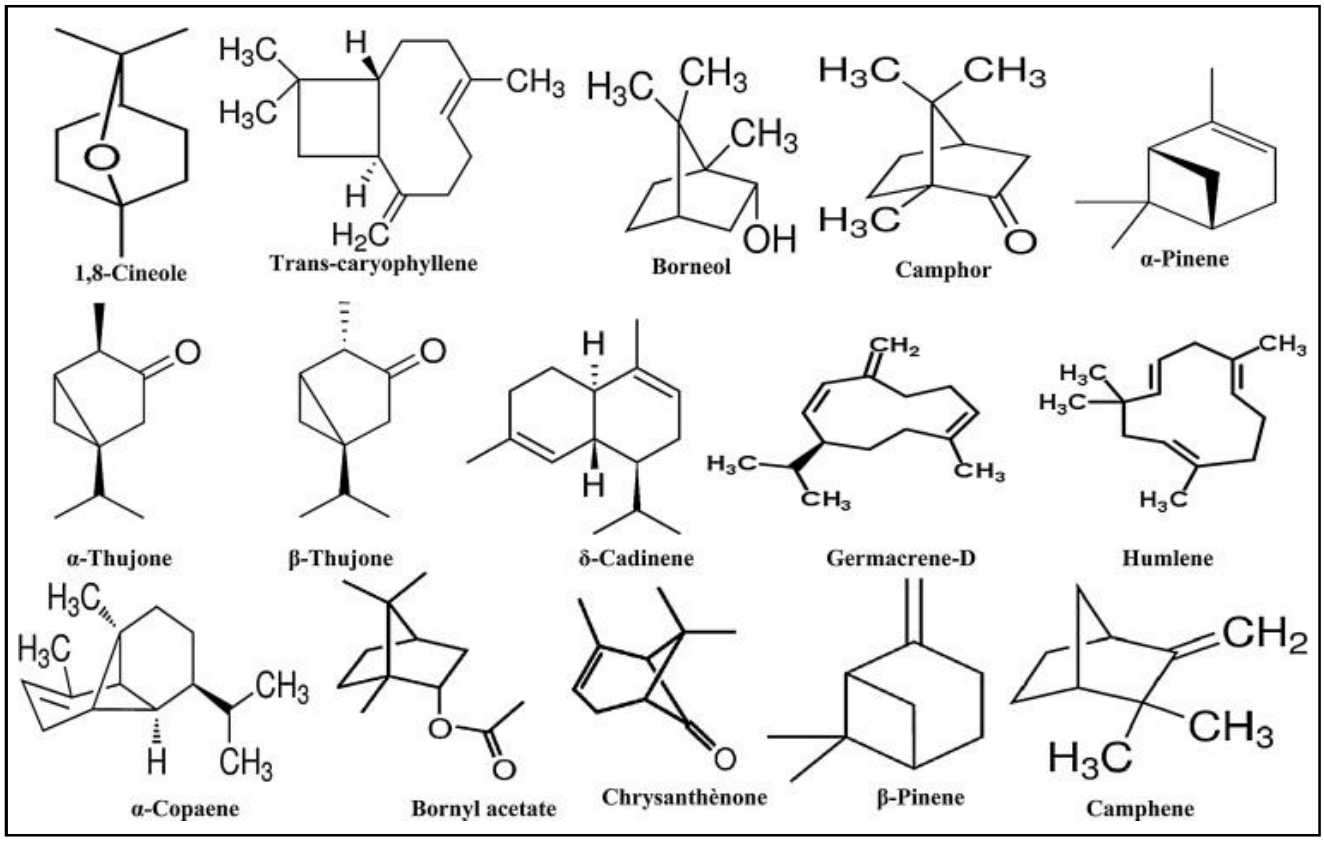

Figure 1: Essential oil components of *Rosmarinus officinalis.*

Darwesh and Alayafi (2018) who evaluated the procedure for shoot induction from the sterilized seedlings of *R. officinalis* seeds cultured them on MS media, transferring the seedlings to MS media modified with abiotic elicitors (Kinetin and BA) in addition to the biotic elicitor (coconut water) to avow the accrual of the tallest shoots and highest number of shots at a level of 3.0 mg/l BA + 5.0 ml/l coconut water and 5.0 mg/l Kinetin, respectively, while the former concentrations produced the highest number of leaves with the highest phenol content divulged at a concentration of 3.0 mg/l. The highest anthocyanin content way fared at a level of Kinetin 3.0 m/l with coconut water 5.0 mg/l.

2.5 Commercial protocol

El-Zefzafy *et al.* (2016) studied the physiological and phytochemical responses of *R. officinalis* with natural and synthetic auxin on *in vitro* callus production and induction. Auxins (IAA or

NAA) and cytokinins (6-benzyl amino purine or TDZ) were used to examine callus induction, callus fresh, dry weight, and callus size. The mass of the highest callus induction and growth was noticed at an intervention of NAA 0.125 + 6-benzyl amino purine 1.0, and this indication was similar to that of Masoody and Stanica (2013), who found that the combined effect of BA and NAA greatly affected the appearance of rosemary callus growth (callus volume, callus fresh, and dry weight). The effects of auxins (IAA or NAA) and cytokinins (6-benzyl amino purine or TDZ) on total hydroxycinnamic derived products as rosmarinic acid in *R. officinalis* callus demonstrated that the proposed MS that is supplied with NAA and 6-benzyl amino purine, had a little positive significant effect on rosmarinic acid build-up. However, when compared to shoot tip explants (*i.e.,* control), the impact of 6-benzyl amino purine (at 0.5 and 1.0 mg/l) was particularly noticeable. The maximum rosmarinic acid production (0.487 % dry weight basis) was seen at NAA 0.125 + 6 -benzyl amino purine 0.50, which was marginally higher than that in the plant alone, suggesting that growth regulators had no effect on rosmarinic acid production (El-Zefzafy *et al.,* 2016).

Table 1: *In vitro* regeneration of *R. officinalis*

S.No.	Explant	Medium	Response	References
1.	Single nodal stem segments	MS + 0.2 mgl^{-1} 6-benzyl amino purine + 0.25 mgl^{-1} IPA	80 % Survival rooting successful	Misra and Chaturvedi (1984)
2.	Adventitious shoot	MS + 1 mgl^{-1} 6-benzyl amino purine and 2 Mm Proline	Elite clonal lines were developed	Ronghui, *et al.* (2009)
3.	Leaves	MS + 0.5 mgl^{-1} 6-benzyl amino purine + 0.5 mgl^{-1} 2,4 DMS + 0.5 mgl^{-1} 6-benzyl amino purine + 0.1 mgl^{-1} 2,4 D	Highest callus fresh weight	Noori, *et al.* (2009)
4.	Apical buds	MSBM + 6-benzyl amino purine (8.88 µM) + IAA(2.85 µM)	80 % Survival frequency	Leelavathi and Kuppan, (2013)
5.	Axillary buds	MSBM + 6-benzyl amino purine (8.88 µM) + IAA (2.85 µM; MSBM + 6-benzyl amino purine (8.88µÌ) + NAA (2.68µÌ) + IBA (4.92µÌ)	75% Survival frequency	Leelavathi, *et al.* (2013)
6.	Leaves	MS + 2.0 mgl^{-1} 6-benzyl amino purine + 1.5 mgl^{-1} NAA	Feat of highest callus size (mm^3) callus fresh and dry weight (g)	Al Masoody, *et al.* (2015)
7.	Apical buds	MS + 2.0 mgl^{-1} 6-benzyl amino purine + 2.0 mgl^{-1} NAA	Callus fresh and dry weight (g) was significantly influenced	Al Masoody and Florin (2015)
8.	Young leaves	MS + 1.5 mgl^{-1}6-benzyl amino purine + 1.5 mgl^{-1} NAAMS + 2.0 mgl^{-1} 6 – benzyl amino purine + 1.5 mgl^{-1} NAA	Positive increase in the number of somatic embryos after 45 days	Al Masoody and Florin (2015)
9.	Apex	MS + 0.2 mg/l BA + 0.3 mg/l AIAMS + 8.0 mg/l 2,4D	Multiplication on mediums with low doses of phytohormones	Laslo Vasile, *et al.* (2015)
10.	Shoot tips	MS + 6-benzyl amino purine 1.0+ NAA0.125	Highest callus induction and growth.	El-Zefzafy *et al.* (2016)
11.	Leaves	Media devoid of growth regulators Media + growth regulators (IAA)	The highest acclimatization percentage (64%)	Mascarello, *et al.* (2017)
12.	Leaves	MS medium + 1mg/l CPPUWPM+ 1mg/l CPPU	The highest mean No. of shoot was 2.40 shoots/explant was produced.	Salwa, *et al.* (2018)
13.	Seeds	MS + modified growth regulators the level of 5.0 mg/l Kinetin medium modified to 3 mg/l BA + coconut milk at 5 ml/l.At the level of 3.0 mg/l BA.When coconut water at 5.0 ml/l + Kinetin 3.0 mg/l	Highest number of shoots/explants	Darwesh and Aisha (2018)
14.	Shoot tips	MS + 6-benzyl amino purine + NAA (MS I) 1 mg l^{-1}/0.1 mg l^{-1} and (MS II) 2 mg l^{-1}/0.1 mg l^{-1}	The density of trichome was found to increase by three folds in comparison with *in vivo*.	Valbona Sota *et al.* (2019)

El-Zefzafy *et al.* (2016) affirmed the superiority of the synthetic auxin NAA over the natural IAA through the implicit of the NAA group in the enhancement of the physiological activity of the rosemary callus culture. Sakr *et al.* (2018) substantiated betwixt the three rosemary cultivars *R. officinalis* (C1), *R. officinalis* Pyramidalis "Upright Rosemary" (C2), and *R. officinalis* Angustifolius "Pine scented" (C3) with the highest essential oil percentage (0.95%) in *R. officinalis.* Angustifolius which was 4 times the amount obtained from that of *R. officinalis* Pyramidalis and this cultivar had twice the amount of essential oil of *R. officinalis* (0.10% only) with verbenone as the main component in C1 and C3 while camphor marked as the major in C2. Yet C1 and C3 wayfare in their scents are attributable to the concomitant essences. In accord with De-Mastro *et al.* (2004), the quantity and quality of essential oil vary due to the type variance and environmental impact while the consequential components stick to the standard. Yang *et al.* (2009) set down the development of elite clonal lines of rosemary *via* tissue culture methods for the furnish of natural phenolic antioxidants. Inoculation of clonal lines of rosemary with *Pseudomonas* sp. on the grounds of syntheses of total phenolics and rosmarinic acid put forth 5 classes of clonal lines, thereby establishing the fact microbial elicitors play a vital role in the stimulation of plant secondary metabolites and they are potent in the production of consistent quality.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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