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Promising Current Trends of Plant Biotechnology and Prospective Future for Sustainable Development of Medicinal Plants and Their Applications in Phytotherapy

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Abstract

Plant cell tissue and organ culture (PCTOC) technology is one of the most significant biotechnology branches, called plant biotechnology. It is applied in production and conservation of medicinal plants for phytotherapy, to combat communicable and non-communicable diseases. Plant biotechnology is a proven technique for quick propagation of medicinal plants, particular endangered species. Medicinal plants are the most valuable source of human life saving drugs that play an essential role in health issues mitigation all over the world's population. This current work intends to highlight the outlines of PCTOC biotechnology for enhancement of valuable bioactive metabolites accumulation in plant tissue yields of medicinal plants via elicitation processes using various biotic and abiotic elicitors. This design will deliver mass production of valuable metabolites aggregations, to be used on scale up production for pharmaceutical preparations. Not only that but also, this work elucidates much interest of green and nano-green biotechnology as recent advances in plant biotechnology applications in our life. This study aimed to epitomize the key investigations and advance made in studying plant biotechnology trends, for medicinal plants development and prospective phytotherapy. Plant biotechnology products can be marketed commercially as natural therapeutic medicines and food supplementary.

Introduction

Biotechnology is the use of biology to develop new products, methods and organisms intended to improve human health and society. Biotechnology, often referred to as biotech, has existed since the beginning of civilization with the domestication of plants, animals and the discovery of fermentation. Early applications of biotech led to the

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development of products such as bread and vaccines, with rapid spread of Biotechnology in agriculture, industry, pharmaceutical requirements for medical purposes. This spread has revolutionized humanity due to the improvement of products quantitative and qualitative. Year by year, the biotechnology-based commercial products are increasing (Datta et al. 2021), particularly medical industry which consider as the worthiest sectors economically all over the world. Moreover, a challenge is oriented using biotechnology in the developing countries to promote the human proficiencies and infrastructure to confirm adequate and influential domestic production. The services donated by biotechnology increased globally the life quality and health (Bhatti et al. 2022). Biotechnology provided influential approaches of drugs delivery as new procedures for therapeutics beside the modified crops by genes to be nutritionally enrich along with the functional techniques for environment pollution elimination (Srivastava et al. 2020, Chena et al. 2022). Nowadays the role of biotechnology has become more potent in different aspects of life, economically, medically, industrially and nutritionally. Despite the most important advantages in biotechnology approach globally, there are many constraints limiting biotechnology applications in developing countries. Lately, researchers aimed to evolve the intrinsic biotechnology possibilities, to improve public health through our initiatives for intensive studies, and exploration of further developed research in sake of availability, to the rest of the world, the profits of biotechnology. Our points of view and efforts in this regard involve, more functional drugs and cheaper derived natural resources, efficacious therapies and enriched crop production, nutritional, tasty and quality. This state of art will shed light on one of the most significant biotechnology disciplines, plant cell tissue and organ culture (PCTOC) technology which called plant biotechnology, it is a corpus of approaches designed for the germination and propagation of the plant cells, tissues and organs by using solidified artificial media rich in nutrient elements under aseptic conditions. This biotech provides a suitable phytohormone regime, appropriate explants like buds, meristems, stems, leaves, roots, etc. to be cultured in vitro and undergo to regulated division for gradual development forming complex structures such as hypocotyls, internodes, cotyledons, anthers, shoot tips, stems, roots, leaf disks, and ultimately whole plants. PCTOC biotechnology is based on totipotency hypothesis, which states that each cell has ability to regenerate forming a complete plant (Long et al. 2022). PCTOC is a proven technique for quick propagation, conservation of plant endangered species (Moghadam et al., 2022), enzymes production (Fasim et al. 2021), and supplying an alternative resource of valuable phytocompounds (Chandran et al. 2020).Nowadays, scientists are keen to use PCTOC biotechnology to be applied in production of genetically modified crops (GM crops) that high yields of biomass, nutrition, and micronutrients, and in medicinal plants conservation for phytotherapy, to combat the communicable and non-communicable diseases. Non-communicable (chronic) diseases such as diabetes, obesity, colitis, cancer, cardiovascular, Alzheimer diseases, etc. (Donohue et al. 2023) and communicable (infectious) diseases that are caused by any of pathogenic strains such as bacteria, fungi,

viruses and harmful yeast (Debta et al. 2020). Thence, plant biotechnology approaches play a significant role to conserve and maintain medicinal plants with enhancement their yields of bioactive metabolites accumulation in plant cell/tissue cultures for phytotherapy as alternative natural sources in pharmaceutical and medical scopes. This current work is aimed to epitomize the key investigations and advances made in studying PCTOC technology trends for sustainable development of medicinal plants and the prospective future for phytotherapy.

Medicinal plants are the most valuable source of human life saving drugs that play an essential role in health issues mitigation all over the world's population (Khan et al. 2009). Several ancient years ago, the humanity depended on the conventional medicinal plants which are extensively collected from the wild (Kasagana and Karumuri 2011) as medical herbs and traditional drugs in the developing countries to serve the health care system. So, these plants have been extremely spread worldwide to address an assortment of different health problems. All parts of medicinal plants: leaf, root, fruit, stem, flower, seed or bark is involved in medicinal characteristics (Jitendra et al. 1996) due to their content of organic compounds which possess a significant function as defensive line in plant-environment interaction and. These compounds known as secondary metabolites (SMs) do not directly influence the development or growth of the plant (Hussain et al. 2012), so they are secreted in low amount (Kim et al. 2002). Secondary metabolites are called natural products, phytochemicals and are accountable for medicinal characteristics in plants which they include.

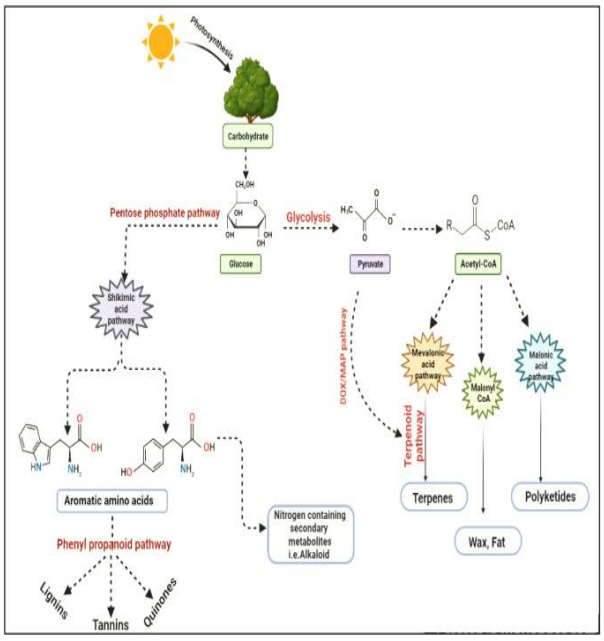

Fig. 1. Synthesis of secondary metabolites in plant (Humbal and Pathak 2023).

Therefore, SMs are defined as bioactive metabolites due to their action as an essential natural source of drugs candidate in pharmacological applications for treatment a lot of chronic diseases such as cancer, inflammations, osteoporosis, diabetes, Alzheimer and respiratory diseases, and infectious diseases caused by bacteria, fungi, yeasts, viruses, etc. (Kabera et al. 2014). It is previously known the differences between primary substances as metabolic intermediates, substantial for plant growth and development, and secondary metabolites as natural protective agents (Fazili et al. 2022), which are produced as derivatives of primary substances through shikimic acid, mevalonic acid and malonate/acetate pathways as shown in Fig. 1. Plant metabolites involve primary and secondary metabolites, the primary metabolites including carbohydrates, lipids and proteins, and the secondary metabolites are classified into three basic groups: phenolics, terpenoids and alkaloids, each group are divided into complex structure sub-classes as shown in Fig. 2.

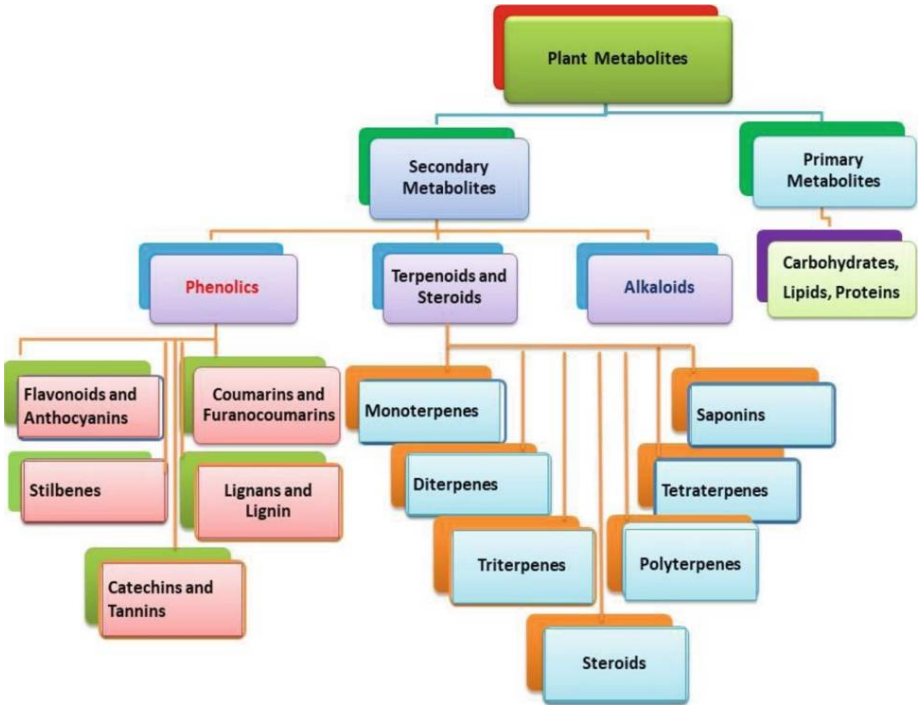

Fig. 2. General classification of plant secondary metabolites (Pandita and Pandita 2021).

The utilization of medicinal plants for pharmaceutical requirements dates back to 4000 years ago due to their safety and less side effects, so over 75% of global populations depend mostly on plant extracts for their health care demands (Khanam and Vayaravel, 2021). Several decades ago, pharmaceutical products were mainly using synthetic compounds from the libraries as medicines discovery source. Although the synthetic

compounds are comparably simple to resupply and produce with good compatibility for the founded high throughput screening platforms, there is a descend tendency in the recent drugs delivering to the market, promoting renewed scientific research in medicines discovery using natural materials, regardless its known challenges. Therefore, the researchers did their best for medicinal plants propagation in vitro to be developed and conserved sustainably using different methods of plant biotechnology techniques as will be highlighted in this study.

The phytocompounds in medicinal plants play a vital role as natural medicines for years thousands, and the reports by WHO revealed that the percentage of people still depend mostly on those plants as traditional remedies is up to 80%.There are many obstacles faced medicinal plants study such as little amounts of their bioactive phytocompounds prepared by chemical procedures, their inexact definition and speciation, and variability of their agricultural conventional protocols (Titanji et al., 2015).Therefore, currently there is a tendency to use different biotechnological methods for medicinal plants in vitro propagation with enhancement their yields of valuable phytocompounds. These biotechnological methods are divided into several tracks including, callus cultures and embryogenesis, organogenesis and plant regeneration, cryopreservation and encapsulation, and elicitation processes.

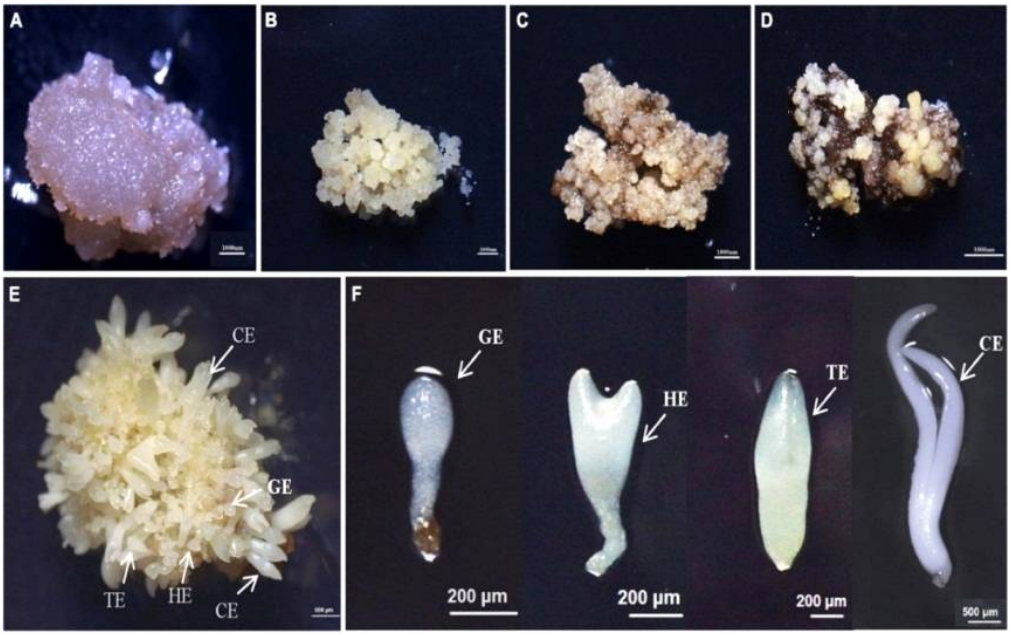

Fig. 3. Stages of Somatic embryos development in hybrid sweetgum SE. (a) NEC: non-embryogenic callus; (b) EC: friable-embryogenic callus; (c) PEM1: 20 days of pro-embryogenic mass; (d) PEM2: 45 days of pro-embryogenic mass; (e) embryogenic callus with somatic embryos; (f) Somatic embryos at different developmental stages (globular embryo: GE, heart-shaped embryo: HE, torpedo-shaped embryo: TE, cotyledonal embryo: CE) (Li et al. 2021).

Callus is massive growth of the cells, callose accumulation is associated with wounding, and it is produced from single cell and then it is proliferated randomly forming assembly of undifferentiated meristematic cells which are totipotent for whole plant regeneration (Nagata and Takebe, 1971). Callus cultures were induced in a lot of medicinal plants researches such as in Papaver orientale, rich in valuable alkaloids, where Zakaria et al., (2011) produced callus from cotyledons-hypocotyls by culture on B5 medium with suitable hormones. Under definite conditions, cells of callus move towards somatic embryos formation, embryogenesis is an operation where sets of somatic cells compose somatic embryos similar zygotic embryos of seeds, this embryo can grow forming seedlings on favorable medium such as in hybrid sweetgum (Qi et al. 2021). Fig. 3 shows somatic embryogenesis via callusing and development stages of somatic ebryos, globular (GE), heart (HE), torpedo (TE) and cotyledon embryos (CE).

Organogenesis includes, direct and indirect regeneration, and addition of plant growth regulators combinations to culture medium coordinate the cellular division for plant regeneration. The excised explants have identical genes with the donor plants, so plant direct regeneration from meristems of shoot or stem or vegetative buds has yielded encouraging findings in medicinal plants Such as Digitalis spp, *Catharanthus and Rauvolfia serpentina* (Perez-Bermudez et al. 2002).

Callus-mediated organogenesis for plant indirect regeneration in medicinal plants was accomplished by different growth regulators application in the culture medium (Tripathi and Tripathi 2003). Cell division, cell growth and tissue differentiation are stimulated by either endogenous growth regulators or exogeneous growth regulators added to the nutrient medium. Fig. 4 illustrates comparison between direct and indirect plant regeneration through microshoot formations derived from vegetative buds of clematis plant cultivar (Mitrofanova et al. 2021).

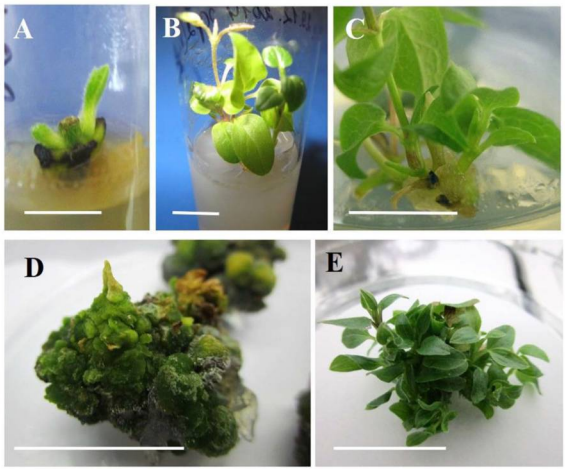

Fig. 4. Direct organogenesis (A, B, C) and indirect organogenesis (D, E) in vegetative buds of clematis plant cultivars (Mitrofanova et al. 2021).

Plant regeneration directly or indirectly were recultured on medium fortified withauxins as primary plant growth regulators, to promote roots, more profus roots were hardened in pots containing peatmoss and perlite as 2:1 in the green house before transplantation in the field. Fig. 5 exhibits protocol of plant tissue culture starting with explants passing through embryogenesis and organogenesis till complete plantlets in the bots for acclimatization stage.

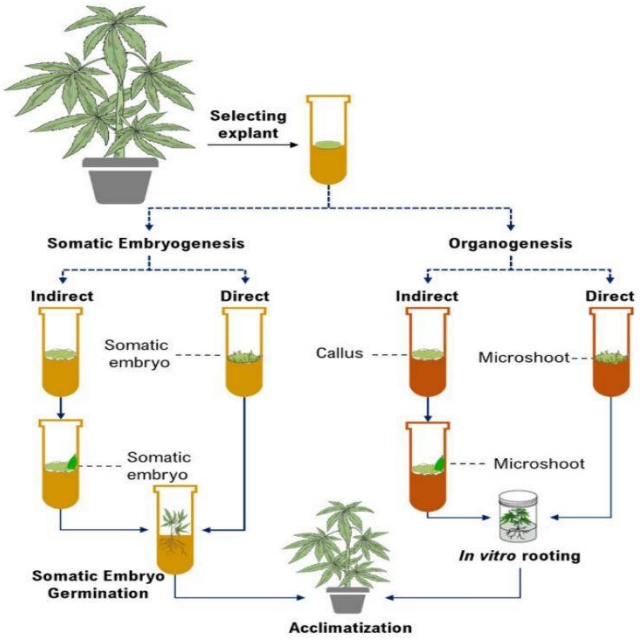

Fig. 5. Schematic representation of plant tissue culture protocol (Hesami et al. 2021).

Cryopreservation is a process to conserve in vitro cultures for a long time, a successful technique for medicinal plants maintenance. Cryopreservation is a long-term preservation procedure in liquid nitrogen at 196°C under zero, where cell division, biochemical and metabolic processes are stopped. A lot of cultures were perfectly stored in liquid nitrogen, and then the frozen cultures can be regenerated forming the whole plants (Bajaj 1991). Subsequently, cryopreservation supplies an opportunity for endangered medicinal plants to be conserved skillfully like in *Hyoscyamus* spp and *Rauvollfia serpentine*, where storage under low temperature is effective for their in vitro cultures producing alkaloids without abnormality either in their alkaloid content or in their fertility (Bajaj 1988). Various explants such as meristems, embryos, anthers, pollens, protoplast and calli were successfully stored by cryopreservation. As well, vitrification process is one of the conservation methods for medicinal plants; it is performed by

exposure plant tissue/organ to cryoprotective agents' solution (CPAs) which consists of high concentrations of dimethyl sulfoxide (DMSO), glycerol, sugar and ethylene glycol (Day et al. 2008). Excessive penetration of CPAs is useful for plant cells when increase the concentration of internal solute which contribute to cell volume maintenance and prevention cell damage (Meryman 1974). Meanwhile, overexposure of CPAs can induce cell damage due to excessive dehydration or the toxic nature of CPAs. The sensitive plants to exposure directly to vitrification solution, owing to osmotic stresses and dehydration intolerance, a loading stage range between 10-20 min are incorporated before incubation with CPAs. This step involves incubation the tissues with CPAs less toxicity/concentration (medium contains 0.4 M sucrose+2 M glycerol), for dehydration tolerance improvement (Nishizawa et al. 1993).Droplet-vitrification is a modified method of the primary vitrification protocol, comprises placing the plant sample with droplet of 1 to 10µl of CPAs, which consists of medium containing 0.4 M sucrose +30% glycerol+15% DMSO+15% ethylene glycol, this solution named plant vitrification solution (PVS) (Kaczmarczyk et al. 2012). The combined plant sample with PVS placed in aluminum foil piece before immersion in liquid nitrogen at -196°C as shown in Fig. 6. This procedure verified higher cooling and re-warming rates, where the less liquid volume lead to high ratio of heat to transfer from and to the sample (Sakai and Engelmann, 2007), as well the cooling rates increased up 130°C/sec (Panis and Lambardi 2005). Thence, the transfer of intracellular water from aqueous state to glassy state is quick, with reduction of water crystallization.

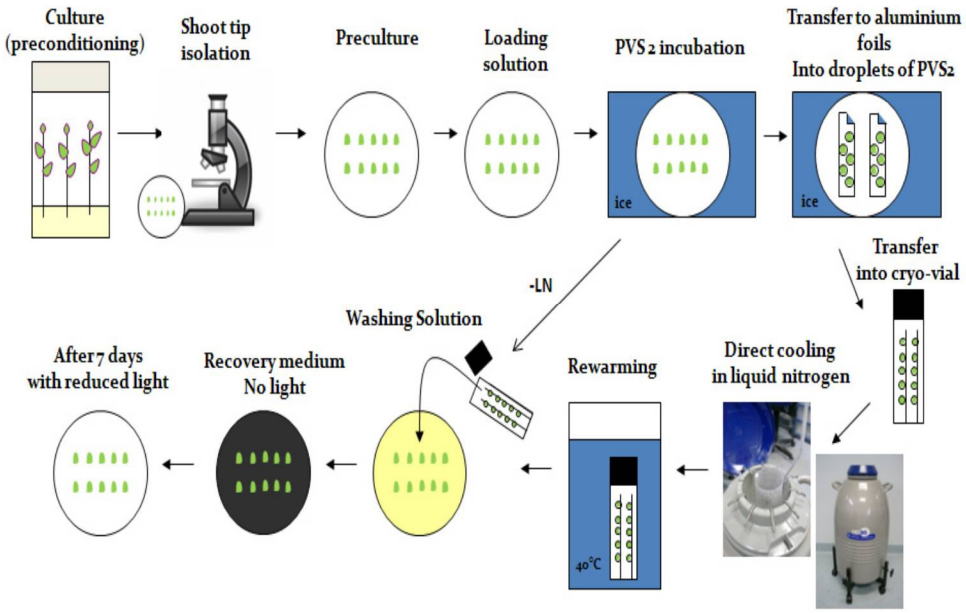

Fig. 6. Cryopreservation by droplet vitrification method (Kaczmarczyk et al. 2012).

Encapsulation process is one of the conservation methods for medicinal plants; it is performed by immersing the plant samples (somatic embryos, micro-tubers, auxiliary buds cell aggregates and shoot duds) in mixture of sodium alginate and calcium chloride. This leads to form encapsulated protocorm-like bodies (PLBs) called artificial seeds which can be cultured as seeds then converted into intact plants. Production of improved artificial seeds is accounted as essential alternate technology for commercial reproduction of many significant crops. This technique is important for mass proliferation of elite plant genotype, alongside an efficient protocol to storage seeds rich in their content of valuable bioactive metabolites (Rihan et al. 2017). (Sarmah et al. 2010)produced alginate beads from axenic leaf explants (six-month age) of endangered orchid (*Vanda coerulea* Griff. ex.Lindl), using 3% sodium alginate with exposure to 100 mM of calcium chloride solution for 30 min, firm was formed lucidity, circularly, and uniform identical beads were appropriate for handling. Authors found that the highest germination percent (94.9%) of the artificial seeds' recovery was achieved when beads were pollinated directly after formation (Fig. 7).

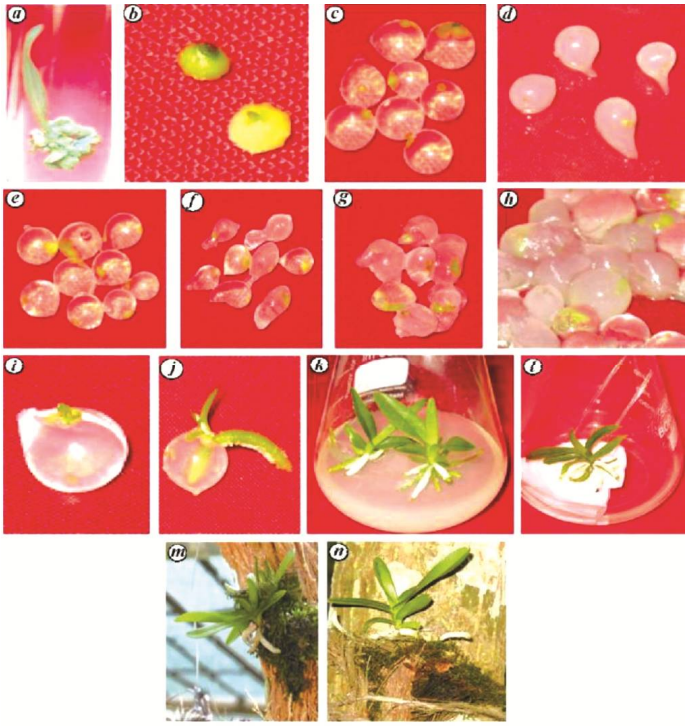

Fig. 7. Encapsulated artificial seeds of Vanda coerulea leaf base.a, Protocorm-like bodies (PLB) of Vanda coerulea leaf base. b, Isolated PLB from leaf base. Encapsulated seeds in c, 4% sodium alginateand 100 Mm CaCL2; d, 4% sodium alginate and 75 mM CaCL2; e, 3% sodium alginate and 100 mM CaCL2; f, 3% sodium alginate and 75 mM CaCL2; g, 2% sodium alginate and 100 mM CaCL2; h, 2% sodium alginate and 75 mM CaCL2. i, Germinated alginate beads. j, shoots and roots appearance. k, development of Complete plant from alginate beads. l, Hardening of plants. m, Hardened plants with primary binding substratum. n, Plant established in tree trunk (Sarmah et al. 2010).

Elicitation process is a system that provides an opportunity for plentiful research in bioscience scope of plant cell cultures exploitation to be extracted intensively their content of enhanced bioactive metabolites assemblages. These metabolites are essential promising products for nutritional, pharmacological and medical applications. Elicitation process is being performed by compounds stimulating plant physiological abnormality, are termed elicitors. Elicitors catalyze synthesis and gathering secondary metabolites in plant cells or induce novel metabolites as plant natural response versus elicitor stress in which elicitors act as signal compound of plant protective responses (Radman et al. 2003). Due to the broader definition of elicitors, they are classified into several categories according to their origin; biotic and abiotic elicitors which in turn divide forming many kinds of elicitors according to their structural nature as illustrated in Fig. 8. Abiotic elicitors are classified into chemical compounds of non-biological origin such as heavy metals, mineral salts, etc., or physical conditions such as temperature, light, drought, osmotic stress, etc. Biotic elicitors are classified into exogeneous factors of external substances that have pathogenic origin and endogenous factors of compounds that have been synthesized by plants. Exogenous elicitors are commonly released by any pathogens like yeast extract, polysaccharides of microbial cell wall and bacterial lysates. Endogenous elicitors involve polysaccharides of plant cell wall, intracellular proteins and phytohormones that are synthesized as plant response of stress (Patel and Krishnamurthy, 2013).

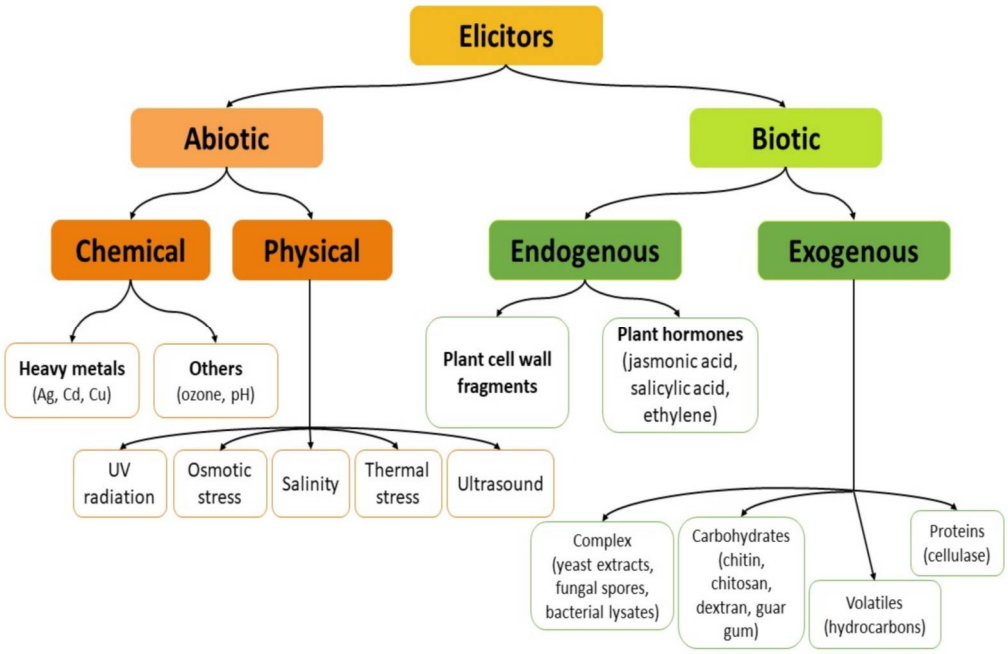

Fig. 8. Schematic diagram of elicitors' classification (Rogowska and Szakiel, 2021).

This study reviews each elicitor application in the culture system of medicinal plants, and its stimulating influences on bioactive metabolites accumulation, here are some of these applications. For physical elicitors, light stimulates the production of gingerol and zingiberene in callus cultures of *Zingiber officinale* (Anasori and Asghari 2008), ultraviolet affected positively to stimulate stibene from *Vitis vinifera* cell suspension cultures (Xu et al. 2015), and exposure to salinity promoted alkaloids, phenols and terpenes in *Plantago ovata*plant (Haghighi et al. 2012). Also, proline and polyethylene glycol (PEG) as osmotic agents enhanced steviol glycosides content in *Stevia rebaudiana* callus and cell suspension cultures (Pratibha et al. 2015), beside the efficient effect of PEG for hypericin and pseudohypericin production in *Hypericum adenotrichum* (Omer and Bengi, 2013). For chemical elicitors, ozone enhanced the production of rosmarinic acid from *Melissa officinalis* shoot cultures (Tonelli et al. 2015), heavy metals such as Cd^{2+} and Cu^{2+} produced high accumulation of shikonin (Mizukami et al. 1977) and digitalin (Ohlsson and Berglund, 1989), as well the betalaines production was stimulated by exposure of hairy root cultures to metal ions (Thimmaraju and Ravishankar 2004), Cd^{2+} and Co^{2+} increased resveratrol in *Vitis vinifera* cell suspension (Cai et al. 2013), and enhancement of betacyanins accumulation has been occurred by treating *Amaranthus caudatus* callus cultures to Cu^{2+} (Obrenovic 1990). In regard of hormonal elicitors, it is revealed that jasmonic acid enhanced higher amounts of Plumbagin in *Plumbago indica* hairy root cultures (Gangopadhyay et al. 2011), methyl jasmonate stimulated silymarin in *Silybum marianum* cell suspension (Firouzi et al. 2013), salicylic acid promoted digitoxin production in *Digitalis purpurea* shoots (Patil et al. 2013),both methyl jasmonate and salicylic acid induced bacoside A compound from *Bacopa monnieri* shoot cultures (Jeyasri et al. 2023) as shown in Fig. 9 and gibberellic acid increased Tanshinones accumulation in *Salvia miltiorrhiza* hairy roots (Yuan et al. 2008).

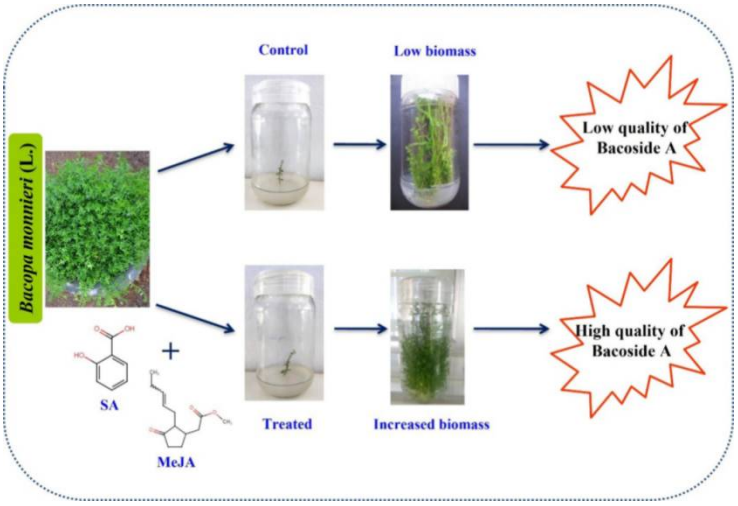

Fig. 9. Enhancement of bacoside A biomass in *Bacopa monnieri L.* (Jeyasri et al. 2023).

In case of biotic elicitors, chitin, pectin and dextran promoted Hypericin and pseudohypericin accumulation in *Hypericum perforatum* shoot cultures (Sonja et al. 2014), yeast extract stimulated Cryptotanshinone and tanshinone IIA production from *Perovskia abrotanoides*a dventitious roots (Arehzoo et al. 2015), *Escherichia coli*, *Bacillus cereus* and *Staphylococcus aureus* induced higher quantities of atropine in hairy root cultures of *Datura metel* (Zahra et al. 2015). In addition, oligogalacturonic acid as polysaccharides derivative from cell wall stimulated ginseng saponin content in cell suspension of *Panax ginseng* (Hu et al. 2003). Success of biomass production and bioactive metabolites accumulation in medicinal plants is influenced by these parameters; elicitor type and concentration, cell line, period of exposure, nutrient composition, and treatment schedule and culture stage. For example, high elicitor dose caused hypersensitive response resulting cell death, whilst appropriate dose is the optimal for induction (Roewer et al. 1992) as occurred in *Gymnema sylvestre* plant, MeJA at concentration over 150 μM caused drastic fall of gymnemic acid accumulation to be recorded 36.3% (Chodisetti et al. 2015), duration of MeJA exposure between 24 and 72 h gave an initial rapid increment for bioactive metabolites quantity in which more than this period may be caused subsequent decrease (Wungsintaweekul et al. 2012), and culture medium composition applied higher amounts of cocaine, chlorogenic and cinnamoylcocaine in *Erythroxylum coca* callus cultures (Docimo et al. 2015). Furthermore, culture age is a vital parameter, plays an essential role for bioactive metabolites production by elicitation, where hairy root cultures of *Withania somnifera* at age 40 days exhibited highest quantities of withaferin A, withanone and withanolide A by treating with SA and MeJA (Sivanandhan et al. 2013).

In the recent era, nanotechnology is termed nanomaterials, is gradually imparting their considerable impact in our life and environment. Since a few decades ago, nanomaterials have infiltrated into each sector of sciences for de novo alternatives creation (Tran et al. 2012). Kinds of nanomaterials, such as nanoemulsions, nanocomposites, carbon nanotubes and nanoparticles, are applied in several disciplines like energy, textiles, electronics and medicines (Khan et al. 2019). Nanoparticles (NPs) defined as particles of matter with sizes between 1-100 nm in diameter, have one dimension or more in their external or internal surface, they are manufactured or natural (Hasan, 2015). NPs are classified based on the provenance into three categories; natural NPs which derived from forest fires, volcanic eruption, mineral processes and photochemical reactions; incidental NPs which emerged from industrial processes; engineered NPs like composites, magnetic and metallic (Nowack and Bucheli 2007). Engineering nanoparticles called commonly nano elicitors or elicitors' nanoparticles (NPs). Elicitors NPs are intelligent nanomaterials because of their unique physiochemical properties and marvelous applications which indicate to their improved features founded on their shapes, sizes and structures. The mentioned features made NPs to be easier solubility, better functionalization and less toxicity (Rai et al. 2018, Joshi et al. 2019). Recently, a lot of researchers applied elicitors NPs in plant biotechnology as novel elicitors to be affected positively on bioactive substances biosynthesis in plant culture

system of medicinal plants. Nanotechnology is paramount technological revolution defined by size, in this sense it involves any nanometer sizes dependent technology, so elicitors NPs has been studied their potential applications in vitro and ex vitro to improve the crops productivity nutraceutical and nutritional, alongside enhancement of bioactive ingredients production in medicinal plants via plant tissue culture protocols and plant biotechnology approach (Rivero-Montejo et al. 2021, Lala 2020). The nanometer size properties confer a large surface area per unit, elevated energy to surface and quantum detention confinement, which in turn lead to improve their biochemical activity. Hence it could be suggested that the significance impact of elicitors NPs in reactive oxygen species (ROS) generation may act as trigger for plant bioactive metabolites. Take into your consideration that the distinguished properties of elicitors NPs are accountable for their unique attitude and environment impacts in comparison to bigger particles in the same type (Marslin et al. 2017). Information about elicitors NPs action as efficient elicitors in metabolites biosynthesis are insufficient, however comparatively little research have been applied on elicitors NPs in production scope of valuable bioactive substances. There are various advantages of elicitors NPs such as tiny size, big surface compared to volume ratio, capability of electron exchange engineering, high surface reactive possibilities, and entrance easy and interaction with different components of plant cells, beside extreme strength and durability, and catalytic activity promotion. Upon the aforementioned elicitors NPs advantages, several studies proved that nanoparticles which have been used in different in vitro cultures as novel elicitors of plant defensive biochemistry are associated by enhancement of bioactive metabolites production (Fig. 10).

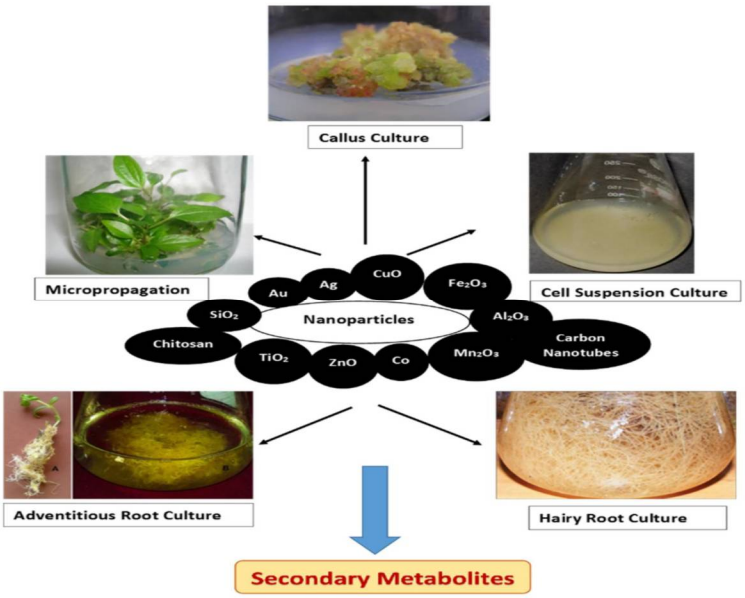

Fig. 10. Schematic representation of secondary metabolites production by elicitor NPs in plant tissue cultures (Lala 2020).

A lot of elicitors NPs including zinc oxide (ZnO), titanium dioxide (TiO2), silicon oxide (SiO2), iron oxide (Fe3O4), selenium (Se-NPs) and copper (Cu-NPs) have received considerable attention due to their non-menace use in the agriculture field (Alabdallah and Hasan 2021, Hashem et al. 2021). Elicitors NPs are produced by particular strategies, including physicaland chemical procedures (Akhtar et al. 2022), and their positive effectiveness on growth and development of plants are variable according to their concentrations, origins, sizes and application time to the crops (Rubilar et al. 2013). It is known that, production of bioactive metabolites by plant cell culture occurred fundamentally in medicinal plants (Hatami et al. 2019), therefore using elicitors NPs in medicinal plants for bioactive compounds induction have been performed to promote therapeutic potential, which is imputed to its carotenoids, coumarins, flavonoids, alkaloids, terpenoids and volatile oil (Muley et al. 2009). Here are some researchers who proved through their recent investigations the significant role of nanoparticles as novel elicitors to augmentation the bioactive metabolites accumulation in several medicinal plants. Singh et al. (2018) found an increment quantity of total phenols and flavonoids content in *Withania somnifera L.* shoots and roots using CuO NPs in their culture system, Karakas 2020 improved production of tryptanthrin and indigo in *Isatis constricta in vitro* plantlets by Ag NPs elicitation and Mosavat et al. (2019) who confirmed success of the elicitation process for thymol and carvacrol production using ZnO NPs of 150 mg/l concentration in culture system of *Zataria multiflora* and thymus species (*Thymus kotshyanus* and *Thymus daenesis*). According to the acknowledgement of several researchers regarding consideration Ag NPs as novel and influential elicitor in plant biotechnology to produce bioactive metabolites. Rezaei et al. 2011 and Chung et al. 2018 depicted the influence of Ag NPs in *Cucumis anguria L.* hairy root cultures, and found promotion of biomass accumulation for total phenolic structures such as protocatechuic, vanillic acid vanillic acid, ϱ-coumaric, chlorogenic, hydroxybenzoic acid, syringic, t-cinnamic, ferulic and caffeic acid, furthermore, the authors found high antioxidant, antifungal and antibacterial activity with perfect cancer cell inhibition. Furthermore, TiO_2 NPs are one of the most studies for enhancement the production of bioactive substances, so particular interest has been paid to apply TiO_2 NPs, Kruszka et al. 2020 increased the germination of seeds in okra plants by TiO_2 NPs application, and also Ogunkunle et al. 2020 increased fresh weight in fruits of Abelmoschus esculentus (L.) using TiO_2 NPs

The topic of elicitors NPs in the in vitro cultures is acquiring attention, yet there is little research regard their impacts. In spite of elicitors NPs advantages for enhancing bioactive metabolites, notorious negative impact by doing NPs in plants called toxicity (Siddiqui et al. 2014, Siddiqui et al. 2015). This phytotoxicity of NPs action is highly complex and relies on the chemical, physical characteristics and the interference between the plant and the environment (Tripathi et al. 2017), also the toxic effects of NPs may depend on particles size and dose. A lot of scientists revealed that the primary mechanism underlying NPs toxicity is the induction of ROS which is raised in plants by their exposing to NPs, resulting in further oxidation stress in plant cell/tissue (Rico et al.

2011, Tripathi et al. 2017, Nair et al. 2010). In normal state, ROS may promote metabolic pathways (Hatami et al. 2019), excessive quantities of ROS may subsequently result in damage in proteins and DNA of the cell membrane, lipid peroxidation, leading to possible cell death and inhibition of physiological processes in plants (Yan and Chen, Z, 2019). Principal phytotoxicity are occurring in plant cell by excess production of ROS, which induce alteration of physiology, morphology with genetic and biochemical constitutions in the plant (Tripathi et al. 2017). Morphological changes include grown possible, biomass and upgrowth of seeds, which are generally associated with toxic impacts of NPs exposure (Aslani et al. 2014). Here are some of these negative effects of phytotoxicity which have been reported by those of Wang et al. 2016 who found growth reduction of Arabidopsis thaliana by 20% and 80% using 200 and 300 mg/l ZnO NPs, respectively, alongside inhibition of genes expression regard chlorophyll biosynthesis and photosystem structure, Hong et al. 2015 who discovered reduction in root length of *Lactuca sativa*and *Medicago sativa* by 49% with activity promotion of ascorbate peroxidase, and plant size reduction with nutrient content alteration. Furthermore, concerns regard the toxic effects of NPs in aquatic plants have been screened, using CuO NPs in *Chlamydomonas reinhardtii* cause growth inhibition, carotenoids level decrease significantly and increasing of ROS level (Melegari et al. 2013). The overall concept of harmful effects of plant exposure to NPs that plants are hugely consumed by animals and humans, so NPs may be absorbed into plant organs and may bioaccumulate in the tissues which are used later for consumption (Tripathi et al. 2017). It is more necessity to screen the biosafety of elicitors NPs used as novel elicitors in plant biotechnology approach, this biosafety involves in life cycle, exploitation by plants and the entrance directly or indirectly into the food chain (Rico et al. 2011). The main reason of phytotoxicity induced by elicitors NPs in which their toxic impacts in any organism may attribute to release toxic metals as precursor for interaction between NPs and cell membrane surface owing to shape or size of NPs (Kirchner et al. 2005). Although the profit of elicitors NPs application in plant biotechnology approaches is known economically, industrially and pharmaceutically, possible risk subjects need to be accounted, since NPs entrance into human body via oral exposure owing to water, food, air or drugs which involve nanomaterials (Karimi et al. 2018). Hence, it is supposed to research the biosafety issues to decrease the knowledge gap related to NPs' toxic influences upon entrance to any organism and causing oxidative stress, DNA damage, inflammation, proteins damage, fibrosis, carcinogenesis and cell death (Abdal Dayem et al. 2017).

Enhancement of bioactive metabolites accumulation in medcinal plant cultures are strictlly controlled spatially and temporally by biotic and abiotic elicitors. Transcription factors (TFs), a complex network comprising several regulatory genes, control in the spatiotemporal transcriptional coordination of metabolic biosynthesis pathways, TFs have a paramount role to regulate likely the genes involved in all aspects of plant propagation and evolution, beside bioactive ingradients production (Afrin et al. 2015).

Jasmonic acid (JAs) as abiotc elicitor is an ubiquitous elicitor to produce metabolites in plant kingdom in both of angiosperms and gymnosperms (Pauwels et al. 2009) via its essential signaling role across the plant to regulate several cellular activities (Memelink, 2009). In plant response to JAs as hormonal elicitor, TFs act as complex regulatory network to fine-tune the capacity, timing and tissue-particular expression of genes pathway and the subsequent aggregations of bioactive metabolites (Vom Endt et al. 2002). Otherwise, yeast extract (YE) as biotic elicitor is potent inducers of terpenoid indole alkaloids (TIA) production through activation of multiple TFs which in turn is binding with gene promoters of TIA biosynthesis (Patra et al. 2013). Plant response to several signaling molecules appear through plant generation a broad variety of bioactive metabolites in which plants respond to any stress via redirecting primary metabolites (PMs) and enhancing secondary metabolites (SMs) production such as terpenes, tannins, phenols, flavonoids, etc. Biochemical and physiological processes occur in plant cells as response to the stimulus (elicitor), mechanism of elicitors action in plant cells is shown in Fig. 11 which illustrates the consecutive reactions that take place throughout elicitor-stimulated defense responses. The plant cell membrane includes receptors which detect the elicitor and catalyze a series of reactions in the cell thorough phosphorylation and dephosphorylation across plasma membrane, increment of calcium concentration in cell cytosol with outflow of potassium and chloride ions, and stream of protons enter the cell, this cell state results in acidified cytoplasm and extracellular alkalinization. These sequential alterations activate several signaling pathways, for example, pathway of mitogen-activated protein kinase (MAPK), that leads to NADPH oxidase activation, which stimulates reactive oxygen species (ROS) production (Humbal and Pathak, 2023). This operation triggers early defense genes expression to provide bioactive metabolites accumulation as lasting defense versus the elicitor (Ramirez-Estrada et al. 2016).

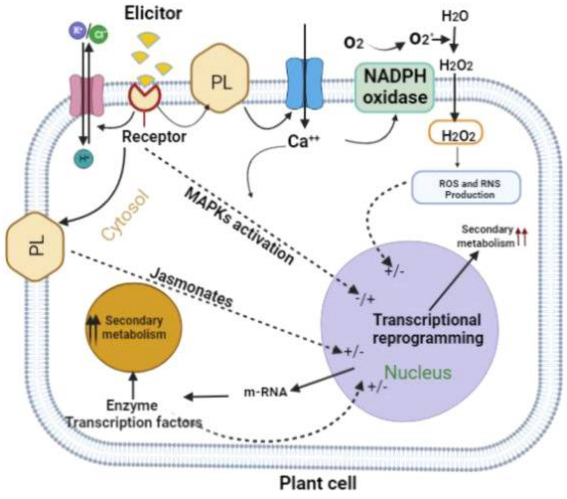

Fig. 11. Schematic diagram of generic mechanism of elicitors action (Humbal and Pathak 2023).

A bioreactor is apparatus in which organisms like plant tissues, or microbe cells are cultivated and aids in production of their desirable yields in a contained environment. Bioreactors play a vital role in bioprocess engineering and are considered as a key step for commercial production of bioactive compounds through plant biotechnology. Bioreactor technology has become a gainful manufacture worldwide. Chemical and biochemical transformations are occurring in the reactor which is enclosed by unit operations that perform physical changes for products recovery through medium preparation. There are different aspects of bioprocess engineering for plant cell cultures derived products dependent on bioreactor types and optimal strategies for bioactive substances production (Su et al. 2019). Bioreactors characterize by these essential properties, optimum control for cell suspension cultures scale up under precise parameters, possibility of constant regulation action for bioreactor operation conditions at different stages, easy of handing for inoculation and harvest during culture, enhancement of nutrients uptake through immersed culture procedures which promote multiplication rate and bioactive substances yields (Tripathi and Tripathi 2003). Furthermore, using bioreactor systems with different cultivation conditions may affect positively morphogenesis and biomass aggregations of plant in vitro cultures, grown in bioreactors. Gaseous atmosphere deficiency in bioreactor system allows precise control of oxygen delivery and carbon dioxide exchange, to improve transfer processes of nutrients and dissolved O_2 through the system. As well the morphogenesis and biomass aggregations are affected by optimization of carbohydrates, growth regulators and minerals in the liquid medium (Gatti et al. 2017). Upon the advantages of bioreactors technology mentioned above, some scientists reported that the progression of plant biotechnology in agriculture, pharmacy and medicine sectors is associated with some applications of modulated bioreactor systems (Yancheva et al. 2019). Bioreactors give superior conditions for large-scale valuable ingredients production from medicinal plants to be applied on commercial scale in several industries (Kieran et al. 1997), for example production of sanguinarine in significant quantities from *Papaver somniferum* cell suspension cultures (Park and Yoon 1992), production 500 mg/l/day of saponin from Ginseng in vitro root cultures (Charlwood and Charlwood 1991), and production of ginsenoside from *Panax ginseng* adventitious roots (Hahn et al. 2003). Production of plant bioactive metabolites via in vitro culture by bioreactor apparatus was performed by Wawrosch and Zotchev1 2021 as shown in Fig. 12. (A) represents in vitro cultures such as shoots, roots, callus, hairy roots.(B) represents multiplication of primary in vitro cultures as first selection for establishment of liquid cultures. (C) represents selection of the highest yield lines with optimization the culture conditions including the nutrient composites, light, temperature, aeration, agitation and inoculum density, as well as elicitation processes across precursor feeding. (D) represents bioreactor design which depends on culture pattern, stirred tanks, bubble column, spray reactors or temporary immersion for organ culture.

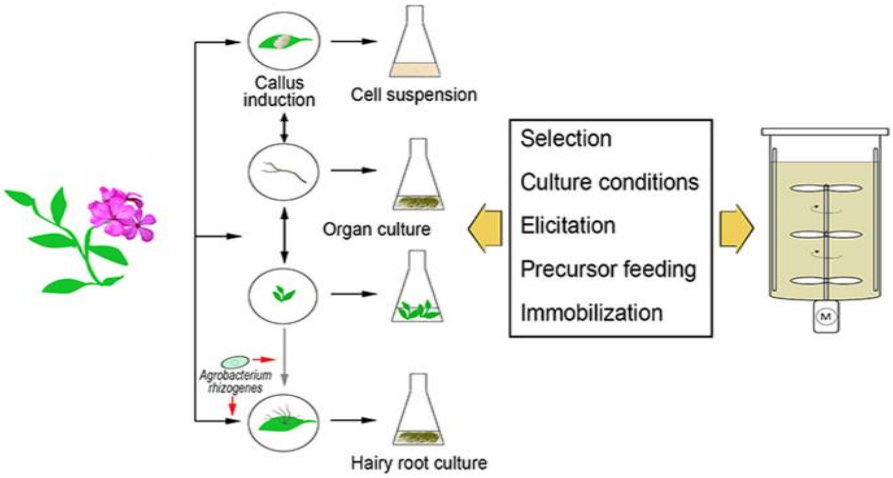

Fig. 12. Schematic representation of bioactive metabolites production from plant in vitro cultures via bioreactors technology, A: in vitro cultures, B: liquid cultures, C: yield lines, D: bioreactor (Wawrosch and Zotchev1 2021).

As a result of bioreactor system benefits which including quick cultures growth, better transfer of gases and nutrients, scalability of the process and decrease labor charges, system of bioreactor allows high grade of cultivation process automation with keeping on constant and precise environmental conditions. Bioreactors have been used globally in production scale-up for plant bioactive metabolites to be applied in pharmaceutical and industrial sectors (Fig. 13).

Fig. 13. Industrial scale-up production of plant bioactive metabolites through bioreactors technology (Photobioreactor Wikipedia).

As known, biotechnology is accountable for the changes of living organism and their metabolites to produce many products that are helpful for the humanity. Biotechnology is used extensively in several aspects of the human life; industrial, nutritional, pharmaceutical and medical, so biotechnology categorized generally according to the applied field into various types such as white biotechnology, green biotechnology, blue biotechnology, red biotechnology, brown biotechnology, purple biotechnology and yellow biotechnology. Owing to enlarged range of biotechnology applications, highlighting by colors was used to distinguish the major research areas, such as white color expresses to microbes-based industry, green color expresses to agriculture, blue color expresses to marine or fresh water, red color expresses to pharmaceuticals, brown color expresses to desert biotechnology, purple color expresses to inventions and patents, and yellow color expresses to insects derived drugs (Barcelos et al. 2018). This part of work will highlight ton the recent advancements of plant biotechnology such as green biotechnology and nano green biotechnology. Green biotechnology is related to our specialty, all biotechnological techniques regarding crops, plants, agriculture fields, plant cell and tissue culture, etc., are the main subjects that were subcategorized of green biotechnology. The benefits of green biotechnology involve improvement of nutritional quantity and quality for the crops, obtaining of plants drought-resistant and diseases-resistant, development of transgenic crops, hybrid varieties and biofuels plants, and increasing of bioactive metabolites productivity of medicinal plants with reduction their production costs(Yashveer et al. 2015). Scientists seek currently through green biotechnology to decrease the reliance of agriculture on chemical and mechanical procedures via using fewer offensive practices to the surrounded environment, beside contribution of better food emergence and plant productivity increment of their metabolites in less cost (Silveira et al. 2005).From the other promising modern technologies, nano green biotechnology, it is a technique involving in nano formula by biogenic nanoparticles synthesis of various metals such as silver, zinc, titanium, carbon, etc. Biosynthesis of NPs became more popular in comparison of chemical procedures to reduce the pollution in the surrounding environment; advantages of biogenic synthesis are attributed to raw materials availability with their less cost (Rauwel et al. 2015). Biosynthesis methodology of NPs lies in using living organisms like bacterial or plant cells as provider source of bioconversion Nano green 5 (Mohanpuria et al. 2008). Plant extracts are used as simple and safe alternative materials to produce NPs of the elements on a large-scale (Iravani 2011). From the significant metal ions which have been used in biogenic nanoparticles synthesis, silver ions that possess unique features to can be applied in various applications such as elicitors, antimicrobials and cosmetic products (Iravani et al. 2014, Srikar et al. 2016). Ag NPs have an efficient influence to promote bioactive metabolites (flavonoids, tannins and phenols) accumulation in callus cultures of both of Juniperus procera (Salih et al. 2022) and Citrus *reticulata L* (Anum et al. 2019). Augmentations of the bioactive metabolites in medicinal plants lead to raise therapeutic activity (Muley et al. 2009). Based on the aforementioned potent role of Ag NPs in

bioactive compounds enhancement, Ag NPs were biosynthesized according to the procedure described by Ahmed et al. 2016 and Ashraf et al. 2016. A volume of 100 ml of *Phoenix dactylifera* leaf extract was mixed with 50 ml of 1 mM aqueous silver nitrate (AgNO3) solution in ratio 2:1, v/v, then the mixture was heated at 80°C for 20 min, preliminarily change was detected across the chromatic conversion from yellow to dark brown forming silver nitrate nanoparticles (Ag NPs). The mixture was then decanted into tubes (15 ml) before centrifugation for 25 min at 4500 rpm, the formed pellets were collected with discarding the supernatant (Fig. 14). For confirmation the good performance of biogenic Ag NPs, many techniques were used to screen Ag NPs properties; UV-visible spectrometer (200-800 nm), FTIR (Fourier Transmission Infrared spectrometer) to detect the functional group, DLS (Dynamic Light Scattering) to identify the surface charge, SEM (Scanning Electron Microscope) to measure surface morphology, size and silver nanostructures distribution, and X-ray spectroscopy to measure energy-dispersive.

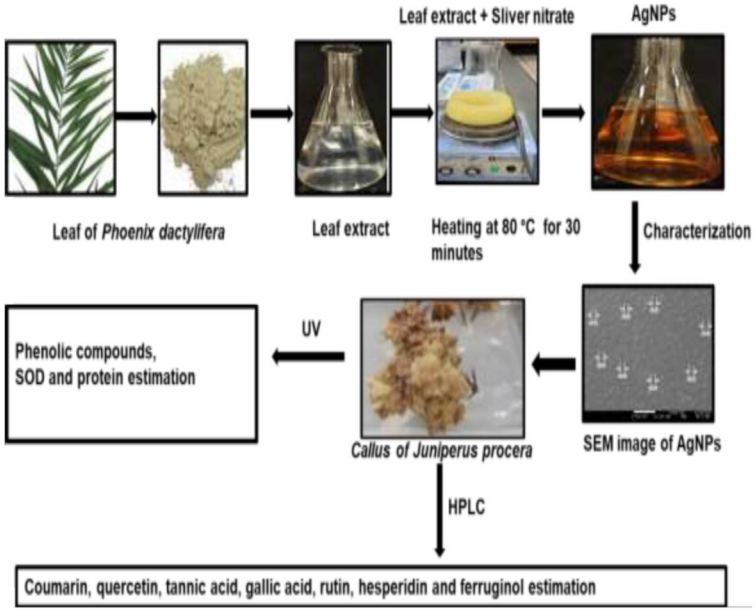

Fig. 14. Steps of biogenic silver nanoparticles (Ag NPs) synthesis (Salih et al. 2022).

The main idea of biogenic Ag NPs synthesis depends on the excitation of surface plasmon oscillations in Ag NPs, this excitation induces change of the color from yellow to brown. The chromatic conversion of the reaction indicates to reduce Ag^{+} formula to Ag° formula in Ag NO_3 solution, confirming Ag NPs formation (Khalil et al. 2014). Subsequently, to be sure of nanoparticles synthesis biologically, there are two steps; the color change as the first sign to form NPs and screening the characterization of NPs by several techniques (DLS, UV, FTIR and SEM) to confirming the shape, surface charge,

morphology, size and functionalization (Banerjee et al. 2014). Mode of action of biogenic NPs in plant cell is attributed to effect of NPs on the biochemical and physiological processes through metabolic process, hormone signaling and electron transport chain (Paramo et al. 2020). As a result of the previous findings regarding the significance of biogenic nanoparticles in enhancement of the plant bioactive metabolites with consideration the less-cost of production, simple and safe method, it is recommended to use biogenic NPs via nano green biotechnology to be serve as the optimal model for enhancement the production of bioactive metabolites in medicinal plants in vitro.

Plant biotechnology applications serve many industrial concerns through either new products delivery or modulation of existing products to be employed in several businesses. Biotechnological methodologies gave a lot of agronomic traits such as crop yields increment and increasing of the bioactive metabolites production in medicinal plants, these agronomic traits are mainstay for industrial products and phytotherapy. Some of biotechnological applications in our life such genetically modified (GM) crops which have been mentioned by Srivastava 2018 who reported that the genetically modified (GM) crops helped in crop yields improvement including biomass feedstock's, food functionality and nutrition, and micronutrients in plant foods such as zinc, iron and β-carotene. Therefore, GM crops can be served as a tool for improvement the nutrients in foods via biotechnology techniques. Not only that but also, plant biotechnology strategies provided essential tools for phytocomponents production to be extended commercially for biosynthesis several high-value compounds of significance to chemical, food and pharmaceutical industries. Interestingly, several techniques of plant biotechnology were employed to produce considerably pigments from different in vitro cultures (callus, organ and cell suspension cultures) for industrial production. This in vitro technology allowed the production scale up of pigments such as anthocyanins which ordinarily are not present in field-grown plants. Production scale-up of these pigments is still one of the greatest challenges for scientists in plant biotechnology to be enabled for considerable production of pigments to be used in food manufacturing processes as natural chromatic pigments instead of chemical dyes Simões et al. (2012). Here are some of these research; Youssef et al., 2021 elevated the productivity of some phytocompounds of *Antigonon* leptopus; the total pigments such as carotenoids, chlorophyll a, b and anthocyanins in shootlets using 0.1 mg/l IBA+0.4 mg/l 2iP; phenols, flavonoids and antioxidant activity in shootlets using 0.6 mg/l 2iP. Also, addition of 1 mg/l TDZ+2.5mg/l NAA to callus culture medium enhanced their active compounds of pigments, phenols and flavonoids structures. Mohamed et al. 2021 enhanced diosgenin compound as bioactive secondary metabolites in *Balanites Aegyptiacae* callus cultures by adding 300 mg/l of tryptophan to calli medium, with consideration of the extract biosafety on the normal human cells. Khalifa et al. 2022 found that callus cultures of *Silybum marianum* L contained the highest phenolic amounts by their exposure to 25 Gy γ-radiation with phenylalanine (4 mg/l) and the maximum flavonoids content by exposure calli to 25 Gy γ-radiation with phenylalanine (1 mg/l), as well the authors detected 11 substantial flavonoids in callus

cultures of *Silybum marianum* L. Mamdouh and Smetanska 2022 obtained the highest productivity of antioxidants, flavonoids and phenols in cell suspension cultures by cultivation leaf explants of *Lycium schweinfurthii* on MS-medium supplemented with 2 mg/l NAA. Lashin and Elhaw 2016 detected the biggest alkaloids content in the regenerated plantlets of *Physalis peruviana, and* the biggest amounts of total saponins and tannins in callus cultures from the seeds. Owis et al. 2016 offered an alternative resource of *Calligonum polygonoides* L. for massive quantitative of secondary metabolites content through callus, cell suspension cultures and the differentiated shoots of *Calligonum polygonoides* L. Atteya et al. 2021 improved the production of chlorophyll a, b and carotenoids in the in vitro plantlets of *Aloe vera* using the combinations of NAA and BAP in culture medium, and increased phenols, flavonoids and antioxidants using the treatment of 0.1 mg/l NAA+3 mg/l BAP, so they recommended to use the in vitro technique for *Aloe vera* plant reproduction with increasing its content of phytocompounds. Concerning of phytotherapy using medicinal plants, the historical development relevant to herbs-derived natural product medicine detection, accompanying challenges of natural product-based medications discovery are seriously discussed in several research. In this regard, a glance of promising plant biotechnology approach to cure several diseases such as Alzheimer disease and microbial infection. For Alzheimer's disease (AD), it is a neurodegenerative disease, it is the fifth leading reason of death in patients have or up 65 years old (Reitz 2012). Its symptoms appear in form of behavior deterioration, memory lack, thought lateness, performance weakness and severe depression (Squire 1992). Radad et al. 2011, Chen et al. 2012, Aglawe et al. 2021, Marde et al. 2021 and John et al. 2022 illustrated that Alzheimer's disease depends on tau aggregation and amyloid deposition which return back to Misfolded protein in cerebrum, inducing oxidative stress-produced damage. It is known that acetylcholine (ACh) is remarkable for cognitive performance among neurotransmission systems, individuals have less ACh concentrations are the most susceptible to AD injury. Based on significance of cholinergic in AD genesis, cholinergic neurons depletion, and acetylcholinesterase activity are abnormalities lead to AD symptoms (Upaganlawar et al. 2021, Kale et al. 2022, Francis 2005). Some drugs and acute psychological stress are reasons of amnesia and Alzheimer disease, allopathic drugs are prescribed for AD treatment, but they induce harmful side effects. Subsequently, medicinal plants are proven their tremendous adequacy as natural perfect source of drugs to address memory deficit and AD in fewer side effects (Akram and Nawaz 2017). Specificity of phytocompounds for cerebrum receptors indicates to the influential role of herbal drugs in neurological illnesses management, through their action as special cholinesterase inhibitory, this lends support in AD therapy (Lundstrom et al. 2017, Kale et al. 2022). From these medicinal plants which met a renewed scientific interest to AD remedy; Ginseng containing ginsenoside, *Ginkgo biloba* containing ginkolides, *Glycyrrhiza glabra* containing glycyrrhizic acid, Pistacis vera containing flavones, Moringa olifera

containing quercetin, Phyllanthus acidus containing terpines and Curcuma longa containing curcumin (Fig. 15).

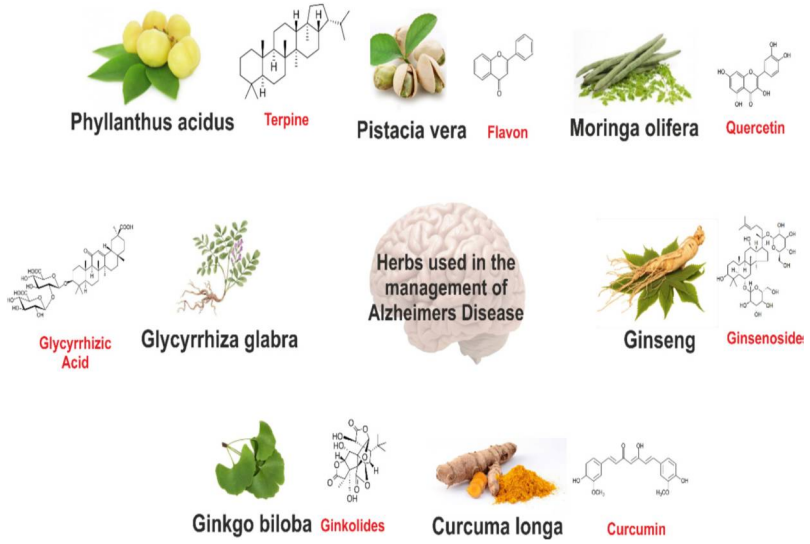

Fig. 15. Different phytocompounds of medicinal plants for Alzheimer's disease management (John et al. 2022).

These phytocompounds can be serving as important neuroprotective potentials as anti-amyloid-beta aggregation and acetylcholinesterase inhibitory activity Moeini et al. 2019. Therefore, Plant biotechnology approach must be activated for the *in vitro* propagation of the aforementioned valuable medicinal plants with enhancement their content of bioactive metabolites (ginsenoside, ginkolides, glycyrrhizic acid, flavones, curcumin, quercetin and terpines) that are responsible of Alzheimer disease remedy. For the microbial diseases, extracts of medicinal plants grown in vitro through plant biotechnology approach were screened and confirmed their efficiency as antibiotics via their actions against several strains of microbial strains. Butanol extract of *Jatropha curcas L*. hypocotyl callus recorded the highest antibacterial effect for *Escherichia coli*, followed by ethylacetate fraction, then water extract (Soliman et al. 2018). Extract of *Calendula officinalis* callus exhibited very close results to these antibiotics; erythromycin, ampicillin, penicillin G, chloramphenicol and novobiocin for *Staphylococcus aureus* and *Bacillus cereus* inhibition, with remarkable the most effective of *C. officinalis* on bacteria positive gram (Çetin et al. 2017). Also, Faraz et al. 2020 confirmed the potent inhibitory efficiency of *Oroxylum indicum L* callus extract against *Staphylococcus aureus* and *Micrococcus luteus*, Magdoleen et al. 2020 reported high inhibition of *Escherichia coli* using hypocotyl callus extract of fenugreek more than seed-extract and Shirsat et al. 2021who revealed that *Bacillus subtilis*, *Klebsiella pneumonia* and *Escherichia coli* were susceptible to methanolic extract of *Caesalpinia bonducella* calli except for *Staphylococcus aureus*. On the same

approach, Al-Saleh et al. 2019 affirmed the best action of microshoots and calli extracts of *Ammi visnaga L* against bacteria in comparison of the extracts of field-grown plant, and Abaka et al. 2020 confirmed stronger antifungal efficiency of *Balanites aegyptiaca* callus extracts than the seed extracts, hence they recommended to use extract of *Balanites aegyptiaca* callus as a good material of therapeutic substances for fungal related disease. On basis the previous investigations about the significant role of plant biotechnology approach for development the valuable medicinal plants as promising alternative natural drugs, phytotherapy were applied using plant biotechnology products in medical sector to be marketed commercially as natural therapeutic medicines and food supplementary (Fig. 16).

Fig. 16. Different types of plant biotechnology products as alternative natural medicines and diet supplements (Products of Plant Biotechnology, Wikipedia).

The potential of plant biotechnology is the axis of the development of many agroindustry fields, and its methodologies have enabled the prompt evolution of several products, commercial processes and economic interests in various sectors of the life; industrial, nutritional, pharmaceutical and medical. The potent role of biotechnology in maintaining the scarce, endangered and threatened medicinal plants, as well enhancement the plant bioactive metabolites is achieved significantly via plant cell, tissue and organ culture (PCTOC) biotechnology techniques. Currently, it is consulted from the advanced references the updated information, to exhibit new potentialities of PCTOC biotechnology in drug research and progression. The modern scientific approach for reproduction medicinal plants in vitro is performed, to guarantee the continuous

productivity of their valuable bioactive metabolites qualitatively and quantitively. Plant biotechnology pathway is one of the most significant trends for medicinal plants mass production across the biotechnology techniques to proceeding towards modern procedures for augmentation the valuable phytocompounds to be extracted profusely for fulfill the pharmaceutical requirements and drugs disability to save the necessary needs for medical purposes service. As well as plant biotechnology can be an efficient catalyst in marketing crops, which can create work chances, gain foreign exchange, to warranty better life for all trendy societies.

Based on all previous collected reports regarding the plant biotechnology approach in medicinal plants development for prospective phytotherapy, here is a glance of some suggestions and intellects about this subject, it is necessary to interest considerably of plant cell tissue and organ culture (PCTOC) technology research. This approach of plant biotechnology opens wide future prospects for promising applications in different aspects of our life. So, it is suggested to exploit PCTOC technology for universal investment, economically, industrially, pharmaceutically and medically. This thought will promote the research field to reinforce the concept of employing the scientific research foundations and methodologies to serve the community distinctly. Here is my idea in this regard; through plant biotechnology techniques aforementioned, Egyptian community can benefit in several sectors particularly, nutritional, pharmaceutical and medical fields. This benefit can be verified using plant cell tissue and organ culture biotechnology for conservation the endangered and threatened medicinal plants, and enhancement their bioactive metabolites accumulation, to recovery mass production of enriched in vitro cell cultures, to service the nutritional, pharmacological and medical purposes. The promising and valuable medicinal plants those are endangered particularly in Sinai; *Thymus vulgaris*, *Lavandula stoechas*, *Juniperus phoenicea, Calotropis procera, Capparis spinosa, Vitex-Angus castus, Origanum Majorana, etc*. Interestingly, the other attempts which it will be must perform, are the acclimatization of whole in vitro plantlets of the enhanced valuable endangered medicinal plants in the green house before transplantation in the fields. This leads to mass propagation of rich agriculture seedlings, to reconstruction much area of our Egyptian lands such as deserts and Sinai. Furthermore, it could be designed herbariums rich high ingredients value, to be sufficient for fulfill the pharmacological requirements and drugs deficiency, to save the necessary needs for the medical objectives service.

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