Secondary metabolites in date palm

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Introduction

Date palm (*Phoenix dactylifera* L.) is widely grown in the hot arid regions mainly in the Middle East and North Africa, and provides nutrition, food security, and raw material to the food industry; and considered as a tree of life. It makes a significant contribution towards the creation of equable microclimates within oasis ecosystems and thus enabling sustainable agricultural development in saline and drought affected areas. The rich fruit plays an important role in the nutrition of human population. Moreover, this crop has a great potential as a source of renewable energy, an alternate source to the fossil energy, by producing bio-fuel since its fruits high carbohydrates, 44-88% total sugars, and production of secondary metabolites that are useful to pharmacy, nutrition, agro-food, and may have potential to serve as attractant or repellent to insects attacking date palm trees.

As an overall defense strategy, plants consistently synthesize, accumulate and utilize a wide range of secondary metabolites: quite many have been used worldwide as medicines for curing various human diseases, nutraceutical, agro-food, and perfume. International trade in medicinal plants has become a major force in the global economy and the demand is increasing in both developed and developing countries. Most medicinal plants are harvested from the wild. The quality and consistency of the products are most challenging issues facing the plant-based medicines. The production of medicinal metabolites in plants is affected by plant genotype, cultivation, harvesting, processing and distribution. Medicinal plant preparations may also be contaminated with microbes and soil contaminants such as heavy metals, herbicides, pesticides, and other agricultural chemicals which can cause qualitative and quantitative changes in the levels of medicinal metabolites. *In vitro* cell culture and controlled environment production systems offer an excellent opportunity for the selection and seasonal independent propagation of elite lines with specific, consistent levels of medicinal metabolites with minimum contamination. Additionally, the plant materials produced by *in vitro* techniques allows efficient application of the emerging analytical methods, such as metabolomics.

Date palm as a source of secondary metabolites

Date fruit is a rich source of sugar, nutrients and pharmaceutical secondary metabolites such as phenolic, citric acid, oxytetracycline and ethanol as well as essential oils, polyphenols and dietary fibers. The phenolic compounds, hydroxycinnamates, Gallic acid derivatives, monohydroxybenzoic acids, flavones and anthocyanins are widely distributed in date palm. Phenolic detected in dates are known to exhibit antiviral, antibacterial, antifungal, antiulcer, antitumor and immuno-modulatory properties, making them a remedy for certain diseases and prevention of chronic inflammations, and inhibitory to pathogens and parasites. The antioxidant activity of date palm has been attributed to phenolic compounds. Dates are also rich in carotenoid and provitamins. The major carotenoid pigments are lutein and β-carotene. Provitamin a value also varied with cultivar and ripening stage. Studies on the saturated fatty acids in the flesh and seeds of dates revealed the presence of saturated fatty acids, including capric, lauric, myristic, palmitic, stearic, margaric, arachidic, heneicosanoic, behenic and tricosanoic acids. Unsaturated fatty acids included palmitoleic, oleic, linoleic and linolenic acids. Phytosterols are beneficial in lowering the blood levels of low-density lipoprotein LDL, the so-called *bad* cholesterol; involved in blocking the absorption of dietary cholesterol into

the bloodstream and in inhibiting the reabsorption of cholesterol from bile acids in the digestive process, thus reducing the amount of cholesterol returned to the bloodstream. Alpha-tocopherol (vitamin E) is often used orally to treat deficiencies, and in preventing cardiovascular diseases, diabetes and its complications, and benign prostatic hyperplasia. This vitamin is also administered against angina, thrombophlebitis, intermittent claudication, hypertension, and to prevent ischemia-reperfusion injury after coronary artery bypass surgery. Alpha-tocopherol reduces the risks of various cancers, Alzheimer's, and Parkinson's diseases and other dementias. Vitamin E is also used against allergies, asthma and other respiratory problems, as well as digestive or circulatory diseases. Additionally, vitamin E is used topically against dermatitis, aging skin, preventing skin ulceration often caused as a consequence of chemotherapeutic drugs.

In vitro culture of date palm

In vitro techniques such as somatic embryogenesis and organogenesis are suitable for large-scale plant multiplication of vegetative propagated crops, and biomass production for secondary metabolite production. The success of these techniques is highly genotypic dependent, however, have successfully been applied for plant propagation in wide ranging crops including date palm. Micropropagation via direct organogenesis is widely used for rapid clonal propagation of elite genetic material of date palm. The performance of micropropagated date palm seems to be better than conventionally grown plants in terms of yield, early flowering time, and quite uniform in fruit quality and physical properties. Micropropagation has an advantage of using low concentrations of plant growth regulators, consequently callus phase is avoided. The duration of culture period is limited by frequent subcultures for maintaining and providing shoot cultures for plantlet production. However, the highest number of subcultures must be determined before starting the fresh cultures from the mother plants. Somatic embryogenesis is induced for producing somatic embryos in many date palm cultivars, grown in date palm growing countries. This technique is improved to enhance plant regeneration rate through partial desiccation of somatic embryos. These studies have contributed to understanding the effects of a variety of medium components and culture conditions leading to enhanced plant regeneration. Matured somatic embryos could be compared with zygotic date palm seeds in terms of phytochemical quality and quantity and any new chemicals produced. Since zygotic and somatic embryos behave similarly and high quality phytochemicals produced in somatic embryos could be up-scaled by growing somatic embryos in a bio-reactor.

Cell suspension cultures are well established from friable callus and used to induce somatic embryogenesis for the development of somatic embryos, secondary metabolites, protoplasts, in vitro mutagenesis and cryopreservation. In addition, cell suspension cultures grow very well in temporary immersion bioreactor (TIB) for high output of date palm somatic embryos and shoot proliferation. Also, plant cell and somatic embryogenic cultures can be entrapped or encapsulated in alginate beads for a certain period of time to allow secondary metabolites to accumulate in plant cells and harvest secondary metabolites. Initially fast growing plant cells are selected depending on the plant type. The exposure of plant cells to osmotic stress or ultra violet rays or sequential treatment with abiotic and biotic stress would enhance the accumulation of metabolites in plants cells; scale up plant cell biomass production in a bioreactor for large-scale production of compounds. Plant cell biomass harvest can is readily done at the stationary phase of cell growth and extract accumulated compounds. Initially fast growing plant cells are selected depending on the plant type. The exposure of plant cells to osmotic stress or ultra violet rays or sequential treatment with abiotic and biotic stress would enhance the accumulation of metabolites in plants cells; scale up plant cell biomass production in a bioreactor for large-scale production of compounds. Plant cell biomass harvest can is readily done at the stationary phase of cell growth and extract accumulated compounds.

Cryo-storage or cryopreservation is widely used for long-term storage of *in vitro* cultures of genetic material under ultralow temperatures, usually at -196^{o}C in the liquid nitrogen. In vitro storage of selected elite callus and plant cell lines is highly desirable for future uses. They can also be stored for short-term at low temperature. Cold storage of shoot cultures and plantlets facilitate year round supply genetic material. Moreover, plant cells would lose their specific trait during subsequent cultures depending o0n the genotype.

Somatic cell hybridization

Somatic cell hybridization is used to produce somatic hybrids by fusing protoplasts of donor and recipient, which can be sexually incompatible plant species. We can produce different type of somatic hybrids such as asymmetric, symmetric, cybrids, and somato-gameto hybrids in several crop plants. This technique is suitable for partial genome transfer, organelle transfer, virus resistance, cytoplasmic male sterility, herbicide resistance, and other various traits. Date palm protoplasts are isolated by enzymatic treatment of fine cell suspension or leaf mesophyll or pollen, and roots. Proto-calli are formed after 1-2 weeks of culture and transferred to shoot regeneration medium. Well-developed shoots are rooted on root initiation medium. However, plant regeneration from proto- calli as well as the production of somatic hybrids is yet to be realized to enhance production of secondary metabolites and even produce new compounds. In tobacco, somatic hybrids showed an increase in nicotine content. In a somatic cell fusion product of carrot and tobacco, carrot showed nicotine content without change in the morphology of asymmetric somatic hybrid. This was done by partial genome transfer from tobacco to carrot. Therefore, we could produce somatic hybrids by two different plant species and even sexually incompatible.

Hairy root culture

Hairy roots, transformed with soil bacterium *Agrobacterium rhizogenes*, characterized by the extensive formation of extensive formation of adventitious roots at or near the site of infection. The ability of the bacterium to incite hairy root is encoded by a large plasmid called the root-inducing (RI) plasmid. The rhizogenicity of *A.rhizogenes* infections distinguishes hairy root tumors from the related disease, crown gall, which is incited by strains of *Agrobacterium* that harbor tumor-inducing (Ti) plasmid. Hairy roots are suitable for the production of secondary metabolites because of their stable and high productivity in hormone-free culture conditions. A number of plant species including many medicinal plants have been successfully transformed with *A. rhizogenes*. Transformed root cultures have a potential source of high-value pharmaceuticals and have a great potential to increase the alkaloid accumulation by modification of technique. The selection of high productive root lines offers option to enhance the productivity. Elicitors and modification of culture conditions have been shown to increase the growth and the alkaloid production in some cases. Hairy root cultures can be grown in a bioreactor for scale-up production of desirable secondary metabolites having a great commercial interest, either in pharmacy or agro-food or nutraceutical.

Shoot cultures

Shoot cultures are another way to produce biomass for the extraction of secondary metabolites. They can be produced on solid culture medium containing agar or gelrite gelling agent. Shoot cultures are sub-cultured on the fresh culture medium at 3-4 week interval depending on shoot growth. Another approach to produce them is in RITA bioreactor or Temporary immersion system, which is quite efficient and ideal in shoot biomass production. Prolific shoot cultures are also

produced by transgenic approach using *Agrobacterium*-mediated transformation system containing cytokine producing genes. Transgenic shoots could be grown in a bioreactor for large-scale production as well as entrapment of shoot buds or meristem

Biotransformation

Plant cell cultures exhibit a vast biochemical potential for enhancing the production of specific secondary metabolites, especially which do not form and accumulate in vitro cultures. However, biotransformation mediated by plant enzymes or chemical compounds such as steroids, aromatic, and other elicitors. Plant cell cultures and enzymes have the potential to transform cheap and plentiful substances into rare and value added products by change of environment constituents. Plant bioconversion system may be used alone to produce novel chemicals or in combination with organic synthesis. Also, elicitors are used to stimulate to enhance plant metabolite synthesis. Elicitation is a process of induced or enhanced synthesis of secondary metabolites by the plants. The application of elicitors has been considered as one of the most effective methods to improve the synthesis of secondary metabolites in medicinal plants. Plant secondary metabolites are unique sources for pharmaceuticals, food additives, flavors and other industrial materials. Some of the commonly tested elicitors are salicylic acid, methyl salicylate, benzoic acid, chitosan and others which affect production of phenolic compounds and activation of various defense related enzymes in plants. It might be interesting to look at the metabolite profile and identify chemicals responsible for virus resistance in date palm, and use them as elicitors to control viruses in other plants.

Metabolomics and nano technology

Plant Metabolomics is the study of metabolic pathways and processes through the use of analytical methods in model species. The information gained from this research is used to understand how plants grow and carry out functions, as well as improve the quality of food or medicines. The first step is having a large database of encountered and identified metabolites. Other functional genomic techniques such as proteomics or transcriptomics already have such databases established. Currently, it is estimated that only 10% of the metabolites within an *Arabidopsis* leaf has been characterized. This is largely a shortcoming of technology. The most challenging aspect of using metabolomics as a tool is the amount of redundancy. Metabolites are often linked to several enzymes or the appearance of several phenotypes.

The seeds of date palm are rich different types of phytochemicals including polyphenols. Recently, an eco-friendly method for the synthesis of gold nanoparticles (GNPs) from the seed extract of date palm without any extra stabilizing or capping agents. The synthesized GNPs were utilized as a catalyst for the sodium borohydride reduction of 4-nitrophenol to 4-aminophenol. Therefore, there is a great potential of nanotechnology in pharmaceutical industry for improving drug delivery as well as use as elicitors for biotransformation.

Recommendations

1. Establish friable callus system from different explants of different date palm cultivars, and identify cultivars producing prolific callus

2. Establish fine cell suspension, and determine appropriate cell density for the initiation of cell suspension, doubling time of cell suspension, determine duration of growing phase before moving to stationary phase of the cell suspension; and make a cell suspension growth curve.
3. Identify an appropriate cell growth stage for the application of stress, e.g. UV-light treatment so that secondary metabolites accumulate in plant cells in large quantity
4. Develop hairy root culture system in the liquid medium, beginning with 250-500 ml flasks and gradually scale-up to bigger volume
5. Develop shoot culture system in the liquid medium, beginning with 250-500 flasks and gradually scale-up to bigger volume
6. Set up an appropriate bioreactor system for growing plant cells/hairy roots/shoot cultures/somatic embryos in order to scale up biomass production
7. In vitro mutagenesis for the selection of highly productive mutant lines of secondary metabolites
8. Cryo-storage of highly productive cell lines or callus for preventing their losses during continued tissue culture. These cell lines could be stored for a long period of time without
9. Identify trait specific gene for secondary metabolites.
10. Establish genetic transformation system for developing transgenic lines that are highly productive for secondary metabolites
11. Somatic cell hybridization for enhancing secondary metabolite production, and develop set of somatic hybrids producing new metabolites by asymmetric gene transfer.
12. Develop metabolomics system for the identification of metabolites useful for the pharmaceuticals, nutraceuticals, cosmetics, and food industry
13. Identify aromatic compounds that act as attractant to insect and pests, e.g. red palm weevil
14. Date palm seems to be resistant to viruses. Identify compounds making date palm for this trait.
15. Biotransformation: A wide variety of chemical compounds including aromatics, steroids, alkaloids, coumarins and terpenoids can undergo biotransformation using plant cells, organ cultures and enzymes.. Biotransformation efficiencies can further be improved using molecular techniques involving site-directed mutagenesis and gene manipulation for substrate specificity.
16. Establish date palm somatic embryogenesis, which may differ variety to variety or even tree to tree, and develop somatic embryo genic cell suspension and somatic embryos. Somatic embryos are ideal for cryopreservation.
17. Encapsulation or entrapment of somatic embryos and cells for enhancement of metabolite production
18. Deletgene by radiation treatment for undesirable traits, e.g. insect attractants or disease susceptibility.
19. Setting up facilities for plant cell tissue culture, molecular biology, HPLC, GPLC, bioreactors, and metabolomics
20. The quality control of the final product is essential before release to the consumer.