In vitro propagation technique of medicinal plants, a review

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تقنية التكاثر الدقيق للنباتات الطبية في المختبر، دراسة مرجعية

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المستخلص:

تحتوي النباتات الطبية على مواد ثانوية فعالة مثل مضادات الأكسدة ومضادات السرطان ومضادات الالتهابات ومضادات الجراثيم والميكروبات. الطلب المتزايد على المنتجات الطبية بالإضافة إلى الزيادة السكانية والتجارة غير المشروعة هي مؤشرات إلى استنزاف العديد من النباتات الطبية، والكثير منها مهدد بالانقراض في مواقعها الطبيعية. التكاثر في المختبر هو أداة التكنولوجيا الحيوية التي تحل هذه المشكلة. أظهرت ممارسات زراعة الأنسجة أن ثلاثة عوامل هي اختيار النبات والوسط الغذائي والتحكم في البيئة المادية، مهمة في نجاح الزراعة. مجموعة من التقنيات لنمو وتكاثر الخلايا والأنسجة والأعضاء النباتية باستخدام المغذيات العضوية في بيئة معقمة وخاضعة للرقابة. لكل نوع نبات ظروفه المختلفة في الزراعة المخبرية والحفاظ عليها في المزرعة أو تجديد النباتات السليمة من الخلايا المستنبتة. تستعرض هذه الدراسة تقنية التكاثر الدقيق في المختبر عن طريق إنتاج كميات هائلة بواسطة التفرع، التجدير والتأقلم.

الكلمات المفتاحية: *زراعة الأنسجة، الإكثار الدقيق، الموائل الطبيعية، التكاثر والتأقلم.*

Abstract:

Medicinal plants contain active secondary substances such as antioxidant, anticancer, anti-inflammatory, antibacterial and anti-microbial. The increasing demand for medicinal products in addition to the increase in populace and unlawful trade are indications to the reduction of several medicinal plants, many of which are threatened with extinction in their natural sites.

In vitro propagation is a biotechnological tool that solution to this problem. Tissue culture practices have shown that three factors namely explant choice, medium composition and control of the physical environment, are important in successful cultures. A set of techniques for the growth and reproduction of plant cells, tissues and organs using organic nutrients in a controlled and sterile environment. Each plant type has its own different conditions for initiating and maintaining plant cells in culture or regenerating healthy plants from cultured cells. This Study reviews in vitro micropropagation techniques by an achieving mass production by in vitro multiplication, rooting and acclimatization.

Keywords: *Tissue culture, in vitro, micropropagation, natural habitat, multiplication and acclimatization.*

Introduction:

Medicinal plants are plants that are generally and safely used for persons in the treatment of some diseases. In the past two decades, importance in medicinal plants has increased because of their health benefits in terms of safety and cost compared to synthetic drugs. (Thomford *et al*., 2018; Anand *et al*., 2019).

In all world civilizations, medicinal plants have played a large role in health care. Although the total number of flora in the world is not yet certain. However, the proportion of medicinal plants in the world does not exceed 10 % (Schippmann *et al*., 2006). The estimate of the World Health Organization (Bannerman, 1982). Most of the rural population in the world, about 80%, chances its needs for main health carefulness from alternative medicine (Yuan *et al*., 2016). Most of the world's population depends on medicinal plants because of their medicinal substances (Tripathi and Tripathi, 2003). Furthermore, More than 50% of the medicinal plants in the world are included in the red list and this is scary (Paunescu, 2009). Medicinal plants are used in many industries such as (drugs, make-ups, and flavoring) and others because they contain high-value materials, and therefore the demand for them is increasing. (Suneetha and Chandrakandh, 2006).

The World Health Organization (WHO) recorded that a large number of the world's population, especially the countryside, depend on medicinal herbs for their primary treatment (Vines, 2004).

Plants are the main source in the pharmaceutical industry, whether direct way or conversely. (Rout *et al*., 2000). Because Manufacturing these chemical compounds is difficult, not easy, and economically unpracticable (Oksman-caldentey and Inze, 2004). Moreover, a large number of medicinal plants that contain secondary compounds that are valuable are threatened extinction with increased demand (Edwards, 2004). In addition, consumption of herbal medicines is widely spread and increasing.

Therefore, the reason for the decline in plant genetic resources and the loss of natural habitats for medicinal plants is random collecting in large quantities. High-demand plants face great challenges, and still today the world suffers from this problem (Kumari and Priya, 2020). Conversion of land into industrial area, over-collecting, urbanization, pollution, habitat destruction and degradation, and climate change are the leading causing a decline of total plant genetic resources (PGR) (McCune *et al*., 2013). Because there is no regulations to preserve these valuable plants from extinction, are needed to increasing the mass production of medicinal plant by in vitro propagation techniques. *In vitro* culture techniques are now necessary for the production of disease-free plants, rapid multiplication of rare plant genotypes, plant genome transformation, obtaining transgenic plants, breaking dormancy and production of plant-derived metabolites of important commercial value (Sedaghati *et al*., 2019; Debnarh *et al*., 2006; Altpeter *et al*., 2016). In addition, the cultivation of medicinal plants is the most effective way of addressing the gap between supply and demand (Wang, *et al*., 2020). Plant tissue culture technique have great industrial importance in plant propagation, conservation of plant resources as well as the creation of secondary metabolites. This techniques deals new and sustainable opportunities in solving numerous problems in the field of medicinal plant breeding and conservation biology (Chandana, *et al*., 2018; Chandana, *et al*., 2020). Also by tissue culture techniques, it will be easier to produce high number of plant independently off season, weather, or climatic conditions, and without the effect of disease and pests or soil

problems. Moreover, it reduces land labor (Pierik, 1997). Plant tissue culture has been utilized for the last decade. Many protocols have been developed for many crops, shrubs, cut flowers and forest trees (George *et al*., 2008; Pierik, 1997). *in vitro* cultivation of all plant parts, like cell, tissue, and organ for multiplication by using nutrient solutions in an aseptic and controlled environment (Ahmad *et al*., 2013; Shatnawi *et al*., 2011 ; Staden *et al*., 2008 ;Victor, *et al*., 2006). Some examples of culture types in plant tissue culture are meristematic cells, somatic cells, pollen grains, vegetative cells or undifferentiated cells (callus) (George, 1993). Simple screening of plantlets by tissue culture provides unique opportunity for studying many features of plant development and improvement under optimal conditions. (George *et al*., 2008; Honda *et al*., 2001; Shatnawi *et al*., 2006). Therefore, tissue culture techniques became widely used for many plant species (Pierik, 1997; Shatnawi *et al*., 2004). One of the goals of plant tissue culture technique is the speed of reproduction in a short time. Addition to producing plants identical to the mother plant (true to type). (George *et al*., 2008; Hofman *et al*., 2002; Shatnawi *et al*., 2007; Staden *et al*., 2008). The greatest potentials for tissue culture are the selection of agronomical desirable characteristics, increasing yield, disease resistance, water stress and salt tolerance, as well as conservation of genetic resources (Hofman *et al*., 2002; Pierik, 1997; Shatnawi *et al*., 2006). Plant tissue culture techniques are considered the most effective methods for propagating high number of plant species (George *et al*., 2008). Tissue culture enables mass propagation of uniform plants and overcomes the problems of propagation. Hundreds or even thousands of rooted plants will be successfully acclimatized from a few plant materials in a short time. Different levels of various plant growth regulators (PGRs) were used in the previous studies to induce proliferation and rooting *in vitro* for some medicinal plants (Al- Qudah *et al*., 2011; Evenor and Reuveni, 2004; Mostafa *et al*., 2010; Musallam *et al*., 2011; Owies *et al*., 2009).

1- Micropropagation:

Micropropagation *in vitro* is technology fast grow elite plants using new plant tissue culture approaches. Which it is famous for its requests in the agro, horticultural and forestry industries (Li *et al*., 2010). Micropropagation is carried out from plant tissues grown in fields and nurseries using different tissue culture techniques on sterile nutrient media containing different growth regulators and controlled environment (Pierik, 1997). The organogenesis is usually direct the safer way for multiplication of clonal, true-to-type plants (Sandhu *et al*, 2018). *In vitro* propagation of medicinal plants has been documented such as using shoot tips/ axillary buds via organogenesis (Rout *et al*., 2000), production of adventitious shoot (Thomas and Yoichiro, 2009) and somatic embryogenesis (Omar *et al*., 2004). Micropropagation is considered to be easier and rapid for herbaceous plants than woody species (Schwarz and Beaty ,1996). In conventional cultivation many plant seeds do not germinate, flower and produce seed under certain climatic conditions or have long periods of growth and multiplication. Micropropagation assures a sure source of medicinal plants, using lowest space and time (Prakash and Van Staden, 2007). Micropropagation practices have been evolve for many essential species of medicinal plants such as *Swertia chirayita, Cathranthus roseus, Panax, Stevia rebaudiana, Artemisia annua-aremisin, Elettaria cardamomum, Allium chinense, Camellia sinensis*, and so on. (Pradhan *et al*., 2013).

The success of the *in vitro* propagation technique depends on some important characteristics such as genotype, medium, plant growth regulators and type of explants (Kim *et al*., 2017). But still all possible types of explants and hormonal combinations have not been exploited to develop more various and efficient protocols. *In vitro* propagation is more rapid than *in vivo* for many horticultural crops (Schwarz and Beaty, 1996). In addition *in vitro* culture can give virus and disease-free plants (Pierik, 1997).

Little starting material is needed when *in vitro* techniques is used to give thousands or even millions of genetically identical plants from single or few mother stocks (Furmanowa and Olszowska, 1992; Tamura *et al*., 1993). Currently, a large number of protocols are available for the micropropagation of medicinal plants (Debnarh *et al*. 2006; Rizvi and Kukreja 2010; Sarasan *et al*. 2011; Kaul *et al*. 2013; Kun-Hua *et al*. 2013; Bhattecheryya *et al*. 2014; Chen *et al*. 2014; Atanasov *et al*. 2015; Younes, *et al*., 2015; Al-Qudah *et al*., 2011), as well as some commercially important plants, such as *Agave salmiana* (Puente- Garza *et al*. 2017), artichoke (Pandino *et al*. 2017), *Stevia rebaudiana* (Ramírez-Mosqueda *et al*. 2016) and *Moringa oleifera* (drumstick tree) (Juan-jie *et al*. 2017). The technology of micropropagation is generally goes through various stages such as choice of mother plant, beginning of explants, subculture of explants for multiplying, shooting, rooting, and Acclimatization.

1.1 Plant Materials:

Micropropagation start with the selection of plant tissue (explants) from a young, healthy mother plant. From the point of view of true-to-type propagation, explant choice is very important. Meristems or shoot tips (dormant or actively growing buds) can be used as explants due to their genetic stability. Several explants sources were selected in the establishment phase of the medicinal plants, The survival of explants depends on their rate of microbial contamination and of explant browning, which pertain not only to the explants used for culture initiation but also to the physiological stage of mother plants and to the season when explants are collected

(Werner and Boem, 1980; Yepes and Aldwinckle, 1994). Seeds were used as starting material in establishing *in vitro Origanium vulgar* (Arafeh, 1999), *Amygdalus communis* (Shibli *et al*., 1998), *Salvia fruticosa* (Arikat *et al*., 2004), *Achillea fragrantissima* (Younes *et al*., 2015), *Teucrium polium L* (Al-Qudah *et al*., 2011). But in case of *Coffee arabica* (Ebrahim *et al.,* 2007) zygotic embryos were used. Other plant starting materials were used *in vitro*; like shoot tips, lateral buds, primordial leaves explants inoculated on medium with appropriate supplements of the required growth regulatore uses to give shoot regeneration of some endangered medicinal plant (Guo *et al*., 2007). Furthermore, apical and axillary meristems and single node cuttings were used for *Coffee arabica* (Ebrahim *et al.,* 2007). *In vitro* propagation may also be achieved by somatic embryogenesis and cell suspension culture with some important medicinal plants (Shibli *et al*., 2012).

Tissue culture success mainly depends on the age, types and position of explants (Gamborg *et al*., 1976). Because all plans have not equal Totipotency. Large size explants can increase chances of contamination and small explants (meristems) can show virus free (Fowler *et al*., 1993; Staba and Seabrook 1980). Meristem culture were utilized to obtain virus-free plants by culturing. To increase the probability of success, the mother

plant should be *ex-vitro* cultivated under optimal condition to minimize contamination in the *in-vitro* culture.

1.2 Surface Sterilization:

Microbial contamination is a very common problem in plant tissue culture (Fowler *et al*., 1993). For this reason explants is surface sterilized by different reagents then transferred into nutrient medium usually. Sterilization of explants is one of the major steps for successful *in vitro* micropropagation. Explants cleaned by distilled water and sterilized agents like calcium hypochlorite, sodium hypochlorite, ethanol, mercuric chloride, hydrogen peroxide, or silver nitrate are for sterilization (Mihaljevic *et al*., 2013). The selection of sterilizing agent depends on the type of explants depending on the morphological characteristics like hardness and softness of the tissue (Yadav and Singh, 2011). Contamination it is considered more rapid in woody species than herbaceous plants (Niedz and Bausher, 2002; Das and Pal, 2005). Sterilization of laboratory tools carried out by autoclaving, alcohol washing, baking, radiations, flaming and fumigation. A great decrease in bacterial contamination was seen by using an ultrasonic sonicator (Monge *et al*., 2008). Sources of *in vitro* contamination include culture containers, media, explants, tools, the environment of the culture room and transfer area, and operating personnel. The success of propagation *in vitro* mostly depends on the external steriliz of explants because this is critical, also, there are microorganisms that grow on the surface of the plant more rapidly than cultured plants.

Microbial contamination is one challenge facing plant tissue culture practices. (Leifert *et al*., 1994; Kim *et al*., 2017). Wide range of microbes are found on the surface of plants, and these grow faster than the cultured explants. Explant contamination is connected to various causes such as basis of explants and location (Singh *et al*., 2011). The most common problem of micropropagation suffers is pollution of all kinds, whether bacterial or fungal.

1.3 Media Composition:

Seeds and vegetative explants have been used on different types of media with different PGRs. Several types of medium formulation were identified as suitable for medicin plants in tissue culture. Basic nutrient basal media that are most frequently applicable and used include (Murashige and Skoog, 1962) medium is commonly used, Other media used are Gamborg (B5; Gamborg, *et al*., 1968), (LS; Linsmaier and Skoog, 1965) and (NN; Nitsch and Nitsch, 1969)., (SH; Schenk and Hildebrandt 1972), Woody plant medium (WPM; Lloyd and McCown, 1981) , W medium (White, 1963).

In 1962, Murshige and Skoog were able to formulate a revised medium for culturing *Nicotiana tabacum* callus (Vasil and Vasil, 1972), *Salvia fruticosa* Mill (Arikat *et al*., 2004), *Ceratonia siliqua* (Naghmouchi *et al*., 2008), *Morus alba* (Anis *et al*., 2003) and *Coffea Arabica* (Ebrahim, *et al*., 2007). *Thymus vulgaries* was established on (NN) medium (Tamura, 1993). While modified MS medium (MMS) was used in seed germination and establishment of *Origanium vulgare* (Borovec, 1988). In general, Medium contains a macro and micro nutrients and vitamins dissolved in distilled water with carbon source 3% sucrose, solidified with 0.8% Agar powder is not essential media component but is used as gelling agent (Bhojwani and Razdan 1996; Rayns *et al*., 1993). It prevents death of cultured cells due to submerging and lack of oxygen in liquid

medium. The pH was adjusted to 5.8 before adding the agar, followed by autoclave sterilization at 121°C for 20 min. The pH of the medium was adjusted to 5.7 with 0.1M NaOH or 0.1M HCl after the addition of growth regulators, prior to the addition agar. The medium was autoclaved at 121°C, for 20 min. All the cultures were maintained in sterilized culture room at 26 ± 2°C, under 16/8h light regime provided by cool white fluorescent light (60µmol-2s-1 light intensity) and with 55 - 60% relative humidity (Bhojwani and Razdan 1996).

1-4 *In Vitro* shoot proliferation:

Shoot formation is promoted by light, temperature, culture medium, PGRs, explant type, and physiological development of tissue used (Pierik, 1997). In many cases it is possible to place explants directly on a medium that will encourage shoot multiplication, but cultures of some species are best initiated with different PGRs that are required for shoot formation (George, *et al*., 2008). Multiplication rates depend on species, cultivar, auxin /cytokinin ratio and explant type (Iqbal *et al.*, 1999).

For successful plant tissue culture first shoots are formed by culture of explants or callus on media containing PGRs (mainly cytokinin but sometimes auxins also). Different types and concentrations of cytokinin such as 6-Benzylamino-purine (BA), 6-Furfurylaminopurine (kinetin), zeatin, Thidiazuron (TDZ) and6-(gamma,gamma-Dimethylallylamino) purine (2ip), are used for shoot formation.

Ali *et al*., (2010) reported that maximum shoot multiplication *in vitro* was obtained on MS medium supplimented with 1.0 mg /L BA in *Stevia rebaudiana* . Also, Bipasha *et al*., (2010) achieved *in vitro* clonal propagation of *Wedelia chinensis* on MS medium containing (2.0 mg/L BA +1.0 mg/L IAA) for shoot elongation while they used MS medium supplemented with (1.5 mg/L BA+ 0.5 mg/L Gibberellic Acid (GA_3) for shoot proliferation. Maximum proliferation rate in *Achillea filipendulina* was obtained on MS medium supplemented with 1.0 mg/L IAA plus 2.0 mg/L BA (Evenor and Reuveni, 2004). The highest proliferation rate in *Arbutus andrachne* L. was obtained on MS medium complemented with 2.0 mg/L zeatin (Mostafa *et al*., 2010). The best result proliferation of *Achillea fragrantissima* was achieved while used MS medium complemented with 1.2 mg/L kinetin (Younes *et al*., 2015).

In *Senecio macrophyllus* shoot proliferation rate was highest when explants were cultured on MS medium supplemented with 4.4 µM BA in combination with 0.54 µM 1-Naphthaleneacetic acid (NAA) (Trejgell *et al*., 2010). Kinetin was found to be more efficient cytokinins used to induce proliferation in different plant species (Bouhouche and Ksiksi, 2003) while BA and kinetin was found to be more efficient cytokinins used to induce proliferation rate in *Origanium syriacum* (Arafeh *et al*., 2003).

There are many reported on the benefits and problems associated with *in vitro* multiplication of medicinal plants (Bajaj, 1993). Most known species studied were Mentha spp. (Cellarova, 1992) , *Origanium syriacum* (Arafeh *et al*., 2003), *Origanium vulgar* (Borovec, 1988), *Salvia fruticosa* (Arikat *et al*., 2004), *Coffea arabica* (Ebrahim *et al*., 2007), *Teucrium polium* (Al-Qudah, *et al*., 2011), wild *cyclamen* (Al-Majathoub and Karam, 2006), *Gundelia tournefortii* (Owies *et al*., 2009), *Achillea fragrantissima* (Younes *et al*., 2015) and *Artemisia herba alba* (Shatnawi, 2011). *Artemisia herba-alba* (Shibli, *et al*., 2018). Different levels of various cytokinins are being used in previous

studies to induce proliferation, a cytokinin is required and applied either alone or combined with small amounts of auxin (Kahia and Owuor, 1990). Each treatment consisted of five replicates (flasks), with four explant / replicate. Cultures were incubated at growth room and kept under a regime of 16- hr. light, 8- hr. dark and 24 ± 1°C. Data was collected after 4 weeks from culture such as: number of shoots/ explant, shoot height, callus Formation and rooting percentage.

1.5 *In vitro* rooting:

Auxins are main agent in *in vitro* rooting stage as they driving the signal transduction alleyway in plant cells into transferred to roots (Taiz and Zeiger, 2002).

Root formation after producing healthy shoots, plantlets transferred to different media for root formation. Auxins such as Indole-3-butyric acid (IBA), naphthaleneacetic acid (NAA) or Indole-3-acetic acid (IAA) are mainly used in root induction and their effect varies with type and concentration used in different plant species (Pierik, 1997 ; Swamy *et al*., 2002) . NAA and IBA are most commonly used anxins (Bhojwani, and Razdan,. 1996). Different levels of various rooting growth regulators were used in the previous studies to induce rooting. There are many reported associated with *in vitro* rooting of medicinal plants. Most known species studied are *Arbutus andrachne* (Mostafa *et al*., 2010) reported that, The maximum rooting was obtained with 1.0 mg /L NAA. While, *Capparis spinosa* (Musallam, *et al*., 2011) the highest number of roots was obtained at 5.0 mg/L IAA on 1/2 MS media.

Ali *et al*., (2010) found best *in vitro* rooting was reported on MS medium containing 1.0 mg /L NAA of *Stevia rebaudiana* , in *Senecio macrophyllus* rooting was achieved on full- and half-strength MS medium without auxin (Trejgell *et al*., 2010). In *Origanium syriacum* the maximum number of roots was obtained on MS medium supplement with 0.8 mg/L IAA (Arafeh *et al*., 2003). In *Salvia fruticosa* Mill root formation was completely inhibited on MS medium free of auxins or containing high concentrations of IAA, NAA, or IBA, highest roots number were obtained at 0.5 mg/L IBA or 0.5 mg/L IAA (Arikat *et al*., 2004). With 20 replicates (test tube) per treatment and one explant / replicate. Cultures were incubated at growth chamber and saved under a system of 16- hr. light, 8- hr. dark and 24 ± 1°C. Data was collected after 4 weeks from culture such as: rooting percentage, number of roots/ explant, root height and callus formation.

1.6 *Ex Vitro* Acclimatization:

Acclimatization is the final, but necessary, step in all micropropagation schemes (Van-Huylenbroeck and Debergh, 1996). The process of acclimatization is important for all types grown *in vitro* to ensure survival sufficient number of plants survives and grows strongly when moved to the soil (Preece and Sutter ,1991). *Ex vitro* acclimatization was performed for plantlets with well-developed roots. Cotton plugs were removed off vessels and agar was removed by washing the roots gently under running tap water, plantlets were transferred to sterilized plastic cups (5×5 cm) containing sterilized growing medium mixtures of peatmoss and perlite at (1:1, v/v) and adapted to growth chamber conditions by gradual exposure to reduced relative humidity by covered by perforated plastic bags, Each cup were irrigated with distilled water every 2 days for 4 weeks. keep the potted plantlets in growth chamber for 4 weeks and later transferred to normal GH (33 ±1°C) conditions 4 weeks and the survival percentage was recorded (George *et al*., 2008).

Successful acclimatization of regenerated plants in greenhouse on potting mixture was reported. Various cultivars reported to growth well in pots containing soil-less medium of vermiculite, peat, perlite or compost and other on salt: sand soil.

Rooted shoots were successfully re-established in soil under controlled conditions in most studies: For example, *in vitro* rooted *Senecio macrophyllus* plantlets were obtained after four weeks of cultivation on the rooting medium. The author reported 92% survival (Trejgell *et al*., 2010).

(Goel *et al*., 2009; Lal and Singh, 2010) were record about the success of acclimatization and transfer to open field of both *Peganum harmala* and *Celastrus paniculatus* respectively. Moreover, completely rooted resulting from the multiplication of *Albizia lebbeck* were successfully adapted in vessels containing a mixture of sterile soil and sand (1:1) with a survival rate of 60% under open field conditions (Yadav and Singh, 2011).

Rooted plantlets of *Teucrium stocksianum* were successfully acclimatized, with a survival rate of 75–80% (Bouhouche and Ksiksi, 2007). While for *Arbutus andrachne* acclimatization was successful, with a survival rate of 80% (Mostafa *et al.,* 2010). Al Qudah *et al*., (2011) reported that, rooted plants of *Teucrium polium* were successfully acclimatized, with 75% survival and grown in the greenhouse. In addition, Arikat, *et al*., (2004) obtained 80% survival of acclimatized *Salvia officinalis* plantlets. While *Capparis spinosa* acclimatization was successful, with a survival rate of 63% (Musallam *et al*., 2011). The best result achieved of *Achillea filipendulina* were survival rate about 90% after roots successful acclimatized (Evenor and Reuveni, 2004).

Conclusion:

Medicinal plants are potential renewable natural resources and are play a beneficial role in human health care. The medicinal value of these plants contains phytochemicals such as glycosides, alkaloids, volatile oils, flavonoids, tannins, terpenoids, saponins, fatty acid and phenolic compounds as a secondary metabolites. Legislations has failed to stop the indiscriminate harvesting of the plants as the trade is the major source of livelihood for an estimated one million people, mostly the rural poor. In recent years, the requirement for medicinal plants has increased dramatically, which leads to a shortage and depletion of natural resources in the wild. To compensate for this loss, we must use plant tissue culture for the propagation in *vitro*. A huge number of medicinal plants of high economic value and their metabolites have been produced by *in vitro* techniques in a short duration of time compared to conventional approaches.

In conclusion, this study could be the beginning of the road for researchers who have a desire to learn more about the *in vitro* propagation of medicinal plants. *In vitro* cultures allow conservation of rare and endangered plant.

References:

Ahmad, I., Tanveer, H., Irfan, A., Muhammad, N., Muhammad, R and Muhammad, I. (2013). Lethal effects of secondary metabolites on Plant Tissue Culture, American-Eurasian J Agric. & Environ. Sci. 13(4):539-547.

Ali, A., Gull, I., Naz, S. and Afghan, S. (2010). Biochemical investigation during different stages of *in vitro* propagation of (*Stevia rebaudiana* L.), Pakistan Journal of Botany, 42, 2827-2837.

Al-Majathoub, M. and Karam, N.S. (2006). *In vitro* propagation of wild *Cyclamen persicum* Mill. from seedling tissue. Acta Horticulturae, 350, 243-251.

Al-Qudah, T., Shibli, R. A. and Alali, F. (2011), *In vitro* propagation, and secondary metabolites production in wild germander (*Teucrium polium L.*), *In Vitro* Cellular and Developmental Biology - Plant , 47, 496-505.

Altpeter, F. Springer, N. M. Bartley, L. E, Blechl, A. E. Brutnell, T.P. Citovsky, V. Conrad, L. Gelvin, S.B. Jackson, D. Kausch, A.P. Lemaux, P.G, Medford, J.I. and Orozco-Cardenas, M. (2016). Advancing crop transformation in the era of genome editing. Plant Cell 28:1510–1520.

Anand, U., Jacobo-Herrera, N., Altemimi, A. and Lakhssassi, N. (2019). A comprehensive review on medicinal plants as antimicrobial therapeutics: potential avenues of biocompatible drug discovery. Metabolites 9, 258.

Anis, M., Faisal, M. and Singh, S. K. (2003), Micropropagation of mulberry (*Morusalba* L.) through *in vitro* culture of shoot tip and nodal explants. Plant Tissue Culture, 13, 47-51.

Arafeh, R. (1999). Factors affecting *In vitro* propagation, callusing cell suspension culture and secondary metabolites production in sweet marjoram (*Origanum vulgar* L.) and Syrian marjoram (*Majorana syrica* L.) Rafin (Origanum syricum L.), M.Sc. Thessis. Jordan University of Science and Technology, Irbid, Jordan.

Arafeh, R., Mahmoud, M. S. and Shibli, R.A. (2003). *In Vitro* Seed Propagation of Wild Syrian Marjoram (*Origanum syriacum* L.), Advances in Horticultural Science 17(4):241-244.

Arikat, N. A., Jawad, F. M., Karam, N. S. and Shibli, R. A. (2004), Micropropagation and accumulation of essential oils in wild Sage (*Salvia fruticosa Mill.*). Scientia Horticulturae, 100, 193-202.

Atanasov, A.G., Waltenberger, B., Pferschy-Wenzig, A.M., Linder, T., Wawrosch, C., Uhrin, P., Temml, V., Wang, L., Schwaiger, S., Heiss, E.H., Rollinger, J.M., Schuster D., Breuss, J.M., Bochkov, V., Mihovilovic, M.D., Kopp, B., Bauer, R., Dirsch, V.M. and Stuppner, H. (2015). Discovery and resupply of pharmacologically active plant-derived natural products: a review. Biotechnol Adv 33:1582–1614.

Bajaj, Y. P. S. (1993). Biotechnology in agriculture and forestry, (ed). Medicinal and Aromatic Plants –IV. Springer-Verlag Berlin Hiedelberg, edition, 21, 413-426.

Bannerman, R.H. (1982). Traditional medicine in modern health care. World Health Forum 3(1):8–13.

Bhattecheryya, P., Kumaria, S., Diengdoh, R..and Tandon, P. (2014). Genetic stability and phytochemical analysis of the *in vitro* regenerated plants of *Dendrobium nobile* Lindl., an endangered medicinal orchid. Meta Gene 2:489–504.

Bhojwani, S.S. and Razdan, M.K. (1996). Plant tissue culture: Theory and Practice: Developments in crop science Vol. 5. Elsevier, Amsterdam.

Bipasha, A., Safiul, A., Farhana, R. and Mohammed, R. (2010). Simultaaneous shoot regeneration and rhizogenesis of (*Wededlia chinensis*) for *in vitro* clonal propagation. American Journal of Sustainable Agriculture, 4, 65-69.

Borovec, V. (1988), Micropropagation of clones of marjoram (*Origanum vulgare L.*) under *in vitro* conditions. Bull VyzSlech Us ZelOlom, 32, 49–54.

Bouhouche, N. and Ksiksi, T.(2007). An efficient *in vitro* plant regeneration system for the medicinal plant *Teucrium stocksianum* Boiss, Plant Biotechnology Reports 1(4):179-184

Cellarova, E. (1992). Micropropagation of *Mentha L.* In: Biotechnology in Agriculture and Forestry. By Bajaj, Y.P.S. (ed.). High Tch and Microproagation III, Springer-Verlag Berlin Hiedelberg, 19, 262-276.

Chandana, B.C., Nagaveni, H.C., Kumari, Lakshmana, D., Shashikala, S.K. and Heena, M.S. (2018). Role of plant tissue culture in micropropagation, secondary metabolites production and conservation of some endangered medicinal crops. Journal of Pharmacognosy and Phytochemistry, 7, 246-251.

Chandran, H., Meena, M., Barupal, T and Sharma, K. (2020). Plant tissue culture as a perpetual source for production of industrially important bioactive compounds. Biotechnology Reports, 26, e00450, 1-10.

Das, M and Pal, A.(2005). Clonal Propagation and Production of Genetically Uniform Regenerants from Axillary Meristems of Adult Bamboo. Plant Biochemistry & Biotechnology. 14: 185-188.

Debnarh, M. Malik, C.P and Baisen, P.S. (2006). Micropropagation :a tool for the production of high quality plant based medicines. Curr Pharm Biotechnol 7:33–49.

Ebrahim, N., Shibli, R., Makhadmeh, I. and Shatnawi, M. (2007). *In vitro* propagation and *in vivo* acclimatization of three coffee cultivars (*Coffea Arabica* L.) from Yemen. World Applied Sciences Journal, 2, 142-150.

Edwards, R. 2004. No remedy in sight for herbal ransack. New Scientist, 181, 10-11.

Evenor, D. and Reuveni, M. (2004), Micropropagation of (*Achillea filipendulina*) cv. 'Parker. Plant Cell Tissue and Organ Culture, 79, 91-93.

Fowler, M.R., Rayns, F.W. and Hunter, C.F. (1993). The language and aims of plant cell and tissue Cultur. *In Vitro* Cultivation of Plant Cells, Butterworth-Heinemann Ltd, Oxford, Page 1-18.

Furmanowa, M. and Olszowska, O. (1992). Micropropagation of Thyme (*Thymus vulgaris* L.). In: Biotechnology in agriculture and forestry. Springer-Verlag Heildelberg Berlin, 19, 230-234.

Gamborg, O., Miller, R. A. and Ojima, K. (1968). Nutrient requirements of suspension cultures of soybean root cells. — Exp Cell Res. 50: 151-158.

George, E. F., Hall, M. A. and De- Klerk, G. J. (2008). Plant propagation by tissue culture. Plant Cell Tissue and Organ Culture, 93,353-355.

George, E. F., Hall, M. A., & Klerk, G. J. D. (2008). Plant growth regulators I: Introduction; auxins, their analogues and inhibitors. In *Plant propagation by tissue culture* (pp. 175-204). Springer, Dordrecht.

George, E.F. (1993). Plant Propagation by Tissue Culture: The Technology. Exegetics Ltd., Edington, UK.

Goel, N., Singh, N and Saini, R.(2009). Efficient *in vitro* multiplication of Syrian Rue (Peganum harmala L.) using 6-benzylaminopurine pre-conditioned seedling explants. Nat Sci. 7:129–134.

Guo, B., Gao, M. and Liu, C. Z. (2007). *In vitro* propagation of an endangered medicinal plant (*Saussurea involucrata*). Plant Cell Reports, 26, 110-1121.

Hofman, P., Haisel, D., Komenda, J. ,Vonger, M., Tichm, J., Chofer, C.and Papkov, V. (2002), Impact of *in vitro* cultivation conditions on stress responses and on changes in thylakoid membrane proteins and pigments of tobacco during *ex vitro* acclimation. Biology Plant, 45, 189-195.

Honda, H., Liu, C. and Kobayashi, T. (2001). Large-scale plant micropropagation. Advances Biochemical Engineering Biotechnology, 72, 157-182.

Iqbal, R., Ahmad, Z., Shoaib, K. and Akram, M. (1999). Bud proliferation and regeneration in Dumb-cane (Dieffenbachia seguine) leaf callus. Pakistan Journal of Plant Sciences. 5 (1): 29 – 35.

Juan-jie, Z., Yue-sheng, Y., Meng-fei, L., Shu-qi, L., Yi, T., Han-bin, C and Xiao-yang, C. (2017). An efficient micropropagation protocol for direct organogenesis from leaf explants of an economically valuable plant, drumstick (*Moringa oleifera* Lam.). Ind Crops Prod 103:59–63.

Kahia, J. W. and Owuor, J. B. (1990). *In vitro* propagation of disease resistant (*Coffea arabica* L.). Kenya-Coffee, 55, 901-905.

Kaul, S., Das, S and Srivastava, P.S. (2013). Micropropagation of *Ajuga bracteosa*, a medicinal herb. Physiol Mol. Biol. Plants 19:289–296.

Kim, D.H., Gopal, J and Sivanesan, I. (2017). Nanomaterials in plant tissue culture: The disclosed and undisclosed. RSC Adv. 7, 36492–36505.

Komakech, R., Kim, Y.G ., Kim, W. J., Omujal, F., Yang, S., Moon, B.C., Okello, D., Rahmat, E., Kyeyune, G.N., Matsabisa, M.G. and Kang, Y. A. (2020). Micropropagation Protocol for the Endangered Medicinal Tree *Prunus africana* (Hook f.) Kalkman: Genetic Fidelity and Physiological Parameter Assessment. Frontiers in Plant Science. volume, 11.

Kumari, R. and Priya, A. (2020). Optimization of Concentrations of Plant Growth Regulators for *In Vitro* Multiple Shoot Formation and Efficient Root Induction in *Phyla nodiflora* L. (*Lippia nodiflora* L.) An Important Medicinal Plant. Indian J. Sci. Res. 10 (2): 39-44.

Kun-Hua, W., Lin-Xuan, L., Young-Caim W., Mei-Ying, W., Cui, L. and Jian- Hua, M. (2013). Tissue culture of *Sophora tonkinenss* Gapnep. And its quality evaluation. Pharmacogn Mag 9:323–330.

Lal, D and Singh, N. (2010). Mass multiplication of *Celastrus paniculatus* Willd: An important medicinal plant under *in vitro* conditions via nodal segments . International Journal of Biodiversity and Conservation Vol. 2(6), pp. 140-145.

Leifert, C., Morris, C.E. and Waites, W.M. (1994) .Ecology of microbial saprophytes and pathogens in tissue culture and field-grown plants: Reasons for contamination problems *in vitro*. Crit. Rev. Plant Sci., 13, 139–183.

Li, S., Yuan, W., Yang, P., Antoun, M., Balick, M. and Cragg, G. (2010). Pharmaceutical crops: An overview. *Pharm. Crop.* 1:1–17.

Linsmaier T. and Skoog F. (1965). Physiologia Plantarum, 18 (1) 100 - 127.

Lloyd, G. and McCown, B. (1981). Commerciallyfeasible micropropagation of mountain laurel, Kalmia latifolia, by shoot tip culture. Intl. Plant Prop. Soc. Proc., 30: 421-427.

McCune, J.L., Harrower, W.L., Avery-Gomm, S., Brogan, J.M., Czergo, A.-M and Davidson, L.N.K. (2013). Threats to Canadian species atrisk: an analysis of finalized Recovery Strategies.Biological Conserva-tion,166, 254–265.

Mihaljevic, I., Dugalic, K., Tomas, V., Viljevac, M., Pranjic, A., Cmelik, Z., Puskar, B and Jurkovic, Z. (2013). *In vitro* sterilization procedures for micropropagation of 'Oblačinska' sour cherry. *Journal of Agricultural Sciences*. 58**:** 117-126.

Monge, G.G., Gatica, A.M. and Melara, M.V. (2008). Somatic embryogenesis, plant regeneration and acemannan detection in aloe (Aloe barbadensis Mill.). Agronomía costarricense: Revista de ciencias agrícolas, 32(2), 41-52.

Mostafa, S. E., Karam, N.S., Shibli, R. A. and Alali, F. Q. (2010), Micropropagation and production of arbutin in oriental strawberry tree (*Arbutus andrachne*.). Plant Cell Tissue Organ Culture, 103, 111–121.

Murashige, T. and Skoog, F. (1962), A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, 15, 473-479.

Musallam, I., Duwayri, D. and Shibli, R. A. (2011). Micropropagation of caper (*Capparis spinosa* L.) from wild plants. Functional Plant Science and Biotechnology, 5, 17-21.

Naghmouchi, S., Khouja, M. L., Rejeb, M. N. and Boussaid, M. (2008). Effect of growth regulators and explant origin on *in vitro* propagation of (*Ceratonia siliqua* L.) via cuttings. Biotechnology, Agronomy, Society and Environment , 12, 251-258.

Niedz, R.P and Bausher. M.G.(2002). Control of *in vitro* contamination of explants from greenhouseand field-grown trees. *In Vitro* Cell Dev Biol Plant. 38: 468–471.

Nitsch, J.P. and Nitsch, C. (1969). Haploid plants from pollen grains. Science, 163: 85-87.

Oksman-Caldentey, K. M. and Inzé, D. (2004). Plant cell factories in the postgenomic era: new ways to produce designer secondary metabolites. Trends in Plant Science, 9, 433-440.

Omar, R., Abdullah, M.P., Hasan, M, A. and Marziah, M. (2004). Development of Growth Medium for *Centella Asiatica* Cell Culture Via Responce Surface Morphology. *American Journal of Applied Science.* 1(3): 215–219.

Owies, D., Shibli, R. A., Ereifej, K. I and Shatnawi, M. A. (2009), *In vivo* propagation of akub (*Gundelia tournefortii* L.) by seeds. Jordan Journal of Agricultural Sciences, 5, 266-272.

Pandino G, Lombardo S, Antonino LM, Ruta C and Mauromicalem G. (2017). *In vitro* micropropagation and mycorrhizal treatment influences the polyphenols content profile of globe artichoke under field conditions. Food Res Int 99:385–392.

Paunescu, A. (2009). Biotechnology for Endangered Plant Conservation: A Critical Overview. *Romanian Biotechnological Letters*. 14, (1): 4095-4103.

Pierik, R. (1997). *In vitro*- culture of higher plants. Kluwer Academic Publishers. Netherlands, 344 .

Pradhan, S., Paudel, Y.P. and Pant, B. (2013). Efficient regeneration of plants from shoot tip explants of Dendrobium densiflorum Lindl., a medicinal orchid. Afr. J. Biotechnol 12(12):1378–1383.

Prakash, S. and Van Staden, J. (2007). Micropropagation of Hoslundia opposita Vahl--a valuable medicinal plant. South African Journal of Botany, **73**, 60-63.

Preece, J.E. and Sutter, E.G. (1991). Acclimatization of micropropagated plants to the greenhouse and fields. In: P.C. Deebergh and R.H. Zimmerman (eds.). Micropropagation. Kluwer Academic Puplishers, The Netherlands, 71-93.

Puente-Garza, C.A., García-Lara, S and Gutiérrez-Uribe, J.A. (2017). Enhancement of saponins and flavonols by micropropagation of *Agave salmiana*. Ind Crops Prod 105:225–230.

Ramírez-Mosqueda, M.A., Iglesias-Andreu, L.G., Ramírez-Madero, G and Hernández-**Rincón**, E.U. (2016). Micropropagation of *Stevia rebaudiana* Bert. In temporary immersion systems and evaluation of genetic fidelity. S Afr J Bot 106:238–243.

Rayns, F.W., Fowler, M.R and Hunter, C.F. (1993). Media design and use. *In Vitro* Cultivation of Plant Cells, Butterworth-Heinemann Ltd. Oxford, pp: 43-64.

Rizvi, M.Z and Kukreja, A.K. (2010). *In vitro* propagation of an endangered medicinal herb *Chlorophytum borivilianum* Sant. et Fernand. through somatic embryogenesis. Physiol Mol Biol Plants 16:249–257.

Rout, G. R., Samantaray, S. and Das, P. (2000). *In Vitro* Manipulation and Propagation of Medicinal Plants. *Biotechnology Advances*. 18: 91–120.

Sandhu, M, Wani, S.H, Jiménez, V.M. (2018). *In vitro* propagation of bamboo species through axillary shoot proliferation: a review. Plant Cell Tiss Organ Cult. 132:27–53.

Sarasan, V., Kite, G.C., Sileshi, G.W and Stevensonm P.C. (2011). Applications of phytochemical and *in vitro* techniques for reducing overharvesting of medicinal and pesticidal plants and generating income for the rural poor. Plant Cell Rep 30:1163–1172.

Schenk, R.V. and Hildebrandt, A.C. (1972) Medium and Techniques for Induction and Growth of Monocotyledonous and Dicotyledonous Plant Cell Cultures. Canadian Journal of Botany, 50, 199-204.

Schippmann, U., Danna, L and Cunningham, A.B. (2006). A comparison of cultivation and wild collection of medicinal and aromatic plants under sustainability aspects. In: Bogers R.J, Craker L.E, Lange D. (eds). Medicinal and aromatic plants. Springer, New York.

Schwarz, O. J. and Beaty, R. M. (1996). Propagation from nonmeristematic tissues, organogenesis. In: Trigiano R.N, Gray D.J, eds. Plant Tissue Culture Concepts and Laboratory Exercises. Boca Raton, New york, London,Tokyo: CRC Press, 95-103.

Sedaghati, B., Haddad R and Bandehpour M. (2019). Efficient plant regeneration and Agrobacterium-mediated transformation via somatic embryogenesis in purslane (*Portulaca oleracea* L.): An important medicinal plant. Plant Cell Tissue Organ Cult. 136:231–245.

Shatnawi, M. A., Anfoka, G., Shibli, R. A., Al-Mazra'awi, M. S., Shahrour, W. and Arebiat, A. (2011), Clonal Propagation and cryogenic storage of virus free grapevines

(*Vitis vinefera* L.) via meristem culture. Turkish Journal of Agriculture and Forestry, 35, 173-184.

Shatnawi, M. A., Johnson, K. A. and Torpy, F. R. (2004). *In vitro* propagation and cryostorage of syzygium francissi (myrtaceae) by the encapsulation– dehydration method. *In Vitro* Cellular and Developmental Biology - Plant ,40, 403–407.

Shatnawi, M. A., Shibli, R. A., Migdadi, H. ,Obeidat, A., Ereifej, K. and Abu-Ein, A. M. (2006). Influence of different carbon sources on wild pear (*Pyrus syriaca*) growth and sugar uptake. World Journal of Agricultural Science, 2, 156-161.

Shatnawi, M. A., Shibli, R. A., Qrunfleh, I., Bataeineh, K. and Obeidat, M. (2007). *In vitro* propagation and cryopreservation of (*Prunus avium*) using vitrification and encapsulation dehydration methods. Journal of Food Agriculture and Environment, 5, 204-208.

Shibli ,R.A., Sharaf ,S.A. , Kasrawi, M.A and. Al-Qudah,T.S. (2018). *In Vitro* Multiplication of the White Wormwood, *Artemisia herba-alba.* Jordan Journal of Biological Sciences. Pages 265 – 271.

Shibli, R. A., Duwayri, M. A., Sawwan, J. S., Shatnawi, M. A. and Al-Qudah, T. S. (2012). Regeneration via somatic embryogenesis of the endangered wild arum (*Arum palaestinum*). *In Vitro* Cellular and Developmental Biology – Plant, 48:335–340

Shibli, R. A., Jaradat, A., Ajlouni, M., Aljanabi, S. and Khawaldeh, W. (1998). *In vitro* Multiplication of bitter almond (*Amygdalus communis* L.). Damascus University, Journal for Agricultural Sciences, 14, 111-119.

Singh, V., Tyagi, A., Chauhan, P.K., Kumari, P and Kaushal, S.(2011). Identification And Prevention Of Bacterial Contimination On Explant Used In Plant Tissue Culture Labs. International Journal of Pharmacy and Pharmaceutical Sciences, 3 (4):160-161.

Staba, E.J and Seabrook, J.E.A. (1980). Laboratory Culture. Plant Tissue Culture As a Source of Biochemicals, CRC Press, Boca Raton, Pages 1-20.

Staden, J., Zazimalova, E. and George, E. F. (2008). Plant growth regulators II: Cytokinins, their analogues and antagonists. In: George, E. F., Michael, A., Klerk, G. Plant propagation by Tissue Culture. 3rd edt. Springer, Dordrecht, The Netherlands, 206-226.

Suneetha, M. S. and Chandrakanth, M. G. (2006). Establishing a Multi-Stakeholder Value Indexin Medicinal Plants—An Economic Study on Selected Plants in Kerala and Tamil Nadu States of India. *Ecological Economics*. 60(1): 36–48.

Swamy, S.L., Puri, S. and Singh, A.K. (2002). Effects of auxins (IBA and NAA) and seasons on rooting of juvenile and mature hardwood cuttings of Robinia pseudoacaia and Grewia optiva. *New Forest*, 23, 143-157.

Taiz, L. and Zeiger, E. (2002) Plant Physiology (Third Edition). Sinauer Associates, Inc., Publishers, Sunderland, 67-86.

Tamura, H., Takebayashi, T and Sugisawa, H. (1993). *Thymus vulgaris L.* (Thyme): *In vitro* culture and production of secondary metabolites. In: Biotechnology in agriculture and forestry. By Bajaj, Y.P.S (ed). Medicinal and Aromatic Plants IV, Springer-Verlag Heildelberg berlin, 21,413-426.

Thomas, T. D. and Yoichiro, H. (2009). *In Vitro* Propagation for the Conservation of A Rare Medicinal Plant *Justicia Gendarussa* Burm. F. By Nodal Explants And Shoot

Regeneration From Callus. *Acta Physiologiae Plantarum*, **32** (5):943-950.
Thomford, N.E., Senthebane, D.A., Rowe, A., Munro, D., Seele, P., Maroyi, A and Dzobo, K. (2018). Natural products for drug discovery in the 21st century: Innovations for novel drug discovery. Int. J. Mol. Sci. 19(6), 1578.
Trejgell, T., Mahaska, M and Tretyn, A. (2010). Micropropagation of *Senecio Macrophyllus* M. Bieb, Acta biologica Cracoviensia. Series botanica, 52(1):67-72.
Tripathi, L. and Tripathi, J. N. (2003). Role of biotechnology in medicinal plants. Tropical Journal of Pharmaceutical Research, 2 ,243-353.
Van-Huylenbroeck, J. M., and Debergh, P. C. (1996). Physiological aspects in acclimatization of micropropagated plantlets. Plant Tissue Culture and Biotechnology, 2, 136-141.
Vasil, I. K., and Vasil, V. (1972). Totipotency and embryogenesis in plant cell and tissue cultures. *In Vitro* Cellular and Developmental Biology-Plant, 8, 117-125.
Victor, M., Loyola-Vargas, A. and vazquez-Flota, F. (2006). Plant Cell Culture Protocols, Second Edition.
Vines, G. (2004). Herbal Harvest with future: towards sustainable source for medicinal plants. Plantlife International, www.plantlife.co.uk .
Wang, W., Xu, J., Fang, H., Li, Z., and Li, M. (2020). Advances and challenges in medicinal plant breeding. *Plant Sci.* 298:110573.
Werner, E.M and Boem A.A. (1980). *In vitro* propagation of Malling 7 apple rootstock. HortScience; 15:509–10.
White, P.R. (1963). The Cultivation of Animal and Plant Cells. 2nd Edn., Ronald Press Co., New York., pp: 228.
Yadav, K., and Singh, N. (2011). Effect of seed harvesting season and sterilization treatments on germination and *in vitro* propagation of *Albizia lebbeck* (L.) Benth. *Tom*, *18*(2):151-156.
Yepes, L.M., and Aldwinckle, H.S. (1994). Micropropagation of thirteen Malus cultivars and rootstocks, and effect of antibiotics on proliferation. Plant Growth Regul;15:55–67.
Younes, L.S., Shibli, R.A and Al-Qudah, T.S. (2015). *In vitro* Propagation and Acclimatization of *Achillea fragrantissima* Frossk Sch. Bip, Jordan Journal of Agricultural Sciences, Volume 11, No.2-339.
Yuan, H., Ma, Q., Ye, L., and Piao, G. (2016). The traditional medicine and modern medicine from natural products. Molecules 21(5), p. 559.