

Summery:-
employment,
provides a chief
source of

[Science](#), [Agriculture](#)



Summery:-Organogenesis and physical embryogenesis are unit the 2 pathways of selection for fast and all-encompassing propagation of *Phoenix dactylifera*.

These pathways completely used for the micropropagation of best genotypes and have verified their importance for the industrial production of plenty of cultivars. However regeneration through physical embryogenesis and organogenesis in *Phoenix dactylifera* continues to be troublesome to attain for recalcitrant genotypes and is commonly during a weak position by sure physiological disorders. During this review, we tend to report the results of a total of studies applied on *Phoenix dactylifera* micropropagation. It states that regarding various factors that influence every stage of physical embryogenesis and organogenesis. Keywords:-

Phoenix

dactylifera L.; Organogenesis; Somatic embryogenesis Introduction:-

Date palm (*Phoenix dactylifera* L.) is one of the most essential fruit crops cultured in arid and semi-arid regions.

It is circulated throughout the Middle East, North Africa, South Sahel, areas of East and South Africa, Europe and USA (Mazri et al ., 2015), with approximately 150 million trees worldwide (Mazri et al ., 2015). Date palm is refined for its high yield and the high nutrient value of its fruit, for preserving ecosystems threatened by desertification and creating suitable microclimate for agriculture under arid surroundings. In addition, date palm cultivation generates considerable chances for rural employment, provides a chief source of income for farmers and confirms livelihood and food security of the rural areas (Mazri et al .

, 2015). Date palm can be proliferated sexually by seeds or asexually by offshoots. Propagation by seeds cannot be used for the commercial production of best genotypes due to its heterozygous character (Tisserat, B.

1982), and because of the considerable difference between seedlings and vegetatively propagated plants in expressions of production potential, fruit maturation and value, and harvesting time. Propagation by offshoots is a slow procedure that is hampered by the limited number of offshoots produced by a single date palm tree, the low survival rate and the risk of transferring diseases. Propagation of date palm concluded in vitro techniques presents an competent alternative for the conventional methods. Indeed, date palm micropropagation allows fast and large-scale proliferation of uniform and healthy plants, with neither seasonal effects nor the risk of distribution diseases and pests during plant material exchange (Quiroz-Figueroa et al., 2006). Aim of this review is to summarize the literature on date palm micropropagation through somatic embryogenesis and organogenesis, and highlight the main factors affecting each stage of these two micropropagation techniques. Beside this, the main problems come across during date palm micropropagation are described. Date Palm

Propagation Methods:-

Available techniques of

rapid multiplication of date palm have contributed hugely increased demand of date palm fruits worldwide (Jain et al.

, 2011). Traditionally, date palm is proliferated by both sexually through seeds and vegetatively by offshoots that produced from axillary buds located

on the base of the trunk during the juvenile phase in date palm tree. It is very slow for offshoots to progress and that hampers vegetative propagation of date palm plant.

So far, there is no obtainable technique to speed up in increasing the offshoot quantities as well as reduce the time in developing them. Use of offshoots preserve true-to-type character of reproduced genotypes.

Moreover, sexual propagation of date palm is unsuitable for commercial production of true-to-type value-added genotypes. It is due to heterozygous character of date palm seedlings and also their dioecious nature (Jain, 2007a). In addition, half of this progeny is collected from male trees which not distinguished before flowering stage. The female plants produce variable fruits and commonly of inferior quality (Eke et al., 2005).

Additionally, seed propagation method has another drawback that the growth and maturation of seedlings is extremely low, and this is a reason, date palm seedling may begin to fruit after 8-10 years of plantation. Though offshoot propagation is a true-to-type technique, it is not commercially practical for the following causes: Offshoot production is restricted to a relatively short vegetative phase of about 10 to 15 years; Only a limited number of offshoots are formed during this phase (20 to 30 offshoots, depending on variety); Some varieties harvest more offshoots than others (some do not produce offshoots at all); Offshoot survival ratio is low; The use of offshoots improves the spread of date palm diseases and pests; Offshoot propagation is difficult, lengthy, and therefore expensive. In vitro propagation of date palm:-

Use of in vitro techniques

such as somatic embryogenesis and organogenesis is highly proper for large-scale plant multiplication of vegetatively proliferated crops. Success of these techniques highly genotypic dependent, though, have successfully been practical for plant propagation in wide ranging crops including date palm (Jain, 2007a). Micropropagation by direct organogenesis is commonly used for rapid clonal propagation of best genetic material of date palm plant (Khierallah and Bader, 2007).

Performance of micropropagated date palm appears to be better than conventionally grown plants in terms of harvest, early flowering time, and relatively uniform in fruit value and physical properties. Aouine reported plant redevelopment from 30 genotypes of date palm by direct shoot organogenesis. The major concern with this method is somaclonal variation that is dependent on different factors including genotype, explants, plant growth regulators (Jain, 2007a). Moreover, it is highly necessary to maintain genetic fidelity of regenerated plants, which can be studied by many molecular markers. Micropropagation has a benefit of using low concentrations of plant growth regulators, as a result callus phase is avoided. Direct regeneration of vegetative buds reduces the risk of somaclonal variation among regenerants. Duration of culture period is limited by numerous subcultures for maintaining and given that shoot cultures for plantlet production.

However, the highest number of subcultures must be determined before starting the fresh cultures from the mother plants. This is done to prevent or reduce somaclonal variation. Currently, only a few laboratories use this

technique to produce commercially in vitro date palm plants, mainly in Morocco, Saudi Arabia and United Arab Emirates. Micropropagation technique has been used commercially in selected date palm cultivars described advantages and limitations of date palm micropropagation; major advantages are year round availability of plants, quality control, rapid production of plants of elite cultivars, and cold storage of elite genetic material.

Advantages and disadvantages of somatic embryogenesis (Jain,

2007b) Advantages of somatic embryogenesis:- Somatic embryos

originate from a single cell and minimize or eliminate chimera depending on the plant species.

Somatic embryo cell suspension is ideal for mutation induction due to production of direct mutant somatic embryos. Somatic embryos behave like a zygotic embryo in germination. Single somatic embryo can be encapsulated to develop into a somatic seed that could germinate like a normal seed.

This aspect still requires further research for use at a commercial scale. Most suitable approach in woody species for plant regeneration. Somatic embryos can be produced in a bioreactor which could be automated for large scale production of somatic embryos. Somatic embryos are suitable for long term storage by cryopreservation Disadvantages of somatic embryogenesis:-

Somatic embryogenesis is highly genotypic dependent and therefore culture medium modification may be needed for different genotype.

Germination rate of somatic embryos is very poor in most of the crops.

Somatic embryogenic cultures can lose their property if they are not sub-cultured regularly on the fresh culture medium, and that raises the chance of

getting genetic variability. Organogenesis of Date Palm Explant

selection:- The choice of an explant and its disinfection process can affect the success of micropropagation including the date palm.

Shoot tips and adventitious shoots in lateral buds contain more meristematic tissues than other organs, and therefore are frequently used in date palm tissue cultures (Mazri and Meziani, 2015). A successful regeneration of many date palm genotypes has been achieved when shoot tips were used as explants: “Jihel” and “Iklane”, “Mordarsing” and “Khanizi”, “Nabout” and “Khasab” (Al-Khayri, 2007), and “Khalasah”, “Zardai”, “Barhee”, “Zart”, “Muzati” and “Shishi”. Date palm tissue culturing can also be achieved by using explants derived from inflorescences, as was reported for “Barhee” and “Gulistan”. Reynolds and Murashige (1979) induced somatic embryogenesis from zygotic embryos obtained from green fruits that were harvested 2-3 months after pollination. Pinker also used zygotic embryos to induce somatic embryo genesis in “Khistawi”, “Zahdi”, “Barban”, “Asabe” and “Elarous”. Somatic embryos are useful for the micropropagation and large-scale production of date palm plants and may also be used to obtain true-to-type genotypes. Explant disinfection and

preparation:- The main disinfecting agent that has been used for shoot tips is sodium hypochlorite (NaOCl) at a concentration range from 5% to 25% and for spikelets, mercuric chloride (HgCl₂) at 0.1% concentration.

In addition, the use of antioxidants such as 150 mg/l ascorbic acid (for 30 min), 4% polyvinylpyrrolidone (Aslam and Khan, 2009), citric acid at a

concentration of 150 mg/l with 150 mg/l ascorbic acid (soaked overnight), or anhydrous caffeine widely used during shoot tip explant disinfection (Khierallah et al., 2007). Khan and Tabassum (2012) used an effective protocol to eliminate infection from shoot tips: treatment with 5% (w/v) NaOCl containing one drop of a surfactant (Tween-20/100 ml), stirred gently for 30 min, rinsed three times in sterile distilled water (SDW; 5 min each rinse), surface disinfested with 0.

2% (w/v) HgCl₂ for 10 min and then rinsed three times with SDW. Leaf primordia of 6 cm long shoot tips were removed and used as explants and 2 cm long shoot tips with 2-4 intact primordial leaves also served as explants. A similar protocol has been used by Othmani for leaves adjacent to the apex of axillary shoots of cv. "Boufeggous". Fki first washed young leaves with tap water, and surface sterilized them with 0.01% HgCl₂ for 1 h, rinsed three times with SDW, then cut them into 5-10, 10-15 and 15-20 mm long explants. Ledo described a disinfection procedure for zygotic embryos from mature (wine-colored, -2.

17 g) and immature (green, -1.68 g) fruits from "açai" palm, an Euterpe species of palm tree cultivated for its fruit. After being washed under running tap water, fruits were immersed in 40% ethanol, and seeds were excised on a laminar flow bench, immersed in 70% ethanol for 2 min, then in 2% NaOCl for 20 min under agitation, and finally washed four times with SDW (Khokhar, M. I. et al .

, 2017). Adventitious bud initiation:-

The

formation of adventitious buds on date palm explants depends on

many factors such as media components, genotype, and time period of plant material collection. Various culture media were suggested for adventitious bud formation, depending on the cultivar. From offshoot-derived explants, Beauchesne et al.

suggested half-strength Murashige and Skoog (MS) medium supplemented with 1-5 mg/L 2-naphthoxyacetic acid (NOA), 1 mg/L NAA, 1 mg/L Indole-3-acetic acid (IAA) and 0.1-3 mg/L 6-(dimethylallylamino) purine (2iP).

Khierallah and Bader recommended MS medium supplemented with 2 mg/L 2iP, 1 mg/L BAP, 1 mg/L NAA and 1 mg/L NOA for cv. Maktoom. Al-Mayahi suggested MS medium supplemented with 1 mg/L BAP and 0.

5 mg/L thidiazuron (TDZ) for cv. Hillawi. For cv. Zaghloul, Bekheet used MS medium supplemented with 2 mg/L 2iP and 1 mg/L NAA while Hussain et al.

used MS medium supplemented with 4 mg/L IBA and 1 mg/L BAP for cvs. Asil, Hussaini and Zaidi. According to Al-Khateeb, low PGRs concentrations promote the formation of buds while high concentrations induce abnormal growth without bud formation. Studies on adventitious bud formation from inflorescence explants are very scarce. Loutfi and Chlyah indicated that shoot primordia is formed mostly on Greshoff and Doy medium supplemented with 0.5 mg/L NAA, 2 mg/L BAP and 1 mg/L 2iP. In a recent review of the literature, Abahmane reported that the combination of one auxin and two cytokinins is effective for bud formation on inflorescence explants.

As regards to the period of offshoot removal, Beauchesne et al. suggested a period starting from the end of dates harvesting and lasting until the

beginning of flowering. Aissam reported that the explants taken between October and February show the highest buds formation rate, whereas Zaid et al. reported that the best period for the in vitro culturing of offshoot-derived explants is from the onset of flowering. Shoot bud multiplication Many factors influence shoot bud multiplication in date palm, especially the basal formulation of the culture medium, the genotype and PGRs.

Abahmane mentioned that the main basal formulation used is MS at full or half-strength, supplemented with PGRs at low concentrations as compared with the bud initiation stage. Zaid et al. reported that for shoot bud multiplication, NAA, NOA, IAA, BAP and kinetin might be used at 0.5-5 mg/L. Beauchesne et al. suggested half-strength MS medium supplemented with 2 mg/L NOA, 1 mg/L NAA, 1 mg/L IAA, 0.

5 mg/L BAP, 1 mg/L 2iP and 1-5 mg/L kinetin. For cultivar Khalas, Aslam and Khan used 7.84 μ M BAP for high shoot bud multiplication. Khierallah and Bader recommended MS medium with a combination of 1 mg/L NAA, 1 mg/L NOA, 4 mg/L 2iP and 2 mg/L BAP for date palm cv. Maktoom while Khan and Bi Bi found that MS medium containing 0.

5 mg/L BAP and 0.5 mg/L kinetin yields the highest number of shoots per explant in cv. Dhakki. In a previous work on cv. Najda, we found that the best medium for shoot bud multiplication was half-strength MS medium supplemented with 0.5 mg/L NOA and 0.5 mg/L kinetin, which yielded an average of 23.5 shoot buds per explant after 3 months of multiplication.

Mazri recommended MS medium containing 2.5 mg/L IBA and 2.5 mg/L BAP for cv. 16-bis (22.3 shoot buds per explant) while he recommended half-strength MS medium supplemented with 3 mg/L IBA and 3 mg/L BAP for cv. Boufeggous, which showed 22.9 shoot buds per explant.

Al-Mayahis suggested MS medium containing 1 mg/L BAP and 0.5 mg/L TDZ for cv. Hillawi, which resulted in an average of 18.2 buds per culture.

Other factors such as the medium texture, cultivation in bioreactors, explant size and density and carbon source were also reported to affect shoot bud multiplication of date palm. Shoot elongation, rooting and plantlet acclimatization:-

Shoot elongation and rooting may be achieved either on a medium containing PGRs or on a PGR-free medium. Beauchesne et al.

suggested the use of half-strength MS medium supplemented with 1 mg/L NAA, 0.5 mg/L BAP, 0.5 mg/L kinetin and 1-3 mg/L gibberellin for shoot elongation. El Sharabasy et al. reported that the use of 0.1 mg/L NAA has a better effect on shoot elongation as compared to IBA and IAA. The use of liquid medium was also reported to promote shoot elongation.

As regard to shoot rooting, Bekheet recommended 1 mg/L NAA, which showed better results than IAA or IBA at the same concentration. In a previous work on cv. Najda, we compared media with and without PGRs. Our results showed that shoot elongation is fast in media supplemented with PGRs, with high root formation rates.

However, shoots cultured on PGR-free media had wider and greener leaves, and exhibited higher survival rates after acclimatization. This shows that plantlet acclimatization might be influenced by previous culture conditions. Along this line, it has been shown that the texture of the elongation-rooting medium influences the survival rate of plantlets after ex vitro transfer. Indeed, the use of a liquid medium just before plantlet acclimatization showed lower survival rates as compared to a semi-solid medium. On the other hand, increasing the level of sucrose in the elongation-rooting medium increases the survival rate of plantlets during acclimatization. Other factors such as the nature of the substrate and the application of gamma aminobutyric acid were reported to influence plantlet acclimatization (Mazri et al .

, 2015). Conclusions and Future

Prospects:- Micropropagation of date palm either through somatic embryogenesis or through organogenesis was reported for many cultivars, and several factors have been revealed to influence these regeneration systems. Date palm micropropagation presents an efficient way for the large-scale propagation of genotypes resistant to bayoud, a very dangerous disease caused by the fungus *Fusarium oxysporum* f. sp. *albedinis*, which had decimated more than 12 million trees during the last century. Plantlets of bayoud-resistant genotypes are used to rehabilitate palm groves ravaged by this fungus. Micropropagation also allows the large-scale propagation of cultivars of high fruit quality, in order to satisfy the high demand of farmers and consumers.

Despite the numerous works published on date palm micropropagation, research is still needed to optimize culture conditions for the newly selected genotypes and recalcitrant cultivars, to shorten the time needed to produce plantlets, and to reduce the incidence of physiological disorders. It is also important to carry out studies related to the application of somatic embryogenesis to genetic transformation, synthetic seeds production and cryopreservation of embryogenic cultures