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Green House and Field Level Problems of Tissue Cultured Date Palm (*Phoenix dactylifera* L.) Plants: A Review

B. Mullassery[1], S. Kurien[1], C. Umesh[1], S.S. Kurup[2]

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ABSTRACT

Expansion of cultivation demands an uninterrupted supply of quality planting material. The demand-supply chain in the planting material production of date palms, including India, is skewed due to rapid expansion in non-conventional areas because of increasing awareness of its nutritional importance and stress tolerance. Innovative mass multiplication techniques other than the conventional approaches using offshoots have been developed to achieve the projected demand besides obtaining disease-free plantlets. The expansion has also resulted in fresh problems and the objective and crux of this paper is to critically review this aspect. The study is based on extensive search and thorough analysis of the existing available literature. Systematic approach to classify the problems has also been undertaken. Reports of problems in plants derived through conventional methods and those derived by callus mediated organogenesis and somatic embryos is the foundation of this paper. Problems in date palm propagation and cultivation could be broadly categorised as problems observed under *in vitro* culture and abnormalities in the field conditions. Each of the identified problems have been explained to describe the symptoms, the susceptible variety/varieties, the causative factors and any recommended control measures. These problems demand focussed research to develop standard protocols and procedures aimed at overcoming these maladies. The main purpose of this paper is to have a focused consolidated and comprehensive review of the ailing *in vitro* and field level disorders so as to throw light on the pressing problems and define the future lines of investigations.

Key words: Acclimatizations, Field performance, Field-level disorders, *Phoenix dactylifera* L., Somaclonal variations, Somatic embryo abnormalities, Tissue culture.

Date palm (*Phoenix dactylifera* L.) is an important fruit crop cultivated in arid tropical and sub- tropical regions of the world. Traditionally, propagated through offshoots but the two main constraints in the production process and further spread of the crop are their limited availability and low survival rate in the fields. Propagation using standardized TC protocols has enabled date palm growers to cultivate both native and exotic cultivars on a large scale (Abul-Soad and Mahdi, 2010). Micropropagation *via* direct organogenesis is widely used for rapid clonal propagation of elite genetic material of date palm (Khierallah and Bader, 2006). The type and concentration of plant growth regulator, the number of in-vitro subculture cycles, the type of explant and the genotype employed can all affect the phenotypic changes that the date palm plantlets generated using the TC approach may display in an open field setting. According to Bairu *et al.* (2006), the age of the original cultures and the number of subculture cycles kept on a nutritional medium both increase the degree of phenotypic differences in the case of TC-derived plants. In some cases, soma clonal variation results from TC-derived plantlets produced by callus-mediated somatic embryogenesis. Al-Wasel (2001) found that TC-derived date palm cultivars Medjool, Barhee, Sukkary, Toory, Deglat Noor, Khalas and Nabtat-Saif had a high incidence of soma clonal variants, which he attributed to their limited availability and poor field survival rate. Plants grown from callus tissues exhibit the majority of soma clonal variants in TC (Skirvin *et al.,* 1994; Al-Wasel, 2000a; Al-Wasel, 2000b and Ramage *et al.,* 2004). Dwarfism, late bearing, terminal bud bending, fruit set failure (shees) and the production of numerous carpels were among the variations noted. Growth regulators, the kind of explants used in the micropropagatio'n process, the genetic entity or variety, the length of time cultured tissues were kept, the frequency of subculturing and the proliferation rate of cultured tissues are some of the factors that may cause soma clonal variations in TC date palm (Alkhateeb *et al.,* 2006). Different cultivars showed varying degrees and types of variation. In date palm plants, somaclonal changes are divided into two categories: A) transitory (epigenetic variation) and b)

[1]School of Agriculture, Karunya Institute of Technology and Sciences, Coimbatore-641 114, Tamil Nadu, India.
[2]United Arab Emirates University Al-Ain, United Arab Emirates.

Corresponding Author: B. Mullassery, School of Agriculture, Karunya Institute of Technology and Sciences, Coimbatore-641 114, Tamil Nadu, India. Email: bijug30@gmail.com

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permanent (genetically stable variants). Once the causes of these underlying physiological effects are eliminated, plants with epigenetic variation typically recover over time (Skirvin *et al.,* 1994).

Extant literature has been the major source of the subject of presentation. The first author has almost spent his career working on date palm and that too on *in vitro*studies and continues in this line. Added to this is the visits and experience gained in major growing tracts which has also been the major reason that prompted this exercise. The lab has also excellent facilities for the hardening of plants and a farm wherein observational trials of post-planting of TC-derived plantlets are being done. The available literature in this line were classified, categorized and critically analyzed to develop this review.

The disorders were categorized into a) problems observed in the field or field problems and b) problems observed *in vitro* and in hardening.

Disorder	Variety/Cultivar
(A) Problems observed in the field	
Dwarfism	Ajwa, Barhee
Albinism or leaf variegation	Majhool, Anbara
Soma clonal variation	All cultivars
Pollination failure 'Sheiss'	Barhee, Khlass, Sukkary
Parthenocarpic fruit formation	
Apical bud dryness	Khedry
Abnormal growth and development of leaf and fruit strands	Sukhary, Barhee
Failure or delayed fruit set	Anbara, Majhool
(B) *In vitro* abnormalities	
Browning	All cultivars
Vitrification	Majhool
Absence of apical meristem	Khedry
Spiral leaves formation	Khedry and Majhool

Problems of TC plants observed in the field

An earnest attempt has been made to review the problems of TC derived plants under the following major sub heads.

Dwarfism

The restricted growth of date palm plants produced by the TC technique is a characteristic of dwarfism. Date palm plants only very seldom recover from dwarfism and develop normally again. Only in the first- or second-year following field planting, when stem extension takes place, can the majority of dwarf date palm trees be recognized (Fig 1). Younger leaves exhibit the primary symptoms more so than older leaves. Dwarfism causes stunted development by reducing leaf length, structure and canopy size, which impacts leaf function and results in reduced photosynthesis. Additionally, dwarfism drastically weakens date palm plants and lowers the output of their offspring. Additionally, dwarfism has been frequently noted in date palm plants that have undergone callus-regenerated embryogenesis (Cohen *et al.,* 2003). According to Al-Wasel (2001), dwarfism has also been documented in the cultivars Ajwa (Al-Wasel, 2000 a and b). The prevalence of dwarfism varies from 0 to 30%, depending on the variety (Fig 1, 3a, b). According to Al-Wasel (2001), the Khlass cultivar showed



Fig 1: Dwarfism in date palm Cv. Majhool in field.



Fig 2(a, b): Black scorch in date palm Cv. Boufagous.

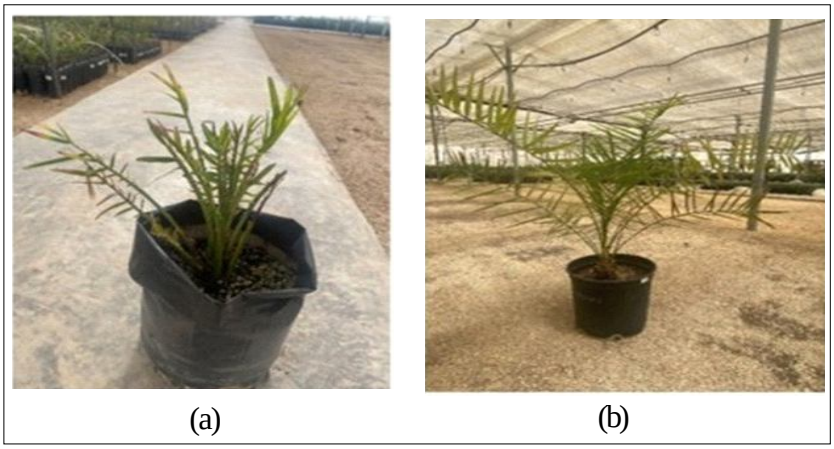


Fig 3(a, b): Dwarfism abnormality appearing in TC plantlets date palm Cv. Khedry (Right, Normal; Left, Abnormal).



Fig 4: Albinism-TC plants date palm Cv. Anbara.

18-24.3% dwarf trees with packed leaves around the trunk, whereas the Barhee trees showed 12-18% dwarf trees. The TC process causes genetic alterations that give rise to the dwarf soma clonal variation. It is challenging to identify this issue in date palm plants early on. Additionally, dwarfism has been linked to black scorch sickness (Fig 2a, b). *Ceratocystis paradoxa* is the pathogen that causes black scorch, often known as Fool's sickness (Amira *et al.,* 2000).

Albinism or leaf variegation

Ability to produce chlorophyll was lost and results in turning from green to variegated colors in some leaves or part of the leaves or the entire plant (Alkhateeb, 2008) (Fig 4); These abnormalities are observed to occur on all leaves but in different forms like stripes or longitudinal yellowing with some wider than the others. However, under field conditions, these plants give almost near to normal yield. In Israel Cohen *et al.* (2007) concluded that this abnormality is prevalent around 3% of some date palm plantations, but this variegation did not extend to all the leaflets and normal fruits were produced by such trees. This abnormality is visible very rarely in date palm orchards therefore its economic significance is considered as negligible.

Soma clonal variation

According to Jain (2001) and Hadrami and Hadrami (2009), soma clonal variants are those that are regenerated from somatic cells and can be employed to introduce new quality features. According to Alkhateeb *et al.* (2006), the primary causes of soma clonal variations in TC date palm plants include growth regulators, the types of ex-plants used in micropropagation, the genetic makeup of the plants, the frequency of subculturing and the rate at which cultured tissues proliferate. In date palm plants, soma clonal differences can be either transient (epigenic variants) or permanent (genetically stable alterations). When the causes of the physiological changes are eliminated, plants with epigenic variations are found to recover in a certain amount of time (Skirvin *et al.,* 1994).

Pollination failure/fruit set failure

Pollination failure (Fig 5a, b) is a major issue affecting the plantations raised through TC date palm plants, which causes major economic losses. The pollination failure is highly noticed in the plantations generated through the somatic embryogenesis technique (Djerbi, 2000; McCubbin *et al.,* 2000). When comparing *in vitro* date palm plants to their offspring of the same culture, Varughese (2000) found anomalies in both fruiting ability and fruit attributes, such as variation in fruit set %, aberrant fruit size and abnormal fruit shape. A female flower typically has three carpels. From those, only one carpel grows to fruit following successful pollination and fertilization; the other two will shrink and die (Alkhateeb and Ali-Dinar, 2002). In the Khalas cultivar, the percentage of dwarf trees with packed leaves around the trunk varied from 18 to 24.3%, whereas in the Barhee, it was 12 to 18%, according to Al-Wasel (2001). According to Ali-Dinar and Alkhateeb (2005), young TC of date palm trees Cv. failed to produce



Fig 5(a, b): Fruit set failure in date palm Cv. Khedry.



Fig 6: Parthenocarpic fruit derived from date palm TC plants.



Fig 7: Apical bud dryness in date palm Cv. Khedry.

fruit normally. Barhee was most likely caused by a number of interconnected factors that resulted in a sluggish pollen tube growth in the early stages of fruit development. These factors may have been made worse by the comparatively high levels of abscisic acid (ABA) atthis time. To determine the reasons behind pollination failure in date palm trees generated from TC, more investigation is required.

Parthenocarpic fruit formation

In commercial fruit culture, seedlessness is frequently seen as a benefit; nonetheless, it is frequently associated by smaller, lighter fruits and occasionally slight morphological abnormalities. According to McCubbin *et al.* (2000), date palm plants cultivated with TC display a variety of typical off-type traits, including variegation, changes in leaf structure and overall plant growth pattern, trees that do not produce inflorescences, or trees that produce parthenocarpic fruits devoid of seeds (Fig 6). Additionally, Cohen *et al.* (2004) noted that some of the undesired date palm genotypes produced aberrant parthenocarpy.

Apical bud dryness

At the beginning of the plant growth in the field, the leaves around the apical bud became dry and subsequently lead to the death of the plant (Fig 7). Abnormal leaf shape, dryness and leaf bending have been observed by Al-Ghamdi (1993) in different date cultivars developed using somatic embryogenesis.

Abnormality in leaf and fruit strands

Date palm one of the major problems observed is the slow development of the pollen tube growth and varied delay in fruit set resulting in incomplete fruit set and further asynchronous fruit development observed within a bunch. The abnormal growth of fruit strands has been observed on certain micro-propagated 'Sukkary' date palm trees (Fig 8a, b), while the twisted growth of fruit strands has been noticed on 'Barhe' trees (Alkhateeb *et al.,* 2006).

Failure or delayed fruit set

As date palm is a dioecious species, separate trees bear unisexual flowers that are either male or female. Natural pollination in date palm is anemophilous and to achieve higher productivity in commercial plantation artificial pollination is an imperative practice (Ortiz-Uribe *et al.,* 2019). In the conventional cultivation one male tree is sufficient for fifty female trees (F.A.O., 2002; Bekheet and El-Sharabazy, 2015).

Several studies related to abnormal fruiting in date palm produced through TC revealed that during the initial years of fruiting, abnormal fruit setting (Gurevich *et al.,* 2005) and development is comparatively high and it declines with increase in age of plants (Cohen *et al.,* 2004). Pollination is a critical process in the production process and it affects the fruit formation, development and yield (Ahmed *et al.,* 2022). In the pollination process, the period



Fig 8(a, b): Abnormal growth of leaf and fruit strands of TC Date palm Cv. Sukkary.



Fig 9: Delayed fruit set in TC date palm Cv. Anbara.



Fig 10(a, b): Phenolic exudation and culture browning.

in which female flowers receive pollen grains is very important as the receptivity period varies with different weather conditions and cultivars (Shahid *et al.,* 2017). Pollination before or after the period of stigma receptivity will not lead to effective fertilization and will result in heavy drop of unfertilized fruits (Muralidharan *et al.,* 2020). In Anbara date cultivars it was observed that only after repeated assisted pollination an acceptable level of fruit set, fruit quality and yield in field conditions was obtained. Maryam *et al.* (2015) reported that pollination should not be delayed for more than 3-4 days (Fig 9). Environmental conditions like temperature also plays a major role and it significantly impacts both the pollination and fertilization processes.



Fig 11: Vitrification in date palm Cv. Khedry.

In-vitro abnormalities observed in culture and during the hardening period

The Al-Rajhi Tissue Culture Lab (Clone Biotech) and research farms in Riyadh, Saudi Arabia, are conducting these observations on the *in-vitro* anomalies of date palm (*Phoenix dactylifera* L.) through direct organogenesis. To enable accurate monitoring and the observation of anomalies, all plants exhibiting growth issues and abnormalities are pooled in a single.

Browning

Date palm offshoots propagated through TC techniques frequently requires special procedure to avoid problems

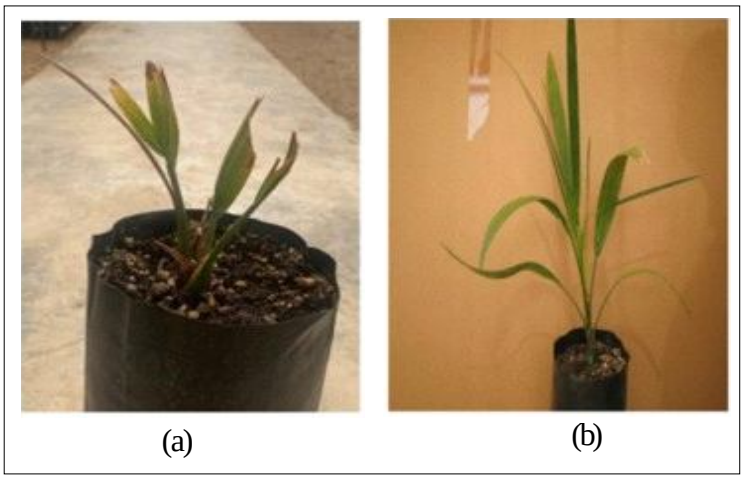


Fig 12(a, b): Absence of apical meristem abnormality appearing in date palm Cv. “Khlass” cultivar propagated through TC (Right, Normal; Left, Abnormal).

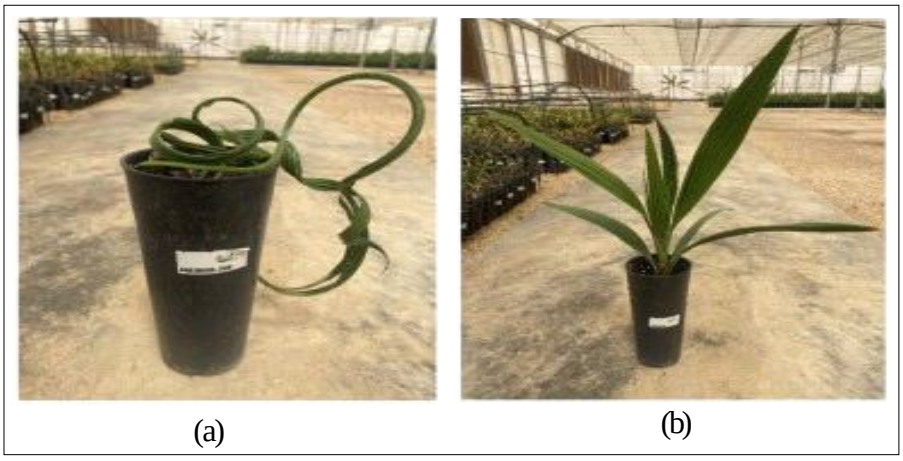


Fig 13(a, b): Spiral leaves appearing in date palm Cv. Majhool cultivar propagated through TC (Right, Normal; Left, Abnormal).

arising on account of exudates. After the inoculation of explants, the introduction medium soon turn brownish becomes darkened (Zaid, 1985) (Fig 10a, b). These released substances are polyphenols that accumulate in the culture, which are immediately oxidized by polyphenol oxidases (Vaughn and Duke, 1984) and formed quinones, highly reactive and toxic to the tissues (Hu and Wang, 1983). By adding activated charcoal to the culture media, harmful brown/black pigments and other unidentified colorless hazardous chemicals will be adsorbed, preventing culture browning (Fridborg and Eriksson, 1975; Fridborg *et al.,* 1978). Leaching of phenolic compounds from agitating the antioxidant solution and properly drying the explants before the inoculation will help reduce the amount of phenolic browning (Meghwal *et al.,* 2000).

Vitrification

In plant TC, vitrification (Fig 11) is a physiological condition marked by an excess of water, slowed growth and the development of glassy, translucent branches. This physiological condition is usually caused by a number of factors, including the use of liquid media, high concentrations of plant hormones, high ammonia concentrations in the culture media, high humidity levels and gases, particularly ethylene, inside culture tubes. This phenomenon can be lessened by taking certain steps, such as using solid medium, lowering the quantities of hormones and ammonium, raising the concentration of agar and using tube caps that provide appropriate gas exchange (Alkhateeb *et al.,* 2006).

Absence of apical meristem

The most dominant abnormality in TC plantlets is the absence of apical meristem which is prominently visible in varieties like Khlass (Fig 12a, b), Majhool, Ajwa, Safawi and Khedry at both primary and secondary hardening stages. The new leaves around apical buds became dry and subsequently led to the death of plants. The real causes of this abnormality are unknown.

Spiral leaves formation

The spiral leaves are generally visible in the primary acclimatization stages. This phenomenon is visible in cultivars like Khedry and Majhool (Fig 13a, b).

CONCLUSION

The progress of *in vitro* propagation of date palm have taken a rapid stride and almost a paradigm shift is observed from the conventional propagation *via* offshoots. The limitation of quality planting material, the high mortality rate during planting and the difficulty in transport are the major reasons for this change. The demand is so high which cannot be met from the supply side. The dearth of planting material can neither be offset by movement across the border which also raises legal hurdles, besides requirements of phytosanitary certification. However, TC has opened out the door to overcoming most of these problems but has equally opened out challenging problems. The large level of variation observed in callus-mediated subculture, mostly due to soma clonal variation, is a major problem that is not confined to date palm alone. High gestation period and cultivation cost, even a small percentage of the variants, can bring about big problems in a commercial plantation. As this problem is already an established one, the safest journey can only be by adopting organogenesis using the apical meristem. The lessons learned by using TC plants in oil palm and the resultant large-scale disaster warrant that this path has to be tread with utmost care, caution and backed by solid scientific field-level shreds of evidence. Hence, it's time to consolidate all known problems and measures to overcome them as date palm propagation and large-scale production attempts are receiving a fresh impetus and the forecast is that it is bound and all set to replace conventional propagation.

Conflict of interest

All authors declared that there is no conflict of interest.

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