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# DIFFERENTIAL *IN-VITRO* RESPONSE OF COMMERCIALLY IMPORTANT GENOTYPES OF DATE PALM (*PHOENIX DACTYLIFERA* L.)

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**ABSTRACT:**

The studies on the *in vitro* response of major varieties of date palms *(Phoenix dactylifera L.)* through direct organogenesis were taken up at Al-Rajhi Tissue Culture Lab, located in Riyadh. The study aimed to assess the response of major cultivated genotypes to *in vitro* culture and categorize them based on the time taken for activation. The explant source used in the study was apical meristem obtained from offshoots. Fourteen genotypes namely Segae, Anbara, Barhi, Majhool, Safawi, Ajwa, Sukkari, Khodry, Khalas, Nabtat Ali, Deglet Noor, Asharasi, Gara and Ruthana were selected for the study. The response was defined as activation of apical meristem with the visible stage of bud formation or new tissue formation in the pre-standardized uniform media and supplements. Based on the initial bud formation response, the genotypes were classified as fast response genotypes, medium response genotypes, and least response genotypes or re-calcitrant types. Segae, Anbara, Majhool, Khodry Safawi and Nabat Ali responded within 300-650 days early responsive genotypes. In Barhi and Khalas the response 650 to 1250 days is mid-responsive genotypes. Deglet Noor, Asharasi, Gara and Ruthana are the least responsive (re-calcitrant) genotypes. The least responsive genotypes were characterized by high phenolic exudation explants. The study underlines that the requirement of each variety or genetic entity is specific and the imperative need to focus on the standardization of media and supplements at the cultivar level with emphasis on overcoming the specific impediments.

**Keywords:** Date palm, Apical meristem, *in vitro* response, Re-calcitrant, Organogenesis, Phenolic exudation

**Key findings**: The differential *in vitro* response of major cultivated genotypes of date palms through direct organogenesis was studied and categorized based on the time taken for activation. The initial response of cultures is related to high phenolic exudation and subsequent browning. High phenolic exudates-generating cultures exhibit a very slow response and low phenolic exudates generating cultures exhibit a fast response on the first bud formation. By identifying the differential varietal response, the genotypes were categorized as fast response, medium response, and least response genotypes.

## 1. INTRODUCTION

Date palm (*Phoenix dactylifera* L.) belonging to the family Arecaceae is one of the most ancient fruit crops in the world characterized by pinnate, "feather-like" gray-green leaves and edible cherished fruit. Most of the other members of the Arecaceae family palms are ornamental or cultivated as oil-yielding plants. It can endure high temperature or heat stress conditions and has been an important crop in tropical belts where other crops fail to come up. Date palm is also one of the most important income-generating crops and a foreign exchange earner, providing livelihood support for millions of rural smallholders. In 2018, date production in Saudi Arabia reached 1.43million tons, from 28 million trees (NCPD 2018). Date fruits are comprehensive nutritional that contains most of the basic components of carbohydrates, proteins, vitamins, and mineral salts. (Al-shahid and Marshall,2003).

Date palm is conventionally propagated through offshoots. The offshoots are limited in number 20 to 30 at the early life of the palm (10 to 15 years from the date of its planting) depending on the variety and on the management practices such as fertilization treatment, irrigation, and earthing up around the trunks, (Nixon and Carpenter, 1978). The major constraint in the spread of date palm cultivation is the acute scarcity of quality planting material and the high cost of offshoot. The demand is so high that it cannot even met by inflow from across the border. The development of mass propagation using the tissue culture method has resulted in the establishment of a large number of tissue culture laboratories and massive expansion of quality date palm plantations in many regions especially Africa and Asia, apart from the Middle East.

Another major field problem in commercial cultivation is the high gestation period to attain productive bearing stage quality date palm plantlets with minimal variations. However, the tissue culture method offers tremendous potential both as a rapid means of propagation also in bringing down the gestation period. Using the tissue culture technique, date palm is propagated commercially through two methods: a) Organogenesis and b) Embryogenesis. Each method has its advantages and disadvantages (Alkhateeb and Ali-Dinar,2002). Using the embryogenesis technique often leads to soma clonal variation and this phenomenon is callus cultures (Skirvin *et al*. 1994; Al-Wasel, 2000 and Ramage *et al.* 2004). Al-Wasel (2001) in a survey study on some tissue culture-derived date palm cultivars obtained from Medjool, Barhee, Sukkary, Toory, Deglet Noor, Khalas, and Nabtat-Saif reported soma clonal variation. Dwarfism, slow growth, morphological abnormality, terminal bud bending, fruit set failure, and supernumerary carpels were the most observed abnormalities. The type and percentage of variations differed between cultivars. In organogenesis, the buds from the meristems develop into plantlets without passing through callus production. However, the plantlets that are produced directly from the tissues of the mother plant, are typically identical to the mother. (Aaouine, 2000, Alkhateeb and Ali-Dinar, 2002, Omer *et al*. 1992). Even though there are many cultivars and landraces in date palms, in-vitro studies have revealed differential responses in these cultivars. Hence this study was taken up with major objectives of a) Screening the commercial varieties' response to in-vitro culture b) Characterizing the response based on the time or duration of the first response c) Categorizing the cultivars based on response and d) Probing the major causes of low response or no-response of cultivars.

## 2. MATERIALS AND METHODS

### a) Materials

This study was carried out in the Al-Rajhi Tissue Culture Laboratory, Ammariyah, in the Riyadh province of the Kingdom of Saudi Arabia during 2017-2021.The commercial varieties were collected from Al-Ammariyah, Al-Qassim and Al-Madina. Anbara, Safawi, Ajwa, Deglet

Noor, Asharasi, and offshoots were collected from Al-Madina Al-monawara. Barhi, Majhool,Khedry and Nabut Ali were collected from Al-Qassim(Buraidha). Sukkary and Khalass were collected from the Riyadh province. (Figure-1)

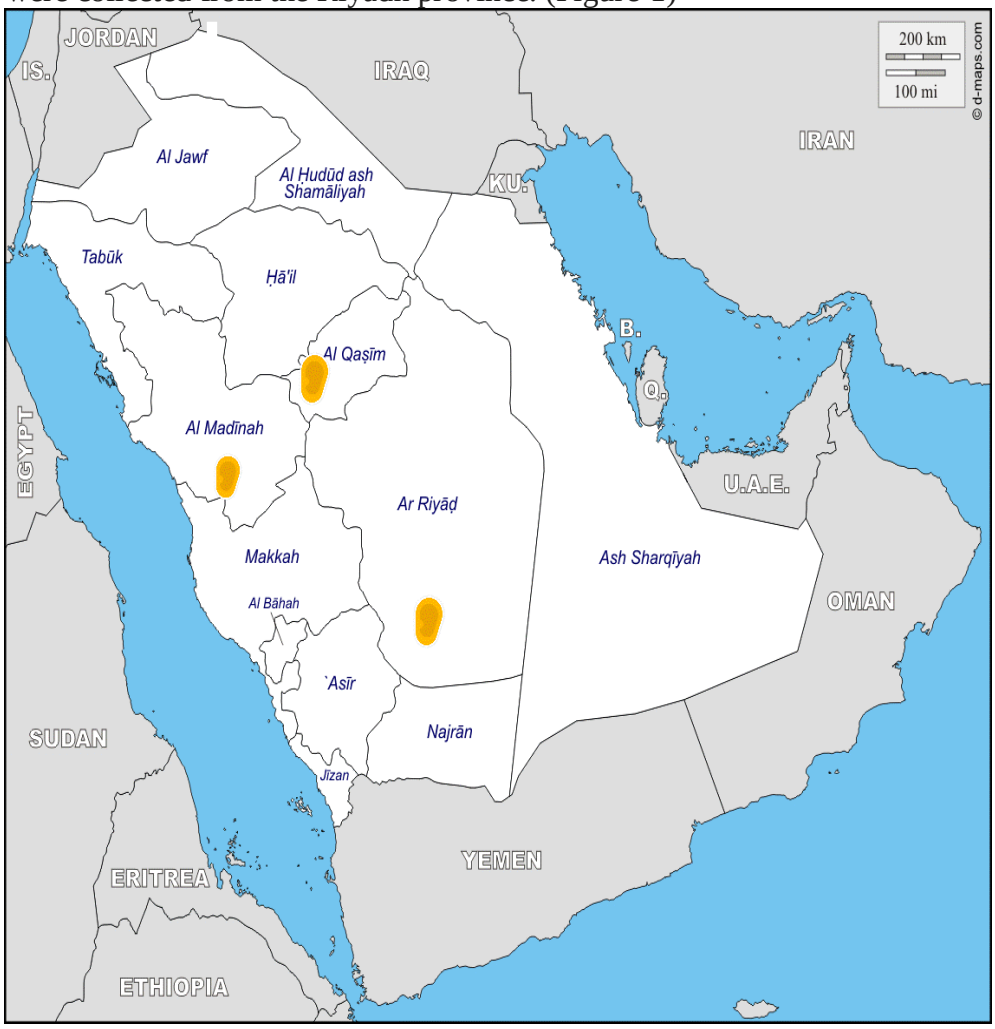



Fig. 1. Geographic location from where date palm cultivars selected for the study

**b) Methods**

Offshoots of 3 to 5 years of age selected that have established their roots and produced a second generation of offshoots from the mother plant were only used in the study. (Figure-2) This was based on the concept that only such offshoots are ready for removal for planting (Nixon, 1966; Nixon and Carpenter, 1978). The size of the offshoot was in the range of 5-6 kg ( Balawy *et al*(2005). Three days before the extraction of offshoots, all the management interventions like irrigation and fertilizer application were withheld.

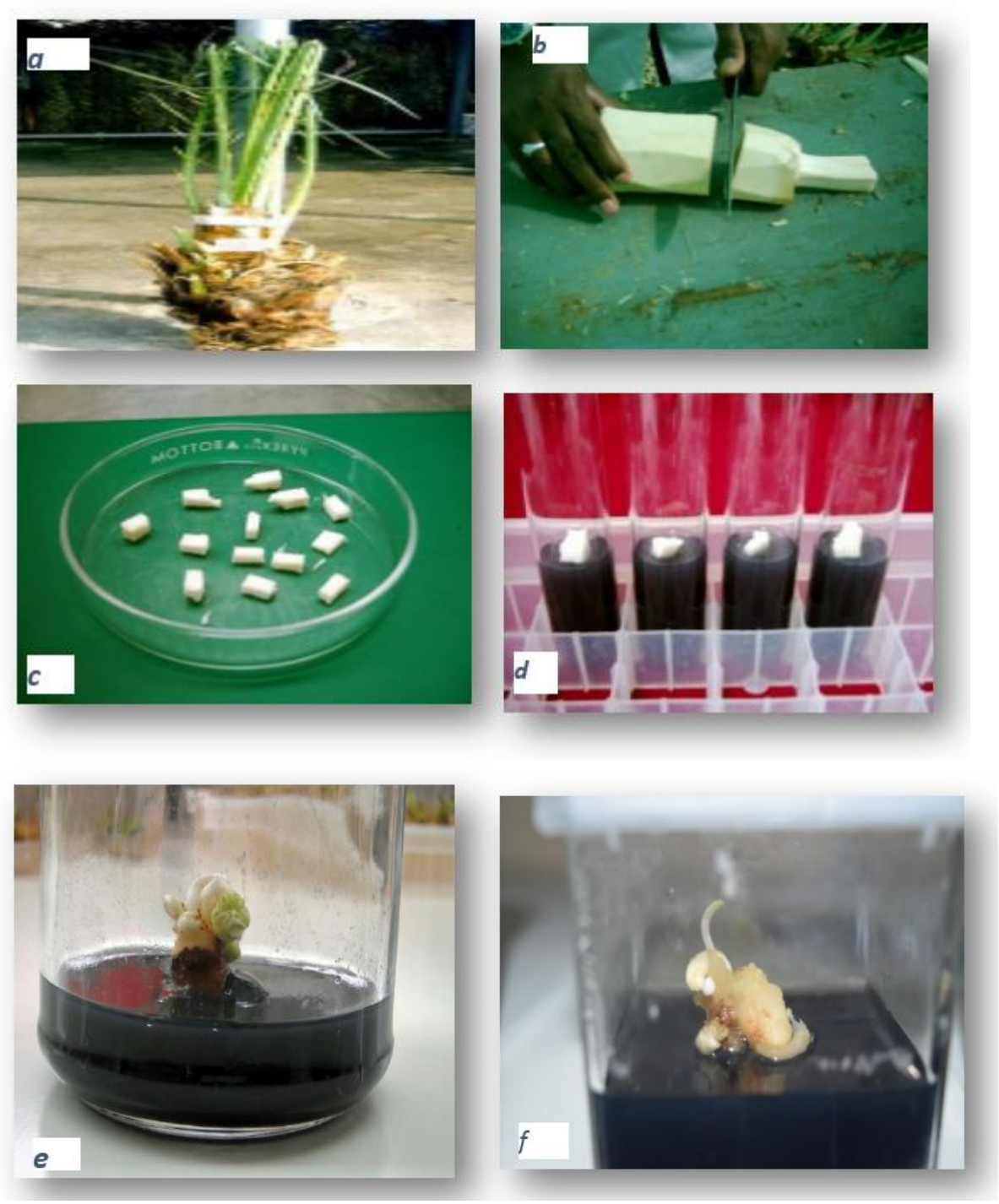



Fig. 2. Organogenesis-Different stages of bud production

a) Offshoot selection
b) Apical Meristem Extraction
c) Explant generation
d) Explants in initiation media
e) & f) New bud formation

The MS basal medium (Murashige and Skooge, 1962) modified with glutamine (200 mg/L), adenine sulfate (20 mg/L), thiamine-HCl (10 mg/L), Ca-pantothenate (10 mg/L), ascorbic acid (75 mg/L), citric acid (75 mg/L), $NaH_2PO_4$. $2H_2O$ (170 mg/L), Agar (Sigma) 6.5g/Lit, activated charcoal (1.5 g/L), and Sucrose (70 g/L)] were used. The pH of the media was

adjusted to 5.7 with 0.1 M KOH or 0.1 M HCl before gelling agent addition. Media were dispensed in glass test tubes (25 x 150 cm) and autoclaved at 121°C for 20 min.

Offshoot preparations were done by using an electric hand saw and a sharp knife, by removing gradually, outer leaves and fibrous tissues at their base until exposure of the shoot tip zone. Ethyl alcohol (70%) was sprayed over the cutter and plant material during the dissection process. The ultimate size of the excised shoot tip should be about 4-5 cm in width and 6-8 cm in length. The selected plant materials were soaked in a sterilized antioxidant solution (150 mg/L each of citric acid and ascorbic acid). Surface sterilizations were implemented twice by soaking in disinfectant Clorox (5.25 % NaOCl) solution 15% for 30 min, 2 drops/ 100 ml solution of Tween 20 (polyoxyethylene sorbitan monolaurate) as a wetting agent was also used. Then it was rinsed in sterile distilled water (Table:1).

The Explants were dissected in a laminar airflow chamber by keeping the axillary buds and apical meristem upright and then transferred to the introduction media. On average, 13-15 explants can be extracted from an offshoot apical meristem. Culture media was transferred to test tubes (25x150mm at 18ml) or a 250ml Majenta box at 80 ml. The media was then sterilized using an autoclave by maintaining it at 121 °C under 1 bar pressure for 20 minutes.

## 3. RESULTS

**Culture Media Standardization**

A modified version of Murashige and Skoog's (1962) (MS) inorganic salts was used for the micropropagation of date palms. The formulation for macro elements was as proposed by Beauchesne *et al*. (1986) which was supplemented with $NAH_2\ PO_4$ (170mg/L), myo-inositol (100mg/L), adenine (30 mg/L), glutamine (200 mg/L), nicotinic acid (1 mg/L), pyridoxine-HCl (1 mg/L), Biotin (1 mg/L), and calcium pantothenate (1 mg/L) which served as the primary media (Al Khateeb 2006; Beauchesne 1983). Different hormonal combinations were tried each cultivar 10 replications. Based on the study, (Table-2) an ideal aseptic protocol has been developed for the cultivars that enhance bud formation in modified MS media with the hormonal combination of NAA(1mg/L), NOA(1–5.5mg/L), 2-iP (0.5-1mg/L), charcoal 1.5g/Lit, sucrose 70g/L and Agar 6.5 g/L. After transfer to introduction media, explants were incubated for 6 to 12 months in a dark room to enhance the new bud formation and to prevent oxidation of phenolic compounds which occurs under light conditions. Introduction cultures were transferred to fresh media every 30 days. The temperature in the dark growth rooms was maintained at 22±1 degrees Celsius (Abahmane *et al*.1999; Anjarne *et al*.2005).

**a) Screening of the commercial varieties' response to *in-vitro* culture**

Depending on the genotype, new bud formation generally requires 9–18 months. Bud initiation is controlled by several factors that may act in concert. Those factors are the culture media components, genotype, and time of plant material collection. Concerning the time of offshoot collection, it was that the best period coincides with the dormancy stage in advance of flowering It was that the best growth and bud regeneration and the lowest rate of tissue browning were obtained when cultures were established between November and April (Figure 3 & 4) (Table 4 & 5). Among these types, Majhool, Ajwa Barhi, Segae, Anbara, Safawi, Sukkari, Khodry, Khalas, Nabtat Ali, Deglet Noor, Asharasi, Gara and Ruthana are very popular in Saudi Arabia.

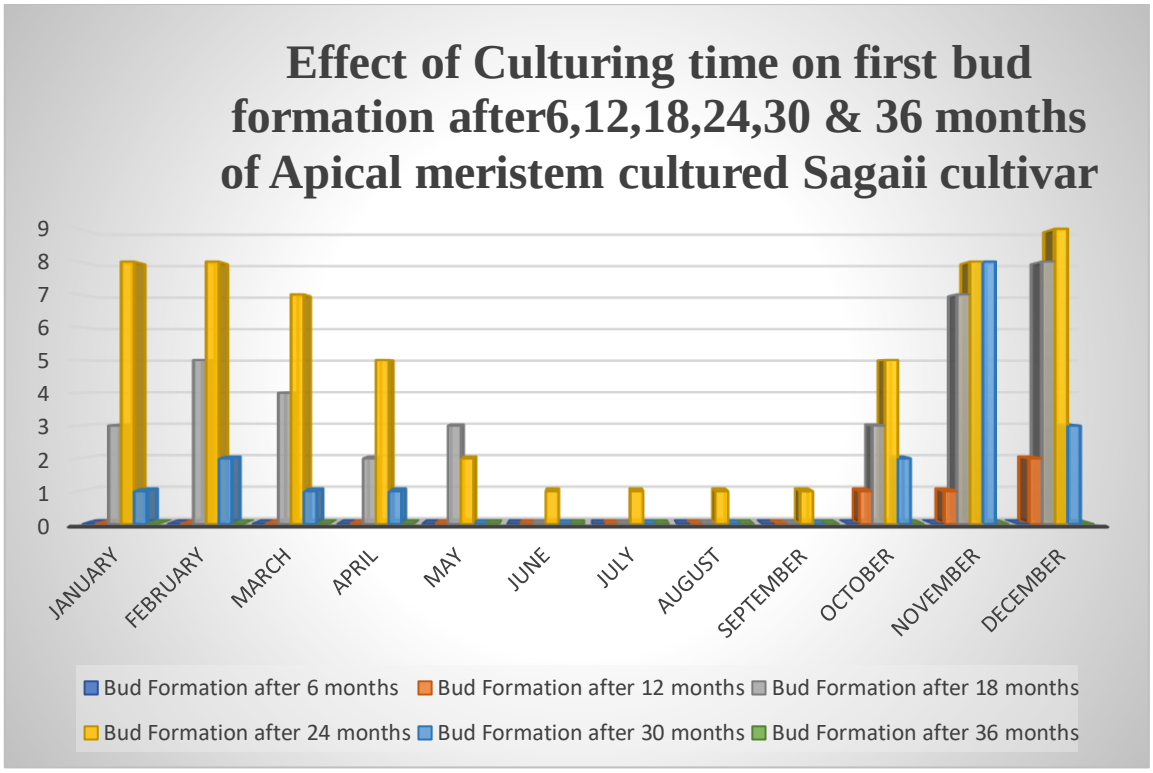



Fig. 3.

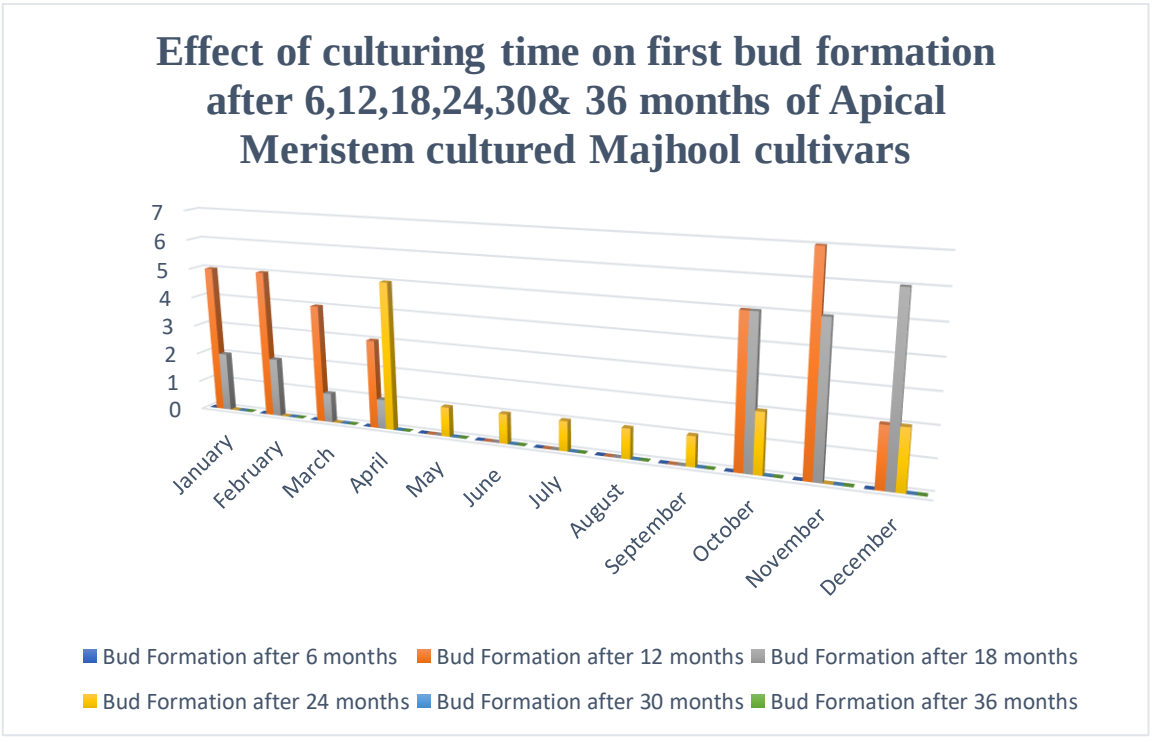



Fig. 4.

**b) Characterizing the response based on the time or duration of the first response**

In each cultivar, ten replications were used to first bud formation response in the initiation media. The first bud formation time varied from 300 days to 1250 days in different cultivars.

**c) Categorizing the cultivars based on response**

The 14 commercially important cultivars' response was defined as activation of apical meristem with the visible stage of bud formation or new tissue formation in the pre-standardized uniform media and supplements. Based on the initial bud formation response, the genotypes were classified as fast response genotypes, medium response genotypes, and least response genotypes or re-calcitrant types (Table 3). Segae, Anbara, Majhool, Khodry Safawi and Nabat Ali showed response within 300-650 days considered to be early responsive genotypes (Figure 5). Barhi and Khalas showed a response in 650 to 1250 days and were categorized as mid-responsive genotypes (Figure 6). Deglet Noor, Asharasi, Gara and Ruthana were the least responsive (re-calcitrant) genotypes. (Figure-7)

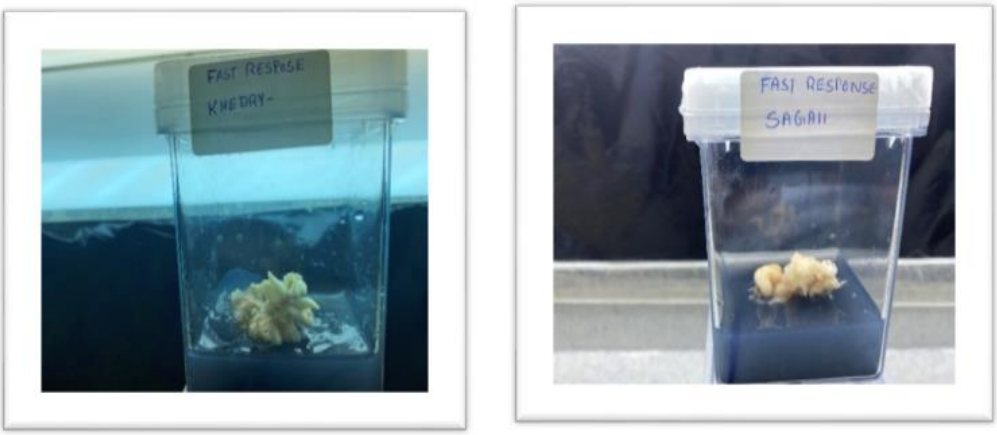



Fig. 5. Fast Response cultivars

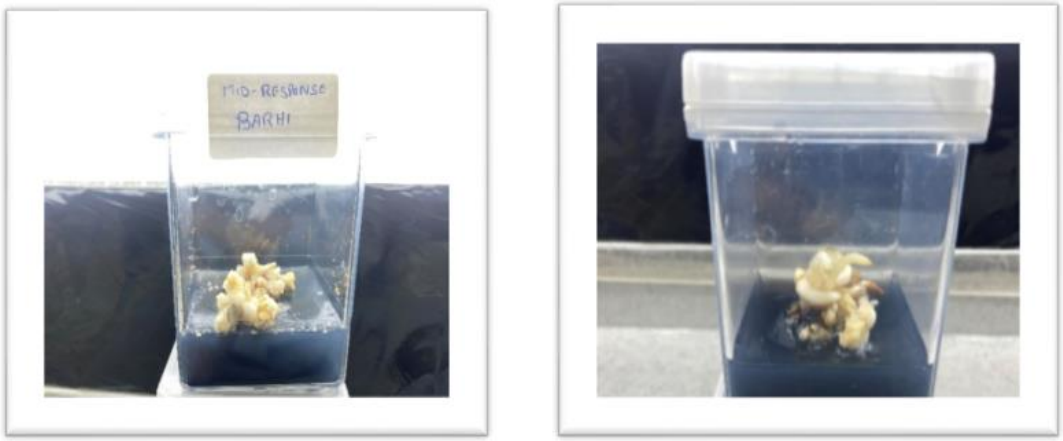



Fig. 6. Medium response cultivars

**Fig. 7.** Least response cultivars

**d) Probing the major causes of low response or no-response of cultivars**

The released polyphenols accumulate in the initial culture medium which turns brown coloration in explants. The main causes of differential response in all these cultivars are due to tissue browning differences in the initial culture growing period. (Figure 8) In this study, early and medium response cultivars show less phenolic accumulation compared to least responsive cultivars. In late cultivars like Deglet Noor, Asharasi, Gara, and Rutana high browning in initiation media (Figure-9). In addition to culture media, some adsorbents like adenine, glutamine, and citrate reduce date palm tissue browning (Rhiss *et al*. 1979), pre-soaking of tissue in antioxidant solutions (100 mg/l ascorbic acid and 150 mg/l citric acid) before their transfer to culture media as reported by Zaid and Tisserat (1983). Activated charcoal was found To reduce the browning. Direct addition of Polyvinylpyrrolidone (PVP-40) in the culture medium at a concentration of 2 g/l reduced date palm tissue browning. Explants with a size of 1.5 to 2 cm were found to reduce the phenolic exudation in introduction cultures. Transferring the cultures to fresh media with 30-day intervals was also found to minimize the culture browning in initiation cultures.

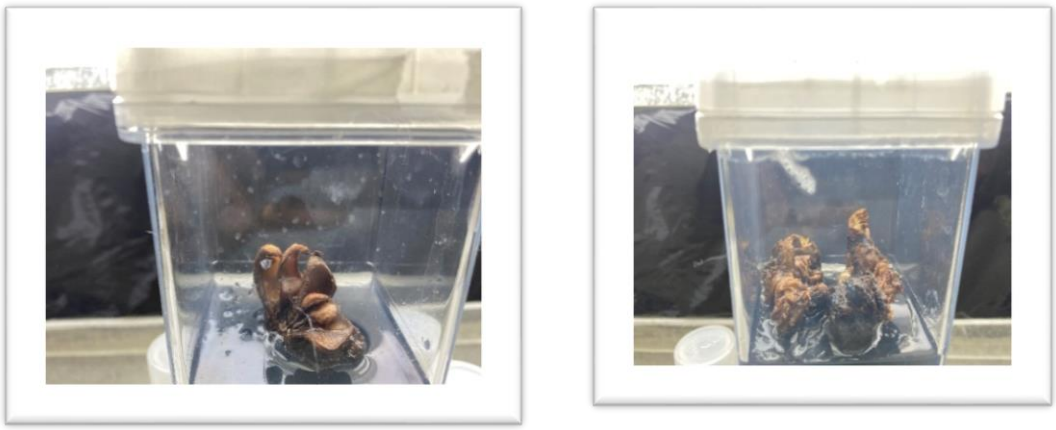
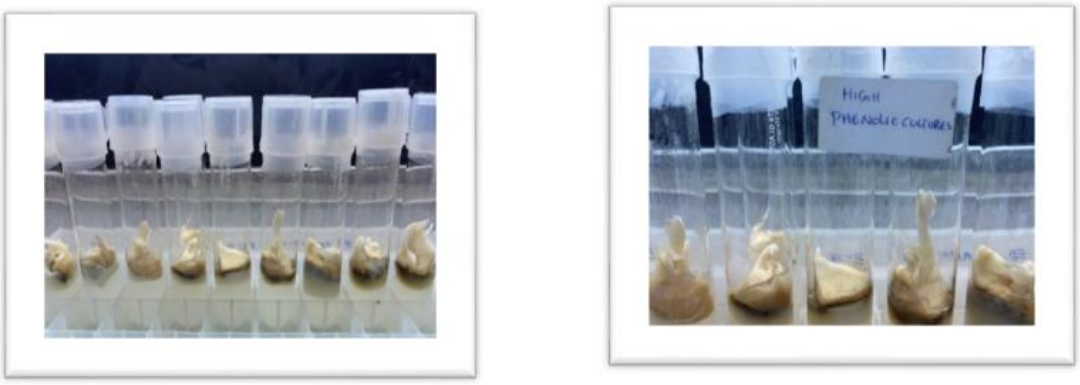


**Fig. 8.** Culture browning

**A**) Asharasi B) Deglet Noor




C) Rutana D) Gara

Fig. 9. High Phenolic exudations in different cultivars

## 4. DISCUSSION

In this study, the initial response of cultures in different cultivars was related to high phenolic exudation and browning of cultures. High phenolic content exhibiting cultivars show very low initial response and less phenolic exudates generating cultivars show fast response on the initial bud generation. In date palm *in-vitro* culture, browning of the explant is the most frequent problem which finally leads to death In this study, direct addition of 2g/L of PVP-40 in the culture medium reduces date palm tissue browning and identical reports have been reported by Beauchense(1983). This finding was also supported by Loutfi and El Hadrami 2005, who stated that Polyphenol accumulation in date palm is known to contain high levels of caffeoyl shikimic acids ranging from 190 to 430 mg/g fresh weight depending on the cultivar. During micropropagation, both somatic embryogenesis (Abohatem; Zouine; El Hadrami, 2011) and organogenesis (Mazri, 2015) encounter this problem. The primary reason is date tissue contains high levels of phenolic compounds.

The results of the current study agree with the results reported by Amin 2001; Al Maari and Al Ghamdi (1998). This study emphasizes the need to fractionate and quantify the phenols in each cultivar as then only we will be able to come out with the intervention measures to overcome these barriers. The study underlines the requirement of each variety or genetic entity-specific and the imperative need to focus on the standardization of media and supplements at the cultivar level with emphasis on overcoming the specific impediments that are suggested as a future line of study.

**Acknowledgment**

The author respectfully acknowledges Eng. Abdulwahab Saleh Al-Rajhi to the favors and liberal support received for the conduct of the study. His constant encouragement and interest are also duly acknowledged.

Table: 1. Tabulated data showing different Treatments & their effects on Date palm Introduction

| TREATMENTS | Cultivar-Majhool | | Cultivar-Ajwa | | Cultivar-Sukkary | | Cultivar-Safawi | |
|---|---|---|---|---|---|---|---|---|
| | Infected | Non-Infected | Infected | Non-Infected | Infected | Non-Infected | Infected | Non-Infected |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Chemical Treatment- (1)Tween 2ml/300ml of water, cleaning using sterile water 3x (2)Ortiva 2ml/1ltr of water after 20 mins clean using sterile water 3x (3)Clorox 33.3ml/1ltr of water. Clean with Ascorbic acid after 15 mins | 3 | 9 | 5 | 8 | 3 | 11 | 6 | 5 |
| Flamming Treatment- Dip Meristem in 70% Ethyl alcohol & flame in Bunsen Burner. Remove the outer portions of the meristem | 2 | 11 | 3 | 9 | 2 | 12 | 3 | 13 |

**Table:2.** Differential response of cultivars in the induction media

| **Sl. No** | **Date palm Cultivars** | **Explant Origin** | **Hormones used in induction Media( mg/L)** | **Time taken for first bud induction (Days)** | **Growth Responses** |
|---|---|---|---|---|---|
| 1 | Anbara | Al-Madina | NAA(1), NOA(1–5.5), 2-iP(0.5-1) | 304 | vegetative buds |
| 2 | Ajwa | Al-Madina | NAA(1), NOA(1–5.5), 2-iP(0.5-1) | 253 | vegetative buds |

| | | | | | |
|---|---|---|---|---|---|
| 3 | Safawi | Al-Madina | NAA(1), NOA(1–5.5), 2-iP(0.5-1) | 391 | vegetative buds |
| 4 | Deglet Noor | Al-Madina | NAA(1), NOA(1–5.5), 2-iP(0.5-1) | 1290 | Shoot proliferation |
| 5 | Asharasi | Al-Madina | NAA(1), NOA(1–5.5), 2-iP(0.5-1) | 1275 | Shoot proliferation |
| 6 | Ruthana | Al-Madina | NAA(1), IBA (1), 2-iP(0.1) | 1305 | Shoot proliferation |
| 7 | Barhi | Al-Qassim | NAA(1), NOA(1–5.5), 2-iP(0.5-1) | 806 | vegetative buds |
| 8 | Majhool | Al-Qassim | NAA(1), NOA(1–5.5), 2-iP(0.5-1) | 310 | vegetative buds |
| 9 | Khodry | Al-Qassim | NAA(1), NOA(1–5.5), 2-iP(0.5-1) | 429 | vegetative buds |
| 10 | Nabut Ali | Al-Qassim | NAA(1), NOA(1–5.5), 2-iP(0.5-1) | 384 | vegetative buds |
| 11 | Gara | Al-Qassim | NAA(1), IBA (1), NOA(1–5.5), 2-iP(0.1) | 1350 | Shoot proliferation |
| 12 | Segae | Al-Qassim | NAA(1), NOA(1–5.5), 2-iP(0.5-1) | 308 | vegetative buds |
| 13 | Sukkary | Riyadh | NAA(1), BA (1), NOA(1–5.5), 2-iP(0.1) | 367 | vegetative buds |
| 14 | Khalas | Riyadh | NAA(1), NOA(1–5.5), 2-iP(0.5-1) | 755 | vegetative buds |

**Table:3 Time duration of different cultivars for first bud formation**

| Cultivar/ Offshoot no | Date of Collection | Number of explants/offshoots (Replications) | First Bud Formation Date | Introduction To Bud Formation Time (Days) | Classification of cultivars |
|---|---|---|---|---|---|
| Sagae-121 | 2/Dec/2017 | 10 | 6/Dec/2018 | 368 | Fast response |
| Sagae-122 | 2/Dec/2017 | 11 | 6/Dec/2018 | 368 | Fast response |
| Sagae-151 | 17/May/2019 | 10 | 15/Sep/2020 | 486 | Fast response |
| Sagae-160 | 18/Sep/2019 | 13 | 19/Jul/2020 | 304 | Fast response |
| Sagae-166 | 18/Sep/2019 | 12 | 19/Jul/2020 | 304 | Fast response |
| Anbara-29 | 24/Mar/2017 | 13 | 27/Jan/2019 | 308 | Fast response |
| Anbara-118 | 19/Nov/2017 | 10 | 29/Sep/2018 | 313 | Fast response |
| Anbara-133 | 7/Dec/2018 | 11 | 8/Feb/2020 | 427 | Fast response |
| Anbara-138 | 17/May/2019 | 10 | 15/Sep/2020 | 486 | Fast response |
| Anbara-158 | 21/May/2019 | 13 | 21/Jul/2020 | 440 | Fast response |
| Nabut Ali-3 | 28/Oct/2017 | 12 | 17/Jun/2019 | 436 | Fast response |
| Nabut Ali-4 | 13/Dec/2018 | 10 | 1/Jan/2020 | 384 | Fast response |
| Nabut Ali-27 | 18/Nov/2018 | 11 | 15/Apr/2020 | 513 | Fast response |
| Nabut Ali-29 | 28/Oct/2019 | 10 | 15/Jun/2021 | 443 | Fast response |
| Nabut Ali-32 | 12/Feb/2019 | 13 | 27/Jul/2021 | 895 | Fast response |
| Majhool-100 | 1/Apr/2019 | 12 | 6/Feb/2021 | 310 | Fast response |
| Majhool-101 | 1/Apr/2019 | 13 | 24/Nov/2020 | 602 | Fast response |
| Majhool-249 | 24/Dec/2019 | 10 | 29/Jan/2021 | 400 | Fast response |
| Majhool-252 | 25/Dec/2019 | 11 | 9/Feb/2021 | 410 | Fast response |
| Majhool-262 | 26/Dec/2019 | 10 | 6/Dec/2021 | 344 | Fast response |
| Safawi-15 | 23/Mar/2017 | 13 | 21/Apr/2019 | 759 | Fast response |
| Safawi-27 | 28/Dec/2018 | 12 | 24/Jan/2020 | 391 | Fast response |
| Safawi-62 | 4/Dec/2018 | 10 | 16/Aug/2020 | 620 | Fast response |
| Safawi-63 | 4/Dec/2018 | 11 | 16/Jun/2020 | 559 | Fast response |
| Safawi-73 | 26/Jun/2019 | 10 | 16/Aug/2020 | 416 | Fast response |
| Ajwa-48 | 12/Apr/2017 | 13 | 3/Jul/2018 | 446 | Fast response |
| Ajwa-50 | 16/Oct/2017 | 12 | 12/Nov/2018 | 391 | Fast response |
| Ajwa-65 | 7/Apr/2018 | 13 | 17/Dec/2018 | 253 | Fast response |
| Ajwa-84 | 5/Apr/2018 | 10 | 12/Mar/2019 | 341 | Fast response |
| Ajwa-106 | 25/Oct/2019 | 11 | 30/Dec/2020 | 430 | Fast response |
| Sukkary-51 | 21/Mar/2017 | 10 | 8/Aug/2018 | 505 | Fast response |
| Sukkary-64 | 9/Mar/2018 | 13 | 18/Jul/2019 | 496 | Fast response |
| Sukkary-74 | 4/Jan/2018 | 12 | 6/Jan/2019 | 367 | Fast response |
| Sukkary-132 | 31/Dec/2018 | 10 | 2/Dec/2018 | 335 | Fast response |
| Sukkary-176 | 21/May/2019 | 11 | 21/Jul/2020 | 440 | Fast response |
| Khodry-1 | 9/Apr/2017 | 10 | 15/Jun/2018 | 429 | Fast response |
| Khodry-2 | 9/Apr/2017 | 13 | 28/Jan/2019 | 630 | Fast response |
| Khodry-3 | 9/Apr/2018 | 12 | 14/Dec/2019 | 611 | Fast response |
| Khodry-7 | 13/Dec/2018 | 13 | 6/May/2020 | 509 | Fast response |
| Khodry-53 | 20/May/2019 | 10 | 8/Jul/2020 | 414 | Fast response |

**Table-3 continued….**

| Cultivar/ Offshoot no | Date of Collection | Number of explants/offshoots (Replications) | First Bud Formation Date | Introduction To Bud Formation Time (Days) | Classification of cultivars |
|---|---|---|---|---|---|
| Khalas-2 | 19/Nov/2017 | 10 | 4/Feb/2020 | 806 | Medium response |
| Khalas-26 | 16/Apr/2017 | 13 | 8/Mar/2019 | 690 | Medium response |
| Khalas-30 | 20/Oct/2018 | 12 | 20/Sep/2020 | 699 | Medium response |
| Khalas-49 | 26/Oct/2019 | 10 | 20/Nov/2021 | 755 | Medium response |
| Khalas-53 | 22/Mar/2018 | 11 | 3/Oct/2021 | 1080 | Medium response |
| Barhi-52 | 14/Mar/2017 | 10 | 23/Aug/2019 | 436 | Medium response |
| Barhi-69 | 21/Mar/2018 | 13 | 8/Feb/2020 | 1054 | Medium response |
| Barhi-105 | 19/Nov/2018 | 12 | 4/Feb/2021 | 806 | Medium response |
| Barhi-190 | 16/Oct/2017 | 13 | 15/Jun/2020 | 977 | Medium response |
| Barhi-220 | 10/Dec/2018 | 10 | 12/Jun/2021 | 1080 | Medium response |
| Asharasi-28 | 11/Dec/2018 | 10 | 15/Jun/2021 | Dead | Least response |
| Asharasi-22 | 16/Apr/2019 | 13 | 20/Sep/2022 | Dead | Least response |
| Asharasi-33 | 20/Oct/2018 | 12 | 4/Jan/2023 | Alive | Least response |
| Asharasi-49 | 26/Oct/2018 | 10 | 25/Sep/2022 | Dead | Least response |
| Asharasi-58 | 14/Mar/2017 | 11 | 8/Feb/2022 | Dead | Least response |
| Deglet Noor | 8/Mar/2018 | 10 | 23/Aug/2021 | Dead | Least response |
| Deglet Noor | 13/Dec/2018 | 13 | 8/Mar/2021 | Alive | Least response |
| Deglet Noor | 28/Dec/2019 | 12 | 16/Aug/2022 | Dead | Least response |
| Deglet Noor | 17/Nov/2020 | 13 | 16/Jun/2021 | Dead | Least response |
| Deglet Noor | 28/Oct/2020 | 10 | 9/Feb/2022 | Alive | Least response |
| Gara-11 | 26/Dec/2017 | 10 | 6/Dec/2021 | Dead | Least response |
| Gara-21 | 26/Dec/2017 | 13 | 6/Aug/2021 | Dead | Least response |
| Gara-23 | 10/Dec/2018 | 12 | 23/Aug/2021 | Dead | Least response |
| Gara-24 | 26/Jan/2019 | 10 | 25/Nov/2020 | Alive | Least response |
| Gara-25 | 24/Dec/2017 | 11 | 9/Dec/2021 | Dead | Least response |
| Ruthana-12 | 22/Feb/2017 | 10 | 6/Oct/2020 | Dead | Least response |
| Ruthana-18 | 9/Mar/2018 | 13 | 11/Mar/2022 | Alive | Least response |
| Ruthana-21 | 7/Mar/2018 | 12 | 23/Apr/2021 | Dead | Least response |
| Ruthana-30 | 28/Oct/2019 | 13 | 3/Dec/2023 | Dead | Least response |
| Ruthana-32 | 7/Mar/2019 | 10 | 16/Mar/2021 | Dead | Least response |

**Table: 4.** Effect of Culturing Time on First Bud Formation after 6,12,18,24,30 36 months of apical meristem cultured Sagai cultivar

| Month | Bud Formation after 6 months | Bud Formation after 12 months | Bud Formation after 18 months | Bud Formation after 24 months | Bud Formation after 30 months | Bud Formation after 36 months |
|---|---|---|---|---|---|---|
| January | 0 | 0 | 3 | 8 | 1 | 0 |
| February | 0 | 0 | 5 | 8 | 2 | 0 |
| March | 0 | 0 | 4 | 7 | 1 | 0 |
| April | 0 | 0 | 2 | 5 | 1 | 0 |
| May | 0 | 0 | 3 | 2 | 0 | 0 |
| June | 0 | 0 | 0 | 1 | 0 | 0 |
| July | 0 | 0 | 0 | 1 | 0 | 0 |
| August | 0 | 0 | 0 | 1 | 0 | 0 |
| September | 0 | 0 | 0 | 1 | 0 | 0 |

| | | | | | | |
|---|---|---|---|---|---|---|
| October | 0 | 1 | 3 | 5 | 2 | 0 |
| November | 0 | 1 | 7 | 8 | 8 | 0 |
| December | 0 | 2 | 8 | 9 | 3 | 0 |

Table: 5. Effect of Culturing time on first bud formation after 6,12,18,24,30 36 months of apical meristem cultured Majhool cultivar

| Month | Bud Formation after 6 months | Bud Formation after 12 months | Bud Formation after 18 months | Bud Formation after 24 months | Bud Formation after 30 months | Bud Formation after 36 months |
|---|---|---|---|---|---|---|
| January | 0 | 5 | 2 | 0 | 0 | 0 |
| February | 0 | 5 | 2 | 0 | 0 | 0 |
| March | 0 | 4 | 1 | 0 | 0 | 0 |
| April | 0 | 3 | 1 | 5 | 0 | 0 |
| May | 0 | 0 | 0 | 1 | 0 | 0 |
| June | 0 | 0 | 0 | 1 | 0 | 0 |
| July | 0 | 0 | 0 | 1 | 0 | 0 |
| August | 0 | 0 | 0 | 1 | 0 | 0 |
| September | 0 | 0 | 0 | 1 | 0 | 0 |
| October | 0 | 5 | 5 | 2 | 0 | 0 |
| November | 0 | 7 | 5 | 0 | 0 | 0 |
| December | 0 | 2 | 6 | 2 | 0 | 0 |

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