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Pollen grain efficiency of two male date palm (*Phoenix dactylifera L.*) genotypes with exogenous application of GA_3 and NAA; *in vitro* and field study *

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Abstract
Two male date palm cultivars, "Ghanami akhdar" and "Jarvis," were studied in order to determine the effects of gibberellic acid (GA3) and naphthalene acetic acid (NAA) on their pollen grain viability, germination, and pollen tube elongation., Different level of each GA3 (10, 50 and 100 mg L-1) and NAA (1, 2 and 3 mg L -1) were used. The viability and fertility of pollen grains were tested by applying same levels to the aqueous pollen suspension using in date palm pollination in the field. The result showed that GA3 at 100mg/L improved date palm pollen germination through accelerating germination rate and pollen tube growth which reflected on the speed of fertilization. Application of 100mg/L GA3 upon pollination, also, led to the best results in term pollen tube growth and fruit set at Khelal stage; this may refer to a relationship between the amount of GA3 in pollen grains and its role in fertilization, fruit growth and development. NAA at these (1, 2 and 3 mg L -1) concentrations can present an effective thinning method in "Medjool". On other hand, no significant differences between cultivars were observed in vitro, but "Ghanami akhdar" outperformed "Jarvis" in vivo. "Ghanami akhdar" can be considering as a recommendable pollinator for "Medjool".

Keywords; Date palm, Pollen germination, Pollen viability, Fruit set, Gibberellic Acid, Naphthalene Acetic Acid.

1. INTRODUCTION

The date palm is considered one of the most domesticated fruit trees in terms of moral value because of its religious importance for many different global societies, as well as for its health benefits and its productive capacity in semi-arid and arid environments (Jain and Johnson, 2015; Krueger, 2021). Date palm plants are categorized as dioecious fruit trees since their male and female blooms grow on different trees. Successful fruit set in fruits bearing seeds is contingent upon the effectiveness of pollination. A fruit's ability to form kernels and grow is dependent on successful pollination, particularly in the case of nuts and drupe fruits with a single seed, such as stone fruits and dates. In date palms, pollination is the process by which pollen grains are

transferred from male to female trees, either naturally or by manual or mechanical means. It is deemed sufficient when 60–80% of the female flowers are pollinated, and this often results in a decent fruit (Alasasfa, M., 2021). Pollination can adversely be affected by, temperature; humidity, pollen grain viability, and ability of female flowers to receipt pollen grain (Alasasfa, 2019). Khalifa et al, (1983), stated that, the big difference in fruit set from one year to another is mainly due to climatic conditions. Several studies on male cultivars have indicated their pollen efficiency. "Jarvis" is an American seedling male date palm that was recognized for its valuable qualities before 1954 and is now included of the Date Palm Germplasm Repository's holdings. Jarvis produces several inflorescences that give enormous amounts of pollen (Krueger, 2021). Pollen from "Jarvis" was found to be the best source for improving fruit physical characteristics. (Mohammadi et al., 2017). From its side "Ghanami akhdar" is a well-known Iraqi cultivar showed the highest pollen germination percentage (87%) and pollen tube growth (323µm) compared to other five cultivars (Aljibouri et al., 1990). In order to increase the efficiency of pollen grains, it was necessary to increase the factors that lead to the effectiveness of pollen grains, and among these studies was the introduction of hormones as a stimulating factor to increase the efficiency of pollen grains.

Gokbayrak and Engin (2017) discovered that GA3 was the most effective plant growth regulator for enhancing pollen germination *in vitro*. Wu et al. (2008), proposed that GA_3 promotes pollen tube growth. According to Prajapati and Jain (2011), GA_3 might play different roles depending on the species tested and the dosage used; it can either help or inhibit *in vitro* pollen germination and tube development. Radovic et al. (2016) found that GAs caused an increase in pollen germination and pollen tube length for many cultivars of almonds. In addition, it was found that GA_3 was suitable for one of two examined species of Bauhinia with related to germination rate (Sanjay Kumar et al., 2016). In term of GA_3 dose, several studies indicated that the germination rate and pollen tube growth were directly stimulated by the high concentrations of GA_3; Qi et al. (2010) investigated the inducing effect of high gibberellin concentrations in kiwifruit, they found that 60-90mg/L GA_3 stimulated pollen germination and pollen tube growth, and 90mg/L was the best concentration. Using GA_3, at high concentrations, significantly improved the percentage of germination as well as pollen tube lengths of two almond (*Prunus dulcis*) cvs (Maita and Sotomayor, 2015). In Chaohong peach, the most suitable concentrations that stimulated pollen germination and tube growth of GA_3 were 25-100 ppm (Xue, et al, 2008), while the most suitable concentration of GA_3 for pollen germination and tube growth of *Syringa oblata* was 100mg/L (Liu, et al 2011). Other studies indicated an inverse relationship; low concentrations stimulate germination and that the concentrations altitude has an inhibitory effect on germination measurements in pomegranate (Gokbayrak and Engin (2018), pistachio (Acar and Sarpkaya, 2010), cotton (Almeida, 1990) and *Arbutus unedo* (Gökbayrak, et al 2020). According to Shenxi, et al, (2004), there were a negative relationship trend between pollen viability and the content of GA_3 and the mediate concentration of GA_3 increased germination and pollen tube growth.

On the other hand, auxin is a hormone that accumulates in pollen grains and governs several aspects of plant flora growth, including apical meristematic differentiation, stamen lengthening, anther maturation, and pollen grain development (Salinas-Grenet et al., 2018). However, a reduction in free auxin levels, particularly in pollen grains, can have a significant impact on pollen grain growth and elongation of the pollen tube, which affects reproduction and the rate of egg fertilization, and consequently seed yield (Gao et al., (2019). NAA is a type of synthetic auxin hormone that is commonly utilized for the vegetative propagation of plants through stem and cutting. The impact of NAA on plant development is significantly influenced by the concentration and timing of its application (Suman, et al., 2017).

The objectives of this study is to examine different level of GA_3 and NAA on germination

percentage, germination rate, and pollen tube elongation of two male date palm cultivars "Ghanami akhdar" and "Jarvis", in addition to studying the viability and fertility of pollen grains by the direct method in the field, which is expressed through the fruit set and to test the metazenic effect of two pollen sources with regard to fruit set parameter.

2. Materials and methods

Location

The field work for this research was performed during the year 2021 at the experimental date palm orchard in Ghor Alsafi Station, National Agriculture Research Center (NARC). The station is located at latitude 31° 02′ N and longitude 35° 3E. It is about 380 meters below sea level. The soil texture is of the soil is sandy loam type. Furthermore, all laboratory works were carried out at the Department of Plant Production, Faculty of Agriculture, Mutah University. Jordan.

Pollen grains collection.

Mature spathes were collected from eight years old date palm males "Jarvis" and "Ghanami Akhdar". The selected male cultivars are traditionally used as pollinators of commercial date cultivars. Fresh mature pollen grains were collected and incubated at 25˚C.

Germination medium preparation

Fourteen treatments were used including two control treatments for each cultivar. *In vitro* germination basal medium consisted of Calcium nitrate dihydrate (0.417 g), Boric acid, (0.200 g), Potassium Nitrate (0.101g), Magnesium sulfate, $7H_2O$ (0.217 g), agar (10 g) and sucrose (200g) per liter. The pH of the media was adjusted at 5.7. This media was sterilized in an autoclave at 121°C and 15 psi for 20 minutes. After sterilization, NAA at concentration of 1, 2 and 3 mg/L and GA_3 at concentration of 10, 50 and 100 mg/L were added to the media. All Petri dishes were incubated at 27 ±2 ˚C for 1, 2 and 3 days.

Experimental set up

In vitro pollen viability, germination and pollen tube growth

Two factorial experiment using Randomized complete block design (RCBD) was carried out. The first involved two different sources of pollinators; "Jarvis" and "Ghanami Akhdar "as the first factor, whereas the second factor was the two plant hormones; NAA at concentration of 1, 2 and 3 mg/L and GA_3 at concentration of 10, 50 and 100 mg/L. Total fourteen treatments, each one contained four petri dishes (replicates), one slides per each petri dish was used as sampling unit at 24, 48 and 72 hours. Slides with germinated pollens were observed under 10× magnification objective of an Evos microscope paired with a Nikon DS – Ri1 camera. The photographs of the observed microscopic fields were taken at a resolution of 1920 × 1536 pixels. Five microscopic fields per slide were photographed.

Pollen viability was determined by staining pollen grain sample taken from each petri dish with 1% acetocarmine. Pollen grain from each microscopic field were counted and graded as viable and aborted in two classes. The pollen grains looking normal and stained red were considered viable, whereas poorly stained or colorless were record as nonviable. The pollen viability percentage was calculated by the following formula according to Iqbal et al. (2019).

Pollen viability (PV %) = (Viable pollen /Total pollen) × 100

Total number of pollen grain and the germinated pollen on each microscopic field were counted at 24, 48 and 72 hours. Pollen grain was considered as germinated if the length of pollen tube is larger than the diameter of the pollen grain. Pollen germination percentage was calculated by using the following formula:

Pollen germination (PG %) = (Germinated pollen /Total pollen) × 100

Absolute pollen viability or the effective germination capacity was calculated using the formula of Visser et al. (1977).

Absolute pollen viability (APV %) = (PV% × PG %) /100

For determination of pollen grain tube length (PTL), 10 pollen tubes were measured from each microscopic field. The length of pollen tubes was measured with a scale of 0.61 µM/ pixel with the help of the software, AxioVision version 4.2. 8.

Fruit set of "Medjool" date palm

The same treatments examined *in vivo* were conducted at pollination time (March, 13, 2021) by using the suspension medium used in aqueous pollination of date palm tree which consist of 2 gm pollen + 3 gm sugar +1L of distilled water. Fourteen uniform" Medjool" date palm trees, 20 years old were chosen to be pollinated. The fourteen treatments were applied on 42 bunches, 3 bunches (replicates) per treatment, 5 strands were marked to measure fruit set percentage at two different stages; initial fruit set 7 days after pollination (DAP) and fruit set at Khelal stage (July 11). The number of fruit and the number of flower scares were recorded and then the fruit set was expressed as the following equation:

Fruit set % = Number of fruit set in the strand / Total number of flower per strand x100.

Statistical analysis.

The MSTATC software was utilized to conduct an analysis of variance (ANOVA) on the experimental data. During the ANOVA, the impacts of various treatments and their interactions were evaluated. To compare means (P=0.05), the Fisher's protected LSD test was employed.

3. Results and discussion

Effect of pollen source (*in vitro*) on pollen viability, pollen germination (PG %), absolute pollen viability (APV %) and pollen tube length.

The effect of pollen source on viability (PV %), germination (PG %), absolute pollen viability (APV %) and pollen tube length (PTL) is shown in Fig.1. The results indicated were no significant differences between the two pollen sources;"Ghanami akhdar" and "Jarvis" in terms of PG% and APV% during the first 48 hours of incubation. but a slight significant superiority of "Jarvis" were noticed in PG and APV values at the third period which recorded 73.60% and 62.58%, respectively; however, "Ghanami akhdar" recorded 66.97% and 57.31% (Fig. 1.b and c). The other two study parameters (PV% and PTL) didn't show any significant differences between the two cultivars along the incubation period (Fig. 1.a and d). This close response of the two studied cultivars may be due to their high vigority and fertility, as they are among the most widely used cultivars for pollinating female date palm trees in the largest dates producing countries.

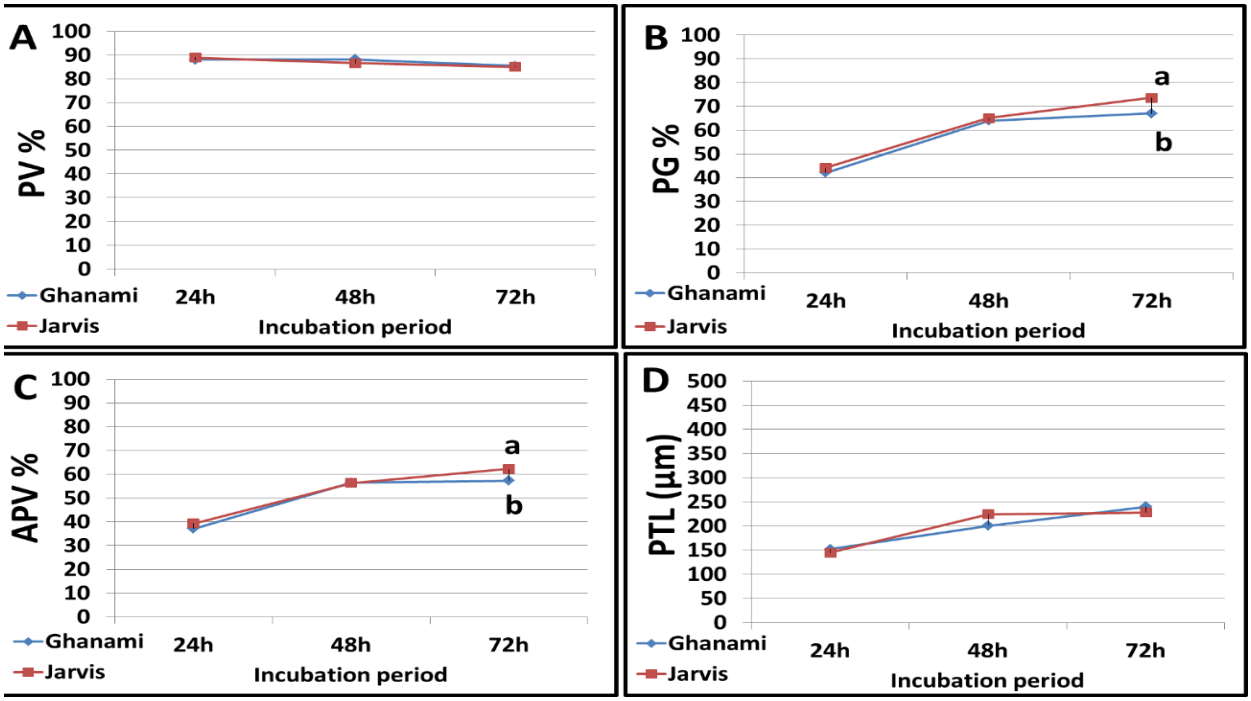

Figure 1: Effect of two pollen sources; "Ghanami akhdar" and "Jarvis" on pollen viability (PV %) (a), pollen germination (PG %) (b), absolute pollen viability (APV %) (c) and pollen tube length (PTL (µm)) (d) at three incubation periods; 24, 48 and 72 hours

Effect of plant hormones (*in vitro*)

Effect of GA3 (in vitro).

Exogenous hormones effects in this study indicated that GA3 at 100mg/L didn't present any inhibitory or enhancement effects at the end compared to control (Fig.2.a~d). However, it accelerated whole in vitro germination process in the early stage. The concentration of 100mg/L was a stimulus to germination, even if it was partially, and it itself was without effect also in other cases, especially in the last stages. There was also inhibition of the germination process by the concentrations of 10 and 50mg/L. Hence, the great importance of such experiments is determining the critical limit for the appropriate hormone concentration for a particular variety.

The use of concentrations 10 ~ 50 mg/L GA3 led to emergence of an inhibitory role of GA3 in the three germination characteristics (PG%, APV% and PTL) in "Ghanami akhdar". The level of 50 mg/L was critical in some cases in "Jarvis". Application of 100 mg/L showed a high effectiveness in accelerating the germination rate and growth rate of the pollen tube in the first 24 hours and did not have any inhibitory role in the later periods (Fig.2.a~d). The germination percentage after applying 100mg/L GA3 reached 66.88% compared to 43.77% in the control treatment during the first 24 hours of incubation (Table 1); and the absolute viability rate reached 59.59% compared to 39.91% (Table 2), while the length of the pollen tube reached 232.5µm compared to 133.9 µm (Table 2). It could be concluded that this treatment increased the speed of germination by 50% and the speed of growth of the pollen tubes by 75%.

According to the findings of Samiee Rad et al. (2016), the impact of growth regulators on the percentage of pollen germination was found to be inconsistent and influenced by the concentration of the material and the compounds present in the medium. The presence of GA3 and NAA without boric acid significantly hindered the germination of pollen. An ideal concentration for one genotype may be toxic for another genotype, and vice versa. According to Patel and Mankad (2014), plant growth regulators may contribute to the growth promoting effects that enhance early emergence of

pollen tubes. This leads us to wonder about what would have happened if the GA3 concentration rose to a higher level than 100mg/L? At least in this study, the concentration of 100mg/L can be considered as the lowest limit that can be built upon in the future with regard to date palm pollen. The higher concentration may lead to an increase in the germination rate and the length of the pollen tube at a constant rate, not only in the early stages of germination. Other factor could be vital; the relationship between medium components and applied hormones. It is known almost that pollen germination mediums were tested on the base of free plant hormones; application of plant hormones may lead to unbalance.

Effect of NAA (in vitro).

In general, it can be concluded from the results that all concentrations of NAA used in this study led to the inhibition of the germination process compared to the control, whether through the germination percentage and rate or through pollen tube growth (Fig.2.a~d). The absence of a definite or linear pattern associated with concentration makes it difficult to predict whether the inhibitory role of NAA is due to toxicity or insufficient concentration, as Samiee Rad et al. (2016) pointed out. According to Gao et al. (2019), the insufficient concentration can greatly decrease growth pollen grains germination and pollen tube elongation. However, excluding the anomalies in the results of the concentration of 2 mg/L, it can be considered that the pattern is mostly proportional to the inhibitory role of NAA directly with the increase in its concentration. Similar results were obtained by Gokbayrak and Engin (2017), who found NAA at 0.5, 1.0 and 2.5 mg L-1.did not induce pollen grain germination for three Vitis vinifera L. cultivars. Furthermore, Xue et al. (2008) and Sotomayor et al. (2012) observed that NAA inhibited pollen germination and tube growth of Chaohong peach and “Carmel” almond, respectively. NAA was found to hinder the peaches pollen germination, and along with the increase of concentration, the inhibition enhanced (Mi, et al., 2012). On the other hand, it was concluded that NAA is necessary to be used; the minimum concentration of 0.5 mg L-1 was sufficient for pollen germination of Tulipa greigii ‘Pinocchio’ (Akçal et al., 2016). The results of the current investigation, in addition to the previous studies may lead to unclear vision because the examined levels were close. However, in Syringa oblate (Liu et al., 2011) and Castanea mollissima (Shigang et al., 2017), it was reported that the optimum concentration of NAA for pollen germination and tube growth of was 10 and 15mg/L, respectively. The last two studies indicated that higher levels may be more effective.

Fig.2.d showed higher significant response of PTL to all GA_3 concentrations in the first 24 hours of incubation compared to control (133.94µm) with superiority of 100mg/L GA_3 (232.48µm). However, a slow significant PTL was shown by NAA treatments compared to GA_3 treatments but not with control. As in PG and APV, after 48 hours of incubation, PTL of control accelerated to the adequate limit (270.01 µm) that removes the significant differences with 100mg/L GA_3 (285.57µm) and 50mg/L GA_3 (241.85 µm), and significantly overcomes 10mg/L GA_3 (210.32µm). NAA treatments continued with significant slow of PTL compared to the control. With the end of the 72 hours of incubation, the longest PTL was recorded at treatment of 100mg/L GA_3 (295.53µm) and control (280.35µm).

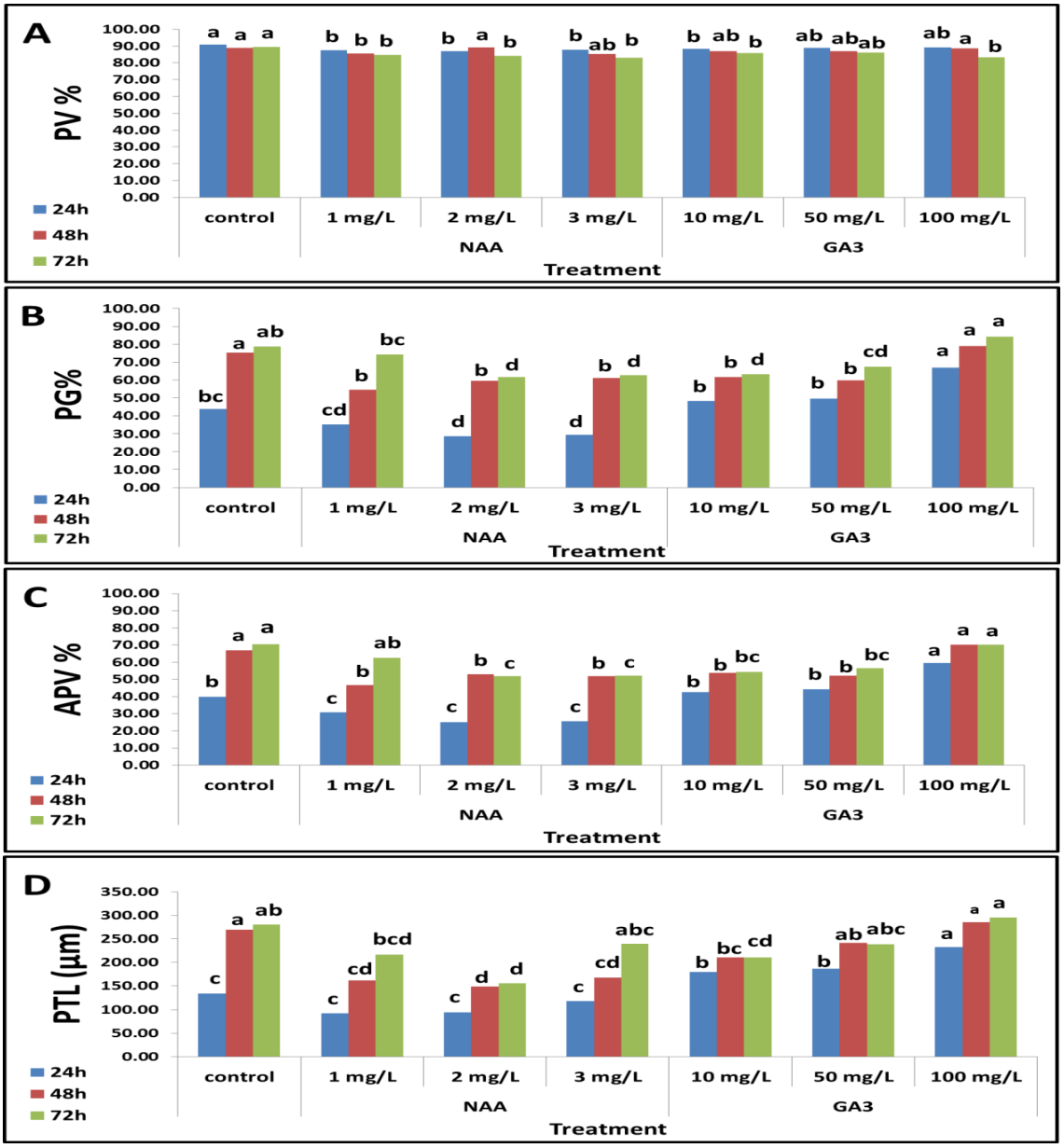

Figure 2: Effect of three concentrations of GA_3at 10, 50 and 100 mg/L and NAA at 1, 2 and 3 mg/L on pollen viability (PV%) **(a)**, pollen germination (PG %) **(b)**, absolute pollen viability (APV %) **(c)** and pollen tube length (PTL (µm)) **(d)** at three incubation periods; 24, 48 and 72 hours.

Effect of interaction between pollen grain source and plant hormones (*in vitro*).

At the end incubation period (72 hours), PV of "Ghanami akhdar" was negatively affected with the increase in the concentration of NAA (Table 1). PV decreased to 83.39% and 83.27% by 2mg/L NAA and 3mg/L NAA, respectively, while all concentrations of NAA negatively affected PV of "Jarvis" without exception. PV of "Ghanami akhdar" was significantly affected by adding 100 mg/L GA_3 (81.64%) compared to control (89.19%). The negative effect of GA_3 was shown at the concentration of 50mg/L (83.58%) in the case of "Jarvis" (Table 1).

The higher concentration (100 mg/L) of GA_3 showed a high significant ability to accelerate PG in both cultivars after 24 hours of incubation (Table 1). After 72 hours of incubation, "Ghanami akhdar" showed a higher significant response in PG% with control and 100mg/L GA_3 treatments;

75.15% and 81.62% respectively. Other two concentrations of GA_3 and all NAA concentrations had a significant negative effect compared to the control. In contrast, "Jarvis" showed a different behavior with wider response involved beside control treatment (82.41%). The two higher concentrations of GA_3; 100mg/L GA_3 (86.94%) and 50mg/L GA_3 (75.93%) in addition to 1mg/L NAA (79.13%) were the best significantly.

Table1: Interactive effect of NAA and GA_3 on *in vitro* pollen viability and pollen germination of two date palm pollen sources

Treatment	Pollen viability (PV%)			Pollen germination (PG %)		
	24h	48h	72h	24h	48h	72h
Ghanami Akhdar						
Control	89.30 $^{a-c}$	89.33 $^{a-c}$	89.19 ^{ab}	40.24^{c-f}	70.82^{a-c}	75.15^{a-e}
NAA 1 mg/L	86.40 ^{c}	85.79 $^{b-d}$	86.58 $^{a-d}$	40.81^{c-e}	60.68^{c-f}	69.28^{b-f}
2 mg/L	86.87 ^{bc}	88.83 $^{a-d}$	83.39 ^{cd}	30.86^{e-g}	68.16^{a-c}	64.64^{d-f}
3 mg/L	87.75 ^{bc}	85.44 ^{cd}	83.27 ^{d}	20.12^{g}	58.27^{c-f}	61.87^{ef}
GA_3 10 mg/L	88.10 ^{bc}	88.56 $^{a-d}$	85.37 $^{a-d}$	43.61^{c-e}	57.79^{c-f}	57.37^{f}
50 mg/L	89.92 $^{a-c}$	89.18 $^{a-c}$	88.50 $^{a-c}$	49.31^{cd}	54.41^{d-f}	58.86^{f}
100 mg/L	88.15 ^{bc}	90.16 ^{a}	81.64 ^{d}	69.53^{a}	77.23^{ab}	81.62^{a-c}
Jarvis						
Control	92.49 ^{a}	88.24 $^{a-d}$	90.00 ^{a}	47.30^{cd}	79.94^{a}	82.41^{ab}
NAA 1 mg/L	88.73 ^{bc}	85.54 $^{b-d}$	82.57 ^{d}	29.59^{e-g}	48.35^{f}	79.13^{a-c}
2 mg/L	86.96 ^{bc}	89.71 ^{ab}	84.64 $^{b-d}$	26.42^{fg}	50.80^{ef}	58.47^{f}
3 mg/L	87.92 ^{bc}	85.14 ^{cd}	83.02 ^{d}	38.44^{d-f}	64.05^{b-e}	63.44^{d-f}
GA_3 10 mg/L	88.43 ^{bc}	85.56 $^{b-d}$	86.05 $^{a-d}$	53.03^{bc}	65.81^{b-d}	68.90^{c-f}
50 mg/L	87.89 ^{bc}	84.65 ^{d}	83.58 ^{cd}	49.67^{cd}	65.50^{b-d}	75.93^{a-d}
100 mg/L	90.12 ^{ab}	87.18 $^{a-d}$	84.86 $^{a-d}$	64.23^{ab}	81.03^{a}	86.94^{a}

Values followed by the different letter(s) in a column are significantly different at p = 0.05 according to Least Significant Difference (LSD).

After 24 hours, APV looked like the trend in PG but with one difference, the absence the high significance of combination of 50mg/L GA_3 with "Ghanami akhdar". APV% was accelerated by100mg/L GA_3 treatments to be 61.27% compared to 35.99% by control in "Ghanami akhdar" and 57.91% compared to 43.83% in case of "Jarvis". Finally, 100mg/L GA_3 treatments recorded the highest APV% values in "Jarvis"(73.69%) and "Ghanami akhdar" (66.71%) without significant differences compared to control treatments in both cultivars that recorded 74.18% and 67.00%, respectively (Table 2).

It is clear from table 2 that GA_3 concentrations showed a considerable ability to accelerate the elongation of the pollen tubes in the first 24 hours. It increased with increasing concentration to reach 260.95µm in "Ghanami akhdar" and 204.00µm in "Jarvis" at concentration 100mg/L compared to 92.13µm and 175.76µm at the control treatments in both cultivars (Table 2), respectively. Results at 72 hours indicated that most treatments had a negative effect on PTL compared to control treatments except in case of 100mg/L GA_3 and 3mg/L NAA in "Ghanami akhdar" and 50 and 100mg/L GA_3 in case of "Jarvis".

Table 2: Interactive effect of different levels of NAA and GA_3 on *in vitro* absolute pollen viability and pollen tube length of two date palm pollen sources.

Treatment	Absolute pollen viability (APV) %			Pollen tube length (PTL (µm)		
	24h	48h	72h	24h	48h	72h
Ghanami Akhdar						
Control	35.99^{c-e}	63.26^{ab}	67.00^{ab}	92.13^{ef}	240.00^{a-d}	279.97^{ab}
1 mg/L NAA	35.29^{c-f}	52.21^{b-e}	60.04^{b-e}	125.79^{de}	164.20^{d-g}	216.03^{b-d}
2 mg/L NAA	26.79^{e-g}	60.59^{a-c}	54.25^{c-e}	93.97^{ef}	133.60^{fg}	148.97^{d}
3 mg/L NAA	17.64^{g}	49.76^{c-e}	51.73^{e}	100.96^{ef}	103.32^{g}	264.46^{ab}
10 mg/L GA_3	38.36^{c-e}	51.14^{b-e}	49.18^{e}	193.89^{bc}	220.75^{c-e}	215.85^{b-d}
50 mg/L GA_3	44.51^{cd}	48.44^{c-e}	52.21^{e}	195.87^{bc}	230.79^{b-e}	229.28^{b-d}
100 mg/L GA_3	61.27^{a}	69.61^{a}	66.71^{a-c}	260.95^{a}	310.75^{a}	323.74^{a}
Jarvis						
Control	43.83^{cd}	70.56^{a}	74.18^{a}	175.76^{b-d}	300.02^{ab}	280.72^{ab}
1 mg/L NAA	26.24^{e-g}	41.38^{e}	65.29^{a-d}	58.22^{f}	160.42^{e-g}	216.92^{b-d}
2 mg/L NAA	23.15^{fg}	45.61^{de}	49.37^{e}	94.60^{ef}	164.39^{d-g}	163.66^{cd}
3 mg/L NAA	33.79^{d-f}	54.25^{b-d}	52.85^{de}	134.23^{c-e}	232.38^{b-e}	214.26^{b-d}
10 mg/L GA_3	46.78^{bc}	56.24^{b-d}	59.37^{b-e}	165.81^{b-d}	199.90^{c-f}	204.99^{b-d}
50 mg/L GA_3	43.68^{cd}	55.63^{b-d}	60.83^{b-e}	175.88^{b-d}	252.91^{a-c}	248.08^{a-c}
100 mg/L GA_3	57.91^{ab}	70.92^{a}	73.69^{a}	204.00^{ab}	260.39^{a-c}	267.32^{ab}

Values followed by the different letter(s) in a column are significantly different at p = 0.05 according to Least Significant Difference (LSD).

3.4 Effect of NAA and GA_3 and pollen source on fruit set of "Medjool" date palm

In this field study, it appears from (Fig.3.a) that "Ghanami akhdar" has clearly and significantly outperformed over "Jarvis" in the percentage of set fruits in both growth stages; 7DAP and 120DAP (Khelal stage), where the percentage in "Ghanami akhdar" was 88.17% and 43.26% in both stages, respectively, compared to 85.07% and 39.95% in "Jarvis", respectively. Which may be due to the stronger metazinic effect of "Ghanami akhdar". The variations in genetic heritage and growth circumstances could also contribute to the differences between the two date palm pollinators. According to many studies, fruit set in date palm is one of the characteristics associated with the metazinic effect of the pollen source, (Sarrwy et al. (2014), Hafiz et al. (2014), Omaima et al., (2015), Salomon-Torres et al. (2017), Shahid et al. (2017), Mohammadi et al. (2017), Iqbal et al., (2019) and El-Hamady, et al. (2010).

The current study findings indicated that the general genotype effect were absent *in vitro* except in case of slightly superiority of "Jarvis" over "Ghanami akhdar" started just at the end of incubation period with related only to PG% and APV%, but genotype effects appeared clearly *in vivo* with superiority of "Ghanami akhdar" over than "Jarvis". The absence of this influence in most parameters *in vitro* and their appearance *in vivo* may be attributed to the higher metazenic effect of "Ghanami akhdar" pollen on characteristics of the tested female date palm which happened due to pollen-pistil reaction as (Swingle, 1928). According to Patel and Mankad (2014), Pollen's germination efficiency could be critical not just in fruit set but also in flower-flower and flower-pollinator interactions. On the other hand, this discrepancy between *in vitro* and *in vitro* may show a relative deficiency in the method of *in vitro* and its ability to simulate the real conditions in the field, and may make it a somewhat invalid method, especially in plants in which the phenomenon

of metazinia is a limited factor such as date palm and may be this is what Read et al. (1993) meant when he indicated that even if the conditions for pollen germination were ideal, they do not fully mimic *in vivo* growth.

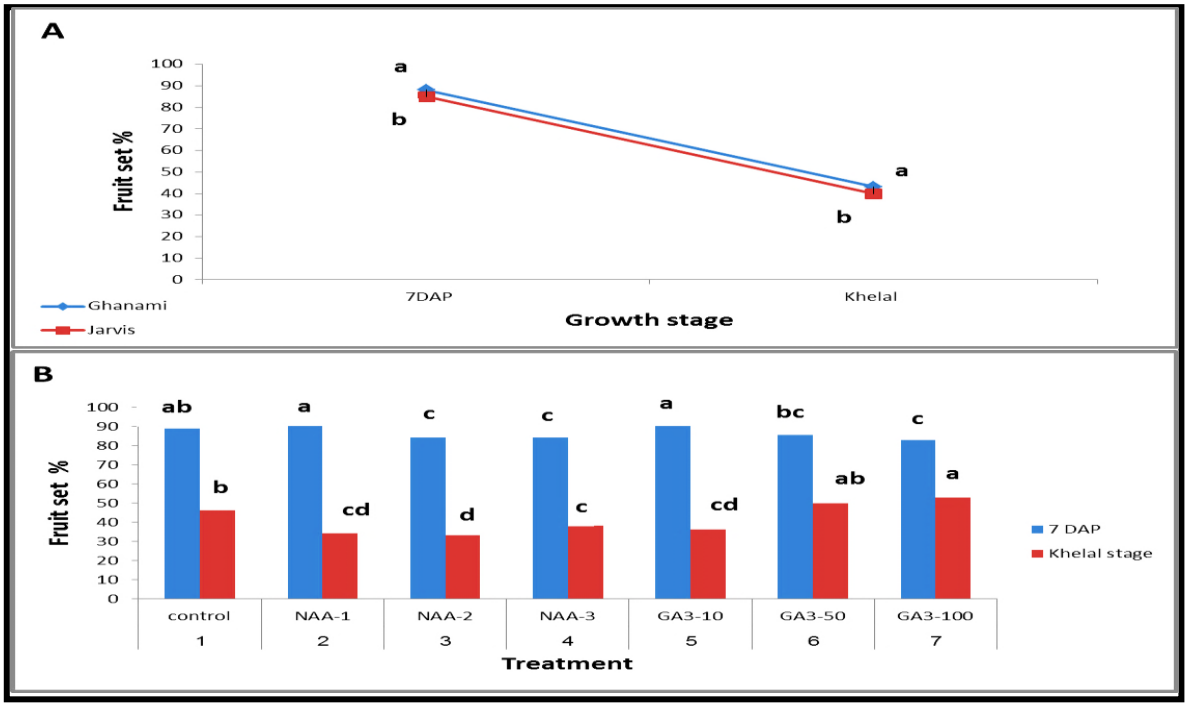

Figure 3: Effect of two pollen grain sources; "Ghanami Akhdar" and "Jarvis" **(a)** as well as NAA at 1, 2 and 3mg/L and GA_3 at 10, 50 and 100mg/L on fruit set % of "Medjool" date palm at two growth stages; 7DAP and Khelal **(b)**.

Looking in some detail at the results of this experiment, the general effect of both genotypes may not reflect the reality. "Jarvis" is correct that it lags behind "Ghanami akhdar", but it had a better response to GA_3, and the reason for its lower general effect is its greater sensitivity to NAA (Table 2), and it may be the most appropriate to compare the performance of the two cultivars with regard to the metazenic effect of pollen is the adoption of the results of the two control treatments for both cultivars, which showed superiority to "Ghanami akhdar" in the early stages of setting fruits, but they showed clear equivalence at Khelal stage.

Table 3: Interactive effect of different levels of NAA and GA_3 and two date palm pollen sources on fruit set % of "Medjool" at two growth stages

Treatment		Ghanami Akhdar		Jarvis	
		7 DAP	Khelal	7 DAP	Khelal
Control		93.52 ^{a}	45.57^{bc}	84.05^{d-f}	46.94^{bc}
NAA	1 mg/L	91.78 ^{ab}	37.34^{de}	88.36^{b-d}	31.49^{ef}
	2 mg/L	86.08^{c-f}	38.21^{d}	82.12^{ef}	28.06^{f}
	3 mg/L	86.91^{b-e}	44.58^{c}	81.77^{f}	31.95^{ef}
GA_3	10 mg/L	91.00 $^{a-c}$	41.67^{cd}	89.71 $^{a-c}$	30.96^{f}
	50 mg/L	84.55^{d-f}	44.44^{c}	86.55^{c-f}	55.41 ^{a}
	100 mg/L	86.24^{ef}	50.99 ^{ab}	84.90^{ef}	54.84 ^{a}

Values followed by the different letter(s) in a column are significantly different at p = 0.05 according to Least Significant Difference (LSD).

With regard to the effect of hormone, there were clear differences in the results of the fruit set % between the early stage and the late stage of growth has been shown (Fig. 3b). The control treatment (88.78%) and the lowest concentration of both GA_3 and NAA (90.36% and 90.07%, respectively) was significantly superior to the rest of the treatments after 7 days of pollination. The results were different in the Khelal stage with the superiority of the two treatments of the highest concentration of GA_3 on the rest of the treatments, where 100mg/L GA_3 recorded 52.91% and 50 mg/L GA_3 treatment 49.93% compared to 46.25% for the control. Auxins and gibberellins are commonly utilized to regulate fruit abscission and enhance the fruit's quality from the time of fruit set to ripening and eventual delivery to the consumer (Suman et al 2017). De Jong et al., (2009) suggested that the levels of AUX and GA genes in ovules are increased After pollination, leading to the activation of AUX and GA response genes. Consequently, these response genes will initiate the growth and development of fruits by controlling both cell division and cell expansion.

In our current investigation, the lack of effect of lower concentrations of both hormones on the percentage of setting fruits in the early stages after pollination may be due to their insufficiency to reach the toxicity level or to the extent that an imbalance occurs in the endogenous hormonal balance and vice versa with regard to the higher concentrations of them (Samiee Rad et al., 2016). Endogenous GA3 at a definite level was a prerequisite for the successful germination of pollen grains. All NAA concentrations significantly reduced the fruit setting percentage, while it was increased by 50~100 mg/l GA_3. Ozga and Reinecke (2003) noted that the hormonal balance fluctuates throughout various stages of fruit development, whereas a single hormone can serve distinct roles at each stage.

Shahsavar and Shahhosseini (2021) found that pollen grains containing higher amounts of gibberellin during pollination on "Piarom" dates can trigger more effective fertilization followed by seed production and larger amount of gibberellin in the fruit as a result. The utilization of exogenous GA3 + diphenylurea + NAA during full bloom has demonstrated its effectiveness in enhancing fruit set by increasing the quantity of pollen tubes that successfully reached the ovaries of pollinated flowers (Williams and Flook, 1980). Al-Samaraie and Al-Falahy (2020) determined that GA3 has a beneficial impact on decreasing the fruit drop percentage and enhancing the weight of the "Braim" date palm fruits. This positive effect can be attributed to the increased rate of assimilation materials moving towards the fruits, resulting in an increase in the overall weight of the fruit bunch. The current study showed that NAA treatments led to a decrease in the percentage of fruit setting as the concentration increased and with a regular behavior during the two growth stages. Fruit set % was about 37~44% by "Ghanami akhdar" pollen and 28~31% by "Jarvis". NAA is commonly used as chemical thinner at petal fall and earlier phenological stages of apple fruitlets, and it is effective at low rates (Wertheim, 2000), it is almost inhibitor at the applied concentrations by hindering most of study parameters *in vitro*. Similar behavior appeared *in vivo*, NAA decreased fruit set percentage over 1mg/L 7 DAP and by all levels 120 DAP (Khelal stage) (Fig.2.a).

The interaction effect between the pollinator and the hormonal treatment is shown in (Table3). 7DAP, the percentage of fruit set in "Ghanami akhdar" significantly decreased at the higher two concentrations of each hormone compared to the control. The treatment 50mg/L GA_3 led to the lowest fruit set (84.55%). On other hand, the lowest concentration of NAA and 10 mg/L GA_3 contributed to a significant improvement in fruit set of "Jarvis" (88.36%and 89.71% respectively) compared with the control (84.05%); the highest percentage of fruit set was observed at concentration 10 mg/L GA_3 (89.71%). The treatment of 3mg/L NAA showed the lowest fruit set

(81.77%) at Khelal stage. The percentage of fruit set in "Ghanami akhdar" increased significantly at the concentration of 100 mg/L GA3 (50.99%) compared to the control treatment (45.57%).

CONCLUSION

It is concluded from this study that GA_3 at 100mg/L can improve date palm pollen germination. Application of this concentration upon pollination also led to the best results in term of fruit set at Khelal, the stage of full fruit size. These findings open the doors to investigate the interactions between indigenous and exogenous GA_3 in the future. This relationship was clear in term of NAA, same behavior *in vitro* and *in vivo* appeared. NAA at these concentrations can present an effective thinning method in "Medjool". On other hand, "Ghanami akhdar" can be considering as a recommendable pollinator for "Medjool". It is advisable to apply GA_3 at rate of 100mg/L to modified BK to accelerate date palm pollen germination and pollen tube growth.

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