

Effect of calcium nitrate and boric acid on pollen germination of some date palm male cultivars

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ABSTRACT

Date palm is one of the most important tropical fruits. It is a dioecious plant which needs artificial pollination for commercial fruit set. Pollen grains play an important role in the size, shape, weight of fruit and time of ripening, as metaxenia phenomenon. Favorable fruit production is related to quality of pollen and its compatibility with a certain female variety. The preparation of suitable medium is necessary for in vitro pollen germination of date palm cultivars. Existence of boron and calcium in medium are necessary for germination and pollen tube growth. In this experiment, the best concentration of boric acid and calcium nitrate was determined for pollen germination of 3 cultivars i.e. Jarvis, Fard and Beraem in a factorial based on completely randomized design, using Brewbaker and Kwack [5] modified medium. Medium containing 50 mgL⁻¹ boric acid and 100 mgL⁻¹ calcium nitrate, was the best for 'Beraem' pollen germination (85.47%). In this medium, the maximum pollen germination of 'Fard' cultivar was 64.49%. However 'Jarvis' pollen had highest germination (77.59%) in the same medium but with a concentration of 150 mgL⁻¹ calcium nitrate. Thus, the different concentrations of B and Ca elements were most effective on date palm pollen germination for the mentioned cultivars.

Keywords: *Phoenix dactylifera* L., Beraem, Fard, Jarvis, boric acid, calcium nitrate, pollen germination.

INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is a Subtropical fruit tree that played a major role in food security and job creation in some region of country. In Iran, date palm has the highest area under cultivation and yields [1]. Date palm is a perennial, monocotyledon and dioecious plant with cluster inflorescence that is covered by a sheath called spathe. It opens naturally for pollen shed and pollination. Hand pollination is essential for fruit set [28]. Size, shape, weight and time of date fruit ripening are affected by pollen grain sources which is known as metaxenia phenomena [7, 16]. The male inflorescences develop rather earlier than female blossoms. So collecting and storing pollens until prepare the female flowers maturity, are needed. Although date palms can be pollinated naturally by wind, but in commercial orchards pollination took place by hand which is very important in production management. Producers always use skilled workers for palm pollination [2]. The utilization of proper pollen to increase yield, reduce costs and increase revenue groves are important. Sometimes, producers are forced to use stored pollen for pollination from previous season, or even stored pollen is usually used in breeding programs [14]. So it should be stored in a good conditions. During this period, some factors such as temperature and relative humidity have a decisive role in pollen longevity [8]. Germination ability and creation pollen tube growth which

carry the male gametes, leading them toward the embryo sac, are sign of compatible pollination [21,23,24]. *In vitro* pollen germination is not quite the same as germinate pollen on the stigma surface. However, it is considered as an index of pollination and ovule fertilization [6, 20]. Pollen can be considered as germinated while it's length is at least equal to the diameter of a pollen grain. *In vitro* pollen germination test is a good method to determine germination percentage, however finding a suitable media for each cultivar is a main problem [12]. As natural conditions, every pollen of an individual male tree requires specific medium for germination [9]. Studying the pollen germination backs to the 1930s. In 1966, Furr and Anrykvaz found the highest *in vitro* pollen germination percentage in date palm with the basis of Brewbaker and Kwack (1964), modified medium. Pollen germination and pollen tube growth are regulated by transferring inorganic Ca^{2+} and K^{+} ions across the plasma membrane [26]. For *in vitro* pollen germination and pollen tube growth boron element (B) is one of the essential factors for [19]. According to Asif *et al* [2], boric acid at 100 mg l^{-1} had the highest effect on date pollen germination. According to FAO (2011), Iran has the fourth position for area under cultivation of date palm and the third position for its production. However, there is a little information about male palm cultivars. So, determination the best medium for *in vitro* pollen germination of 3 male palm cultivars is the main objective of the present study.

MATERIALS AND METHODS

This experiment was done on three male date palm cultivars i.e. Jarvisai ,Fard and Beraem in Date and Tropical Fruit Research Station of Saad Abad in Agriculture and Natural Resources of Busheher (Latitude : $51^{\circ}5'48''$ N., Longitude : $29^{\circ}23'1''$ E and 50 meters above sea level. These male cultivars are used traditionally for pollination of commercial date varieties. The male palm trees were twenty years old in the same horticultural conditions. In March of 2013, the five spaths of each male palm which opened naturally were sampled (Figure 1)



Figure (1) Beram male spathes (before and after cracking) in ambient conditions

Spathes were transferred to a room with 25-30 °C temperature and 30-40% relative humidity. Pollen grains were taken traditionally by shaking the strands on a paper sheet. A mass pollen of each cultivar was dried at room temperature with ambient light.

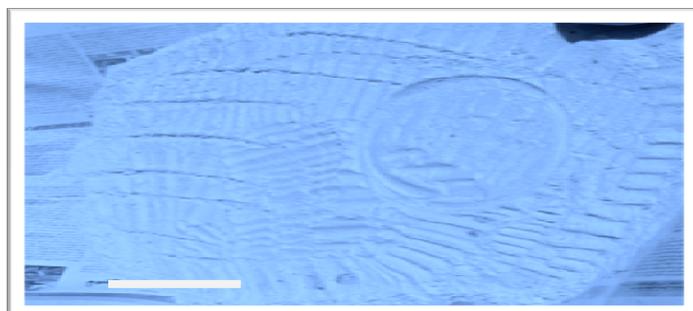


Figure (2) Drying pollen grains in room conditions

In order to find out the best concentrations of calcium nitrate (100, 150, 200 mg l⁻¹) and boric acid (50 and 75 mg l⁻¹), this study was done in factorial based on completely randomized design with three replications. Different concentrations of Ca and B was added to Brewbaker and Kwack [5] medium (10% sucrose, 100 mg l⁻¹ potassium nitrate, 200 mg l⁻¹ magnesium sulfate and 1% agar) and then it was boiled up to 40 minutes. For each replication, 10 ml of culture medium was poured in 90 mm diameter Petri dishes. After cooling, pollen grains were dusted with a very soft brush. All petri dishes were incubated for a bout 24 hours at 28°C. The number of germinated and total pollen grains were counted in each visible area under the optical microscope Nikon Ecllips 90i, using 40 magnification. During the observation, a pollen grain was considered germinated when the pollen tube length was equal to or greater than the diameter of the pollen grain [11].

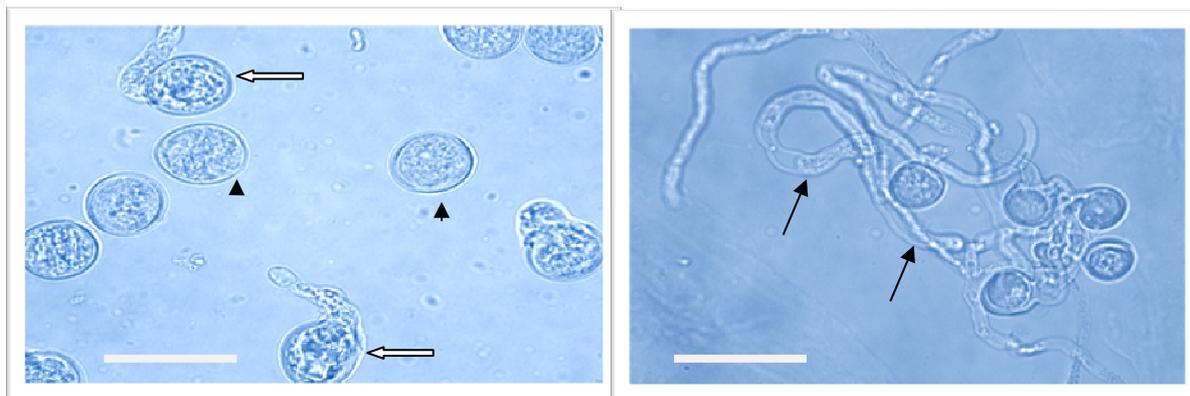


Figure (3) Germinated pollen and pollen tube growth in media (solid , hollow and arrowhead show pollen tube, germinated pollen and non germinated pollen grains respectively).

Percent pollen germination was obtained by dividing the number of germinated pollen on total pollen grains number multiple by 100, [10,25]. Statistical analysis was performed based on MSTATC software and means were compared by LSD at P ≤ 0.01 and 0.05 level.

RESULTS

Analysis of variance of pollen germination for three cultivars is shown in table (1).

Table 1: ANOVA analysis of pollen germination in three date palm male cultivars.

Source of variations	Df	Ms	F	Prob
Calcium nitrate	2	22633.371 **	321.3886	0.000
Boric acid	1	56.2923 **	461.244	0.000
Calcium nitrate × boric acid	2	426.680 **	52.0740	0.000
Cultivar	2	24.166*	2.9493	0.065
Calcium nitrate × cultivar	4	22.0692 **	180.829	0.000
cultivar × boric acid	2	1067.229 **	130.2495	0.000
Calcium nitrate × cultivar × boric acid	4	49.4231 **	404.959	0.000
Error	36	8.194		
c.v			4.71	

Significant level: **1% and *5%

Effects of Boric acid and calcium nitrate on pollen germination are shown in table (2).

Table (2) Effects of boric acid and calcium nitrate concentrations on pollen germination of three date palm cultivars.

chemicals cultivars	Boric acid (mg l ⁻¹)	Calcium nitrate (mg l ⁻¹)		
		100	150	200
Fard	50	64.49 ^d	63.06 ^d	30.26 ^b
	75	35.35 ^e	35.35 ^e	54.76 ^f
Beraem	50	85.47 ^a	70.85 ^c	73.69 ^{bc}
	75	62.56 ^d	65.76 ^d	64.96 ^d
Jarvais	50	54.23 ^f	74.02 ^{bc}	57.57 ^{cd}
	75	60.87 ^{de}	63.44 ^d	77.59 ^b

a: mean percentage of germination at 1% LSD value = 4.740

According to results, in Brewbaker and Kwack modified medium containing 50 mg^l⁻¹ boric acid and 100 mg^l⁻¹ calcium nitrate, the highest pollen germination (85.47%) was related to 'Beraem'. On the other hand, the maximum pollen germination (64.49%) of 'Fard' cultivar was also obtained in the same media. 'Jarvais' with 77.59% germination arranged between 'Fard' and 'Beraem' cultivars. Results showed that each cultivar has specific pollen germination that is related to its genetic makeup, medium components and nutrient concentrations. Although, boric acid and calcium nitrate are necessary for pollen germination, their high levels reduced pollen germination percentages in Fard and Beraem cultivars, however it was not the case for cv. Jarvais.

DISCUSSION

Based on the results, the average percentage of pollen germination of tested varieties were greater than 64%. As Brewbaker and Kwack [4,5] and Mortazavi *et al.* [14, 15] reported, adding boric acid and calcium nitrate to pollen culture media were necessary for convenient date palm pollen germination. Application of boric acid at 50 mg^l⁻¹ and calcium nitrate in 100 mg^l⁻¹ concentrations in Brewbaker and Kwack culture media, could significantly affect pollen germination percentage of Fard and Beraem male cultivars. However the same media, but with 75 mg^l⁻¹ boric acid and 200 mg^l⁻¹ calcium nitrate was the best for Jarvais cultivar, however it wasn't significantly different with the same medium with 50 mg^l⁻¹ boric acid and 150 mg^l⁻¹ calcium nitrate. The results of this experiment are in the same line with Mortazavi *et al.*, [15] report about boric acid, but it was quite different about calcium nitrate. Using 50 mg^l⁻¹ boric acid was the best, but its higher concentration caused lower germination which is in the same line with Mortazavi *et al.*, [15].

Several reports about the effects of boron element on pollen germination and pollen tube growth have been reported [3,27]. In natural conditions, bore is supplied by stigma and style. This element facilitates sucrose uptake and it plays an important role in production of protein at pollen tube [19]. Bore is required for cellular transporting, carbohydrates and indole acetic acid metabolism and has an important role in membrane transported systems [24].

Calcium nitrate is also important for date palm pollen *in vitro* germination. Application of certain concentration of calcium nitrate in culture media increased the pollen germination rates, but its higher concentration could decrease it [15]. Beraem and Fard male palm cultivars had shown the highest pollen germination at culture medium with 100 mg^l⁻¹ calcium nitrate, whereas the maximum pollen germination of Jarvais cultivar was observed at 150 mg^l⁻¹ calcium nitrate. Ca²⁺ plays an important role in plant growth and development cycle such as pollen tube growth and fertilization. Pollen germination requires calcium, and its tube growth in pistil tissue depends on the presence of calcium [13]. Calcium also plays a role in determining the direction of pollen tube growth. [17].

CONCLUSION

Calcium and bore elements are important for pollen germination in date palm. The concentration of these elements is related to the kind of the date palm male cultivar.

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