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Effect of Natural Lipid on Pollen Germination and Pollen Tube Growth on Loquat

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Abstract: The present study evaluated the effects of a natural lipid, Lysophosphatidylethanolamine, (L) on the eight-year old loquat (Sayda) orchard located on coastal Mediterranean region in Hatay. The applications were conducted prior to bloom (Treatment I) and the same branches were treated again after two weeks from Treatment I (Treatment I+II) while the control branches were treated with water. After two weeks from the second application, buds were sampled from all experimental units and pollen viability test were conducted by standard methods. Randomly sampled buds from all treatments were kept on a room temperature to obtain pollen for both varieties and treatments. The pollen were germinated on media having various concentration of sucrose (5, 10, 15 and 20%) and incubated at 10, 15 and 20°C. The Petri dishes were placed on -20°C after 3, 6, or 9 h incubation to consecutively determine their germination rates and pollen tube growths under a light microscope. The results clearly demonstrated that L application before bloom significantly improves pollen germination rate and pollen tube growth although it has no effect on pollen viability. Among the incubation treatments, 20°C gave the most favorable results for both pollen germination rate and pollen tube growth. The highest pollen germination rates were recovered from 20% sucrose concentration while the longest pollen tubes were at 15%. The positive effects of L may be very advantageous for loquat growing especially for the early fruit production regions.

Key words: Lysophosphatidylethanolamine, *Eriobotrya japonica*, pollination, fruit set, Mediterranean, pollen viability

INTRODUCTION

Loquat is an evergreen species from the Rosaceae family, which is grown in subtropical climatic conditions. Unlike other subtropical species, the loquat flowering period is in winter and fruits can be harvested 152-189 days after flowering (Polat *et al*., 2004,2005; Durgac *et al*., 2006). Therefore, loquat fruits can be found for higher prices since there is no fruit on the market when they go in spring months, with the exception of strawberries, some plum and almond varieties which are consumed as immature fruits (Polat, 1996). The total loquat production of Turkey was 12000 tons in 2003 (Anonymous, 2003). The Turkish Mediterranean region has the most suitable ecological conditions for growing this fruit. In 2003, 97% of total loquat production of Turkey was from the costal line of Mediterranean region. On the other hand, loquat production increased by 300% in this region during the last decade.

Loquat flower and fruit development cycle extend during the winter when the average temperature is about 15°C. The tree is rustic but the fruits can be damaged by wind and frost; temperatures below -2°C damage open flowers and small fruits, set fruits can be damaged at a temperature of -1°C. The main problem facing the expansion of this crop in Turkey is the risk of frost and land availability, due to competition with other crops, including citrus, as well as greenhouse for the production of both ornamental and vegetable plants (Caballero and Fernández, 2003).

Loquat is normally pollinated by insects particularly by bees however, due to unfavorable weather conditions during the flowering season bee actives and as a result pollination and fruit set were negatively affected (Eti *et al*., 1990). In extreme conditions bee activities can be severely declined or stopped by cold weather. Frost or cold exposed flowers organs can be damaged or killed. Viability and germination of pollen can also be affected by

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cold (Mellenthin *et al*., 1972; Welsh and Klatt, 1971). In addition frequent rains in this period may cause washing of anthers. For all of these reasons improving pollen germination, pollination and fruit set is highly critical for profitable loquat production in the Mediterranean region in Turkey.

Researchers have been studied the effect of different compounds to improve pollen viability, pollen germination rate and pollen tube growth. For this purpose different types and concentrations of sugars (Singh and Randhawa, 1961), magnesium (Zilenski and Ölez, 1963), calcium nitrate (Calzoni *et al*., 1979), fluorine compounds, gibberellic acid (Chandler, 1957) have been tested. Results from these studies were variable and in some cases these chemicals were not accepted by consumers.

Lipids have been thought to play important roles in membrane structure and critical cellular functions particularly as mediators in signal transduction, cell activation and cell proliferation (Divecha and Irvine, 1995; Ryu *et al*., 1997). Also, recent studies have provided evidence that phospholipids particularly lysophosphatidylethanolamine (L) may act as plant growth regulators by accelerating ripening, retarding ethylene-promoted leaf senescence, softening of some of agrochemicals (Kowan 2006; Ozgen *et al*., 2005a,b; Ozgen, 2005b; Ozgen and Palta, 2002; Ryu *et al*., 1997). The goal of present study is to investigate effect of L application on pollen germination and pollen tube growth in loquat cv. Sayda.

MATERIALS AND METHODS

Experiment 1: Eight-year old loquat, variety Sayda, orchard on the Mediterranean costal region in Hatay, Turkey were used to perform L applications. In the orchard, three trees were randomly chosen. In each tree, three applications and three control branches were randomly picked from all directions of the trees. L (100 ppm) treatment (I) were sprayed on selected branches prior to bloom while the control branches were treated with water. The same branches were treated again after two weeks (Treatment I+II). Two weeks after the second application, buds were sampled from all experimental units. The pollen viability test was conducted on the pollens collected from these treatments by standard methods.

Experiment 2: Randomly sampled buds from all treatments were kept at a room temperature to obtain pollen from both varieties and treatments. The pollen were germinated on media having various concentration of sucrose (5, 10, 15 and 20%) and incubated at 10, 15 and 20°C. The petri dishes were placed at -20°C after 3, 6, or 9 h incubation to consecutively determine their germination rates and pollen tube growths under a light microscope. Observations were made on six different places for each Petri dish. In each region, to determine germination rate, germinated and total numbers of pollen were counted and the ratios were calculated. The length of the five germinated pollen tubes were measured and averaged to determine average pollen tube growth.

Analyses of variance were conducted for a factorial design by GLM procedure of SAS using a split plot where L treatment was whole-plot, incubation temperature was sub-plot and sucrose concentration was sub-sub-plot (SAS, 1990). For main effects, the means were separated by Tukey and using appropriate error terms at 5%. Although pollen germination data was $\sqrt{\text{arcsin}}$ transformed to improve normality, original data were used to present means.

RESULTS

The Analysis of Variance (ANOVA) for the pollen viability test indicated that Lysophosphatidylethanolamine (L) treatments did not affect the pollen viability (Table 1). There were some differences among the means of the two L treatments and two controls; however, these differences were found not to be significant.

The L treatments were found to be highly significant for both pollen germination rate and pollen tube growth at 3, 6 and 9 h (Table 2). Similarly, the Temperature (T) treatments were highly significant for both of the variable tested at all time treatments. However, TxL interaction gave differing patterns for the variables. The interactions were not significant for the pollen germination rate but significant for pollen tube growth at 6 and 9 h. The main factor of Sucrose (S), like L and T, were highly significant for all tests (Table 2). Complex two-and three-way interactions were recovered for the interaction involving S.

Table 1: Analyses of variance and means for Lysophosphatidylethanolamine (L) treatments for pollen viability test (%) on Sayda loquat variety. The L treatments were conducted prior to bloom (Treatment I) and the same branches were treated again after two weeks (Treatment I+II) while the control branches were treated with water

Source	Mean	Standard deviation
Treatment I	91.4	0.1
Control I	84.9	2.8
Treatment I + II	89.3	11.4
Control II	87.0	0.3
Mean	88.1	5.2
ANOVA	sd	Mean square
Treatments	3	10.1^{NS}
Error	4	24.1

^{NS} = Non Significant at 5%

Table 2: Analyses of variance for Lysophosphatidylethanolamine (L) treatments for pollen germination rate and pollen tube growth on Sayda loquat variety. The L treatments were conducted prior to bloom (Treatment I) and the same branches were treated again after two weeks (Treatment I+ II) while the control branches were treated with water. Pollen were germinated on media having various concentration of sucrose and incubated at several temperatures

		Pollen germination rate (%)			Pollen tube growth (μ)		
Source	df	3 h	6 h	9 h	3 h	6 h	9 h
Treatment (L)	2	1597.4**	654.0**	1201.5**	204.4**	443.6**	264.5**
Whole-plot error	15	32.9	27.6	19.7	8.6	26.9	24.2
Temperature (T)	2	3055.9**	2133.6**	1795.5**	29.3	695.2**	648.8**
T×L	4	91.2	23.9	79.4	30.3	65.4*	29.3*
Sub-plot error	30	38.3	33.6	60.7	13.8	4.9	10.0
Sucrose (S)	3	252.8**	318.2**	532.0**	1043.7**	1114.8**	2067.2**
S×L	6	85.6*	81.8**	41.1	11.8	107.8**	162.1**
S×T	6	118.7**	90.8**	36.4	112.4**	115.3**	168.7**
S×L×T	12	95.7**	43.8*	22.9	116.5**	79.3**	139.7**
Error	135	30.1	20.3	39.0	12.7	10.9	14.9

*, **Significant at 5 and 1%, respectively

Table 3: Mean comparisons for Lysophosphatidylethanolamine (L) treatments for pollen germination rate (%) on Sayda loquat variety. The L treatments were conducted prior to bloom (Treatment I) and the same branches were treated again after two weeks (Treatment I+II) while the control branches were treated with water. Pollen were germinated on media having various concentration of sucrose and incubated at several temperatures

	Incubation duration			
Source	3 h	6 h	9 h	Mean
L treatment				
I	88.8a[1]	92.5a	93.7a	91.6
I + II	77.1c	86.6c	87.1c	83.6
Control	84.2b	89.6b	88.8b	87.5
Incubation temperature (°C)				
10	73.6b	83.4c	84.4b	80.5
15	88.9a	91.3b	92.1a	90.8
20	87.6a	93.9a	93.2a	91.6
Sucrose concentration (%)				
5	81.1b	86.6c	85.8c	84.5
10	84.8a	90.4b	91.0b	88.7
15	81.0b	89.1b	90.0b	86.7
20	86.7a	92.0a	92.7a	90.5
Mean	83.4	89.5	89.9	87.6

[1]Mean comparisons were conducted at 5% by LSD and only valid within each main effect

Table 4: Mean comparisons for Lysophosphatidylethanolamine (L) treatments for pollen tube growth (μ) on Sayda loquat variety. The L treatments were conducted prior to bloom (Treatment I) and the same branches were treated again after two weeks (Treatment I+II) while the control branches were treated with water. Pollen were germinated on media having various concentration of sucrose and incubated at several temperatures

	Incubation duration			
Source	3 h	6 h	9 h	Mean
L treatment				
I	31.6b[1]	33.5a	39.4a	34.8
I + II	28.5b	28.6c	35.7b	30.9
Control	28.9b	30.9b	36.5b	32.1
Incubation temperature (°C)				
10	29.0a	27.9c	34.0c	30.3
15	29.9a	30.9b	37.7b	32.8
20	30.2a	34.1a	39.9a	34.8
Sucrose concentration (%)				
5	23.9c	24.8d	28.8d	25.8
10	28.7b	30.8c	36.9c	32.1
15	32.4a	35.3a	43.4a	37.0
20	33.7a	33.2b	39.7b	35.5
Mean	29.7	31.0	37.2	32.6

[1]Mean comparisons were conducted at 5% by LSD and only valid within each main effect

When the main factors for the pollen germination rates (%) were considered (Table 3) for the L treatments, Treatment I gave consistently higher pollen germination rates than both I+II and Control treatments. For the incubation temperature, 20°C was almost in the highest mean group. Similar pattern was observed for the sucrose concentration; 20% was always in the highest mean group.

For the pollen tube growth, the means and their separations were presented in Table 4. The highest mean group was recovered from Treatment I at 6 and 9 h; for the 3 h, the differences were not significant. Similar to pollen germination rate, 20°C was almost in the highest mean group. However, a different pattern was present for sucrose concentration. 15% sucrose concentration was always in the highest mean group.

DISCUSSION

Results of the present study suggested that L application before bloom significantly improves pollen germination rate in loquat cv. Sayda. Present results are consistent with other studies (Ozkan, 1991) where the incubation treatments at 20°C gave the most favorable results for both pollen germination rate and pollen tube growth. The highest pollen germination rate was recovered from 20% sucrose concentration while the longest pollen tubes were at 15%. The pollen viability was not affected by our treatments. Therefore, the differences obtained were caused by other factors but not by the pollen viability.

Synthetic and natural plant growth regulators are used in agriculture as management tools to improve crop performance and enhance quality. Whereas the biochemical mode of action of many of these plant growth regulators is poorly understood and in some cases unknown, most are classified as naturally occurring plant hormones. Phospholipid metabolism and signaling are closely linked to the mechanism of action of plant hormones (Ryu *et al*., 1997). In our experiment L may act

as a plant hormone such as auxins. However, more studies are needed to clarify such an effect on pollen germination and pollen tube growth. Until then, Lysophosphatidylethanolamine may be a good and natural choice for these purposes.

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