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Effects of Sucrose, Maltose, pH and Phloroglucinol on the Germination of Globe Artichoke Pollen *in Vitro*

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Summary

Effects of sucrose and maltose at the concentrations 0.5 and 1.0 M, availability of the phloroglucinol (PG) at the concentrations 0 and 10 µM, and three levels of pH (5, 6 and 7) in a basic medium containing 1.63 mM H_3BO_3, 1 mM KNO_3, 1.27 mM $Ca(NO_3)_2.4H_2O$ and 0.45 mM $CaCl_2$ on the pollen germination *in vitro* of globe artichoke (*Cynara scolymus* L.) were studied. A separate experiment for each type of sugar was carried out. Analyses of percentages for pollen germination showed that main effects of sugar concentrations and availability of PG in the media were significant only in the sucrose experiment where 1.0 M sucrose and 10 µM PG resulted in a germination of 49.8 and 38.9 %, respectively. The main effect of pH however was significant only in the maltose experiment where pH 5 resulted in 29.5 % germination while pH 7 gave only 15.3 %. Regarding the two factor interactions, statistical significance was found only for the combinations of sucrose concentrations x pH and PG x pH in the maltose experiment and sucrose concentrations x PG in the sucrose experiment. The interaction of all three factors was significant only in the maltose experiment where the highest pollen germination was 40.8 % for the combination of 0.5 M maltose x 10 µM PG x pH 6, whereas in the sucrose experiment interaction of the three factors was not significant and the highest pollen germination was 68.9 % for the combination of 1.0 M sucrose x 0 PG x pH 5. Bursting of the pollen tube as well as elongation of the tube in a spiral manner occurred in all the media tested. Overall, more favourable germination was obtained from the sucrose media to which addition of phloroglucinol at 10 µM concentration was useful. The factors influencing pollen germination of the globe artichoke and their relevance for its improvement were discussed.

Zusammenfassung

Einfluss von Saccharose, Maltose, pH und Phloroglucinol auf die *in vitro*-Pollenkeimung von Gemüse-Artischoken. Untersucht wurde, welchen Einfluss Saccharose und Maltose (Konzentrationen 0.5 und 1.0 M), die Zugabe von Phloroglucinol (PG, Konzentrationen 0 und 10 µM) und drei pH-Werte (5, 6 and 7), in einem Grundmedium mit 1.63 mM H_3BO_3, 1 mM KNO_3, 1.27 mM $Ca(NO_3)_2.4H_2O$ und 0.45 mM $CaCl_2$, auf die *in vitro*-Pollenkeimung von Gemüse-Artischoken (*Cynara scolymus* L.) haben. Für jeden Zuckertyp wurde ein separater Versuch durchgeführt. Auswertungen der Pollenkeimung zeigten, dass der Einfluss der Zuckerkonzentration und der PG-Zugabe nur in der Kombination, 1.0 M Saccharose und 10 µM PG mit 49.8 bzw. 38.9 % Keimung, signifikant war. Nur mit Maltose war bei einem pH von 5, mit 29.5 % Keimung (15.3 % bei pH 7) ein signifikanter Einfluss des pH-Wertes nachweisbar. Betrachtete man die Interaktionen zwischen jeweils zwei Faktoren, so wiesen nur die Kombinationen Zuckerkonzentration x pH und PG x pH im Maltose- sowie die Kombination Zucker-Konzentration x PG im Saccharose-Versuch statistische Unterschiede auf. Die Interaktion zwischen allen drei Faktoren war nur im Maltose-Versuch in der Kombination von 0.5 M Maltose x 10 µM PG x pH 6 und der damit erzielten Pollenkeimung von 40.8 % signifikant. Im Saccharose-Versuch war die Pollenkeimung bei der Kombination 1.0 M Saccharose x 0 PG x pH 5 mit 68.94% zwar am größten, doch war dieser Wert nicht statistisch gesichert. Geplatzte oder spiralig wachsende Pollenschläuche traten bei allen getesteten Medien auf. Alles in allem war das Saccharose-haltige Medium, dem Phloroglucinol in einer Konzentration von 10 µM zugefügt war, für die Pollenkeimung am geeignetsten. Die Faktoren, die die Pollenkeimung beeinflussen, und die Möglichkeiten ihrer Optimierung wurden diskutiert.

Key words. *Cynara scolymus* L. – flavonol – phenolic compound – bursting of pollen tube – maltose

Introduction

Globe artichoke (*Cynara scolymus* L.), a member of the *Asteraceae* (*Compositae*), is native to the Mediterranean region where approximately 90 % of the world production is realized. Due to its high nutritious and pharmaceutical value, a special significance is attributed to the globe artichoke (ABAK 1987; NONECKE 1989; ANONYMOUS 2004)

Conventionally, globe artichoke is propagated clonally due to its highly heterozygous nature, but asexual re-

production of the crop is not free of problems. In time, cultivars may loose their typical characteristics because of somatic mutations and the cultivars may become susceptible to attacks from pests and diseases. Therefore, propagation via seeds produced especially through F_1 hybrid breeding programmes has been favoured and increasingly practiced (PECAUT 1993).

Commercial production of F1 seeds requires all aspects of the reproductive processes, i.e., production of pollen, pollination, fertilization, and development of embryos and seeds, to take place to the full extent. Pollen viability is an important characteristic in plants propagated sexually especially in the consideration for selection of male parents suitable for F_1-hybrid breeding programmes (FRANKEL and GALUN 1977; KALLOO and CHOWDHURY 1992).

Reproductive mechanisms of artichoke have been studied and certain difficulties are experienced among which pollen activity, i.e., production, viability, and functionality of the pollen, may pose problems (PECAUT 1993). In a study, using the local cultivar 'Sakiz', where pollen viability tests were carried out via pollen germination *in vitro* using various media, none of the pollen germinated (KELES 1998), whereas in another report using different cultivars, artichoke pollen germination reached up to 19.6 % (BERNAL et al. 2000). To the best of our knowledge, these are the only reports available on the pollen germination *in vitro* of globe artichoke.

Genotypic and environmental factors as well as media characteristics are effective in the induction of pollen germination *in vitro*. While in some species a simple sucrose solution can be satisfactory, even sophisticated media constituents may fail to induce pollen germination in the recalcitrant ones. According to TANAKA et al. (2000), the pollen from certain mutant genotypes with white anthers lacking internal flavonoids is unable to germinate *in vitro*. In such cases, effects of kaempferol and quercetin, flavonol aglycones, have been well established in the induction of incomplete pollen to germinate (GROOT and DE RUITER 1993; NAPOLI et al. 1999). Both flavonols are secondary metabolites widely distributed in pollen throughout the plant kingdom (TAYLOR and HEPLER 1997). Phloroglucinol, a simple phenol and although not as common, is known to be effective in some physiological processes such as in the induction of adventitious root formation in *Rubus in vitro* (JAMES 1979; MODGIL et al. 1999). On the other hand its effect on pollen germination *in vitro* has not been tested and may be of significance similar to the flavonols mentioned above. Pollen of the globe artichoke of the local cultivars of 'Sakiz' and 'Bayrampasa' is creamy white and may be suffering from the same problems as the mutants and it was considered that the induction of the pollen to germinate *in vitro* can be achieved via the use of the phenolic phloroglucinol.

Also the source of sugar and its concentrations are effective in pollen germination *in vitro*. While sucrose is the most commonly used source of energy it also provides the osmoticum necessary. In the isolated microspore culture experiments, where sucrose was replaced with maltose, haploid induction was higher and increasing number of studies are carried out to determine the effectiveness of maltose in this line of research (NAGELI et al. 1999; GUO and PULLI 2000). Therefore, maltose in place sucrose may have a similar positive effect on the *in vitro* pollen germination of species recalcitrant in this respect. Also pH, one of the important factors affecting physiological processes, may play a significant role in the pollen germination if adjusted correctly in the media.

The above considerations, i.e., not only to obtain pollen germination in a local variety of the globe artichoke, but also determination of the effects of the simple phenolic, i.e., phloroglucinol, on the induction of pollen germination stimulated the realization of the present work testing also the effects of pH, and type and concentration of sugars.

Materials and Methods

The experiment was carried out in the summer of 2003 using the globe artichoke (*Cynara scolymus* L.) cultivar 'Sakiz' growing in the experimental fields of the Department of Horticulture, Faculty of Agriculture, Trakya University, Turkey. The plants grew on silty clay loam soil and were irrigated several times. The flowers were available for harvest towards the end of August 2003. Some of the flowers were not harvested and left on the plants to obtain fully opened flowers, which were cut and immediately taken to the laboratory for further manipulation. A modification of the hanging drop method (PRESSMAN et al. 2002) was used and a total of two experiments, depending on the sugar used, i.e. sucrose, or maltose in the basic medium, were carried out. The sugars in each experiment were tested at either 0.5 M or 1.0 M concentrations, osmotic pressures of which being 12.23 atm and 24.47 atm, respectively. Also in combination, effect of the pH at the levels of 5, 6 and 7, adjusted using KOH, and the effect of phloroglucinol (1, 3, 5 Trihydroxy-benzene, SIGMA) was also tested at either nil or 10 μM concentrations. Modified Brewbaker and Kwack medium (1963) containing H_3BO_3, KNO_3, $Ca(NO_3)_2{\cdot}4H_2O$ and $CaCl_2$ at 1.63 mM, 1 mM, 0.12 mM and 0.45 mM, respectively, was used as the basic medium.

The florets at anthesis located in between the centre and the peripheries of the flowers were used throughout the experiment. Practice of otherwise, i.e., random selection of florets from various locations on the flower would have been a source of variation affecting the results since maturation of florets starts from periphery reaching the centre in 2–3 days (FOURY 1967). The pollen from two florets were removed and inoculated into each medium, which was earlier decanted in plastic micro-centrifuge tubes. The tubes, containing small drops of the media following the pollen inoculation, were closed and left to wait upside down and by doing so all the characteristics of the hanging drop method was provided. Following incubation for 24 hours in the media at room temperature of ca. 25 °C, medium with pollen was removed and a drop was placed on a slide on which a small drop of the aceto carmine stain (2 %) was added to give coloration to the cytoplasm of both the pollen and pollen tube. The pollen was considered germinated if the tube was at least the same size as the pollen diameter. Data were obtained from each scope (plot) by counting of nearly 200 pollen depending on the density in the ocular view of the microscope. Both of the experiments were carried out in a three factor, completely randomised plot design with three repli-

cates. The data in the form of percentage germination, following angular transformation, was subjected to the analyses of variance using MSTAT statistical software (NISSEN 1982). The level of significance was α=0.05.

Results

Effects of all three factors tested, sugar concentrations, phloroglucinol and pH, were studied in two separate experiments each using either sucrose or maltose as the source of sugar. For both the sucrose and maltose experiments (Table 1) results showed that the main effects of sugar concentrations and phloroglucinol were statistically significant only in the sucrose experiment whereas the pH main effect was significant only in the maltose experiment. In the sucrose experiment, 1.0 M sucrose resulted in significantly higher pollen germination than the 0.5 M concentration with 49.8 and 20.1 %, respectively, whereas in the maltose experiment the germination percentages were almost the same with 23.5 and 22.9 % for the 0.5 M and 1.0 M concentrations, respectively.

Phloroglucinol main effect was significant in the sucrose experiment and higher germinability was obtained from the media integrated with 10 µM PG in comparison to the control media with 38.9 % and 31.0 %, respectively. In the maltose experiment, on the other hand, pollen germination percentages were lower and the difference was insignificant between the results of 0 and 10 µM PG treatments with 22.8 and 23.7 %, respectively.

The pH main effect was significant only in the maltose experiment where the highest germination was 29.5 % at pH 5, followed by the germination averages of 24.8 and 15.3 % at pH 6 and pH 7, respectively. On the other hand, even though insignificant, in the sucrose experiment, the highest germination percentage was obtained of pH 5 with 39.1 %, followed by 35.5 and 30.1 % at pH 6 and pH 7, respectively.

With regard to two factor interactions, significance was found only for sucrose concentrations x pH (Table 2); PG x pH in the maltose experiment (Table 2) and sucrose concentrations x PG (Table 3). Regarding the interaction between sucrose concentrations x pH, the highest significant pollen germination was obtained from the combination of 1.0 M sucrose and pH 5 with 68.7 % and the lowest was from the combination of 0.5 M sucrose and pH 5 with 9.6 %. On the other hand, regarding the interaction between maltose concentrations and pH, even though statistically insignificant the highest pollen germination was obtained from the combination of 0.5 M maltose and pH 5 with 30.6 %, whereas the lowest germination was obtained from the combination of 0.5 M maltose and pH 7 with 11.6 % (Table 2).

Interaction between the phloroglucinol and pH levels was significant only in the maltose experiment (Table 2) where the highest pollen germination was ob-

Table 1. Main effects of sugar concentrations, phloroglucinol and pH level on the germination of globe artichoke pollen *in vitro* both in sucrose and maltose experiments (Values in parentheses show angular transformation).

Experiment	Sugar concentration (M)		Phloroglucinol level (µM)		pH		
	0.5	1.0	0	10	5	6	7
Sucrose	20.1 (22.6)^{b}	49.8 (44.7)^{a}	31.0 (30.0)^{b}	38.9 (37.4)^{a}	39.1 (35.0)	35.5 (33.4)	30.1 (32.6)
Maltose	23.5 (27.4)	22.9 (24.2)	22.8 (26.5)	23.7 (25.1)	29.5 (32.5)^{a}	24.8 (26.9)^{b}	15.3 (18.1)^{c}

Means followed by the same letter(s) are not significantly different at α=0.05
Maltose pH main effect 5 % LSD: 6.29

Table 2. Interaction effects between sugar concentrations (SC) and pH levels and phloroglucinol availability and pH levels in the sucrose and maltose experiment on the germination of globe artichoke pollen *in vitro* (Values in parentheses show angular transformation).

Experiment	Parameter	pH 5	pH 6	pH 7
Sucrose	SC (M)			
	0.5	9.6 (13.8)^{d}	19.2 (20.6)^{d}	31.4 (3.3)^{c}
	1.0	68.7 (56.2)^{a}	51.9 (46.1)^{b}	28.9 (32.0)^{c}
	PG (µM)			
	0.0	35.3 (31.9)	30.5 (27.2)	27.2 (30.9)
	10.0	43.0 (38.1)	40.6 (39.5)	33.1 (34.4)
Maltose	SC (M)			
	0.5	30.6 (33.4)	28.4 (31.6)	11.6 (17.3)
	1.0	28.4 (31.6)	21.3 (22.1)	19.0 (18.9)
	PG (µM)			
	0.0	29.8 (32.8)^{a}	9.3 (14.4)^{b}	29.3 (32.4)^{a}
	10.0	29.2 (32.1)^{a}	40.4 (39.3)^{a}	1.3 (3.9)^{b}

Means followed by the same letter(s) are not significantly different at α=0.05
Sucrose experiment: Sucrose concentrations x pH: 5 % LSD: 9.26
Maltose experiment: PG x pH: 5 % LSD: 8.89

Table 3. Interaction effects between sugar concentrations (SC) and phloroglucinol levels in the sucrose and maltose experiment on the germination of globe artichoke pollen *in vitro* (Values in parentheses show angular transformation).

Experiment	SC (M)	Phloroglucinol 0µM		Phloroglucinol 10µM	
Sucrose	0.5	6.7	(11.8)^{c}	33.4	(33.4)^{b}
	1.0	55.3	(48.2)^{a}	44.3	(41.3)^{a}
Maltose	0.5	20.4	(26.7)	26.6	(28.1)
	1.0	25.1	(26.4)	20.7	(22.1)

Means followed by the same letter(s) are not significantly different at α=0.05
Sucrose-experiment: Sucrose concentration x PG: 5 % LSD: 7.56

tained from the combination of 10 μM PG and pH 6 with 40.4 % which was followed by the combination of 0 PG and pH 5 with 29.8 %. Even though statistically insignificant, the highest pollen germination in the sucrose experiment was from the combination of 10 μM PG and pH 5 with 43.0 % and the lowest was from the combination of 0 PG and pH 7 with 27.2 % (Table 2).

Interaction between sucrose concentrations and PG levels was significant and the highest germination was obtained from 1.0 M sucrose x 0 PG with 55.3 %, whereas the lowest was from the combination of 0.5 M sucrose and 0 PG with 6.7 % (Table 3). In the maltose experiment however the interaction between the maltose levels and the PG concentrations was not significant, and the highest germinability was obtained from the combination of 0.5 M maltose and 10 μM PG with 26.6 %, on the other hand, the lowest was from the combination of 0.5 M maltose and 0 μM PG with 20.4 % (Table 3).

The triple interactions was significant only in the maltose experiment (Table 4) and 0.5 M maltose together with 10 μM PG at pH 6 resulted in the highest pollen germination with 40.8 %, which was followed by the combination of 1.0 M maltose x 10 μM PG x pH 6 with 40.1 %. No pollen germination, on the other hand, was obtained from the combination of 1.0 maltose, 10 μM PG and pH 7. In the sucrose experiment, however, interaction between the three factors was not significant and the highest pollen germinations were obtained from the triple combinations of, in descending order, 1.0 M sucrose x 0 PG x pH 5 and 1.0 M sucrose x 10 μM PG x pH 5 with 68.9 and 68.5 %, respectively. The lowest pollen germination was observed in the combination of 0.5 M sucrose x 0 PG x pH 6 (Table 4).

Even though statistically insignificant the highest average germination percentages were obtained from the media containing 1 M sucrose at pH 5 both in the absence and presence of the PG with 68.9 % and 68.5 %, respectively. The PG main effect was significant and addition of PG in the sucrose media significantly increased the pollen germination from 31.0 to 38.9 %. The effect of pH in the maltose experiment was significant and pH 5 resulted in the highest pollen germination with 29.5 %.

Although pollen germination and pollen tube development was achieved, bursting of the pollen tubes at a high frequency (exact figures were not determined) was observed (Fig. 1A). Instead of a pollen tube development through a straight line (Fig. 1B), which was the case only in a small proportion of the pollen, outer coatings of most of the pollen tubes burst displaying either a spiral development or massing of the cytoplasm immediately next to the pore where the pollen germination occurred.

Discussion

With the approach adopted here pollen germination of the cultivar 'Sakiz' of the globe artichoke was achieved. Phloroglucinol tested in the germination of globe arti-

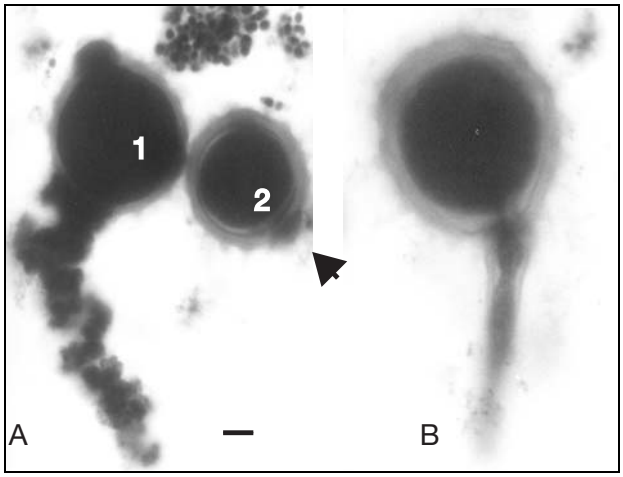

Fig. 1. A, B. Germination and tube development from the globe artichoke pollen. A: (1) outer coating of the pollen tube was burst but still developing in a spiral manner; pollen and tube cytoplasm densely stained with acetocarmine. (2) an acetocarmine stainable pollen without pollen tube but with a small protrusion from the pore (arrow); B: Normal pollen germination and tube development (pollen diameter approx.=90 μ; bar: 20 μ).

Table 4. Interaction effects of sugar concentrations (SC), phloroglucinol availability, and level of pH on the germination of globe artichoke pollen *in vitro* in the sucrose and maltose experiment (Values in parentheses show angular transformation).

Experiment	SC (M)	PG (μM)	pH 5	pH 6	pH 7
Sucrose	0.5	0	1.7 (7.4)	1.2 (3.7)	17.1 (24.1)
	0.5	10	17.5 (20.3)	37.1 (37.5)	45.7 (42.5)
	1.0	0	68.9 (56.3)	59.7 (50.7)	37.3 (37.6)
	1.0	10	68.5 (56.0)	44.0 (41.5)	20.5 (26.3)
Maltose	0.5	0	24.8 (29.7)^{abc}	16.0 (23.5)^{c}	20.6 (26.9)^{bc}
	0.5	10	36.4 (37.0)^{ab}	40.8 (39.7)^{a}	2.7 (7.7)^{d}
	1.0	0	34.8 (35.9)^{abc}	2.6 (5.4)^{d}	37.9 (37.8)^{ab}
	1.0	10	21.9 (27.3)^{abc}	40.1 (38.9)^{ab}	0.0 (0.0)^{d}

Means followed by the same letter(s) are not significantly different at α=0.05
Maltose concentration x phloroglucinol x pH: 5 % LSD: 12.58

choke pollen *in vitro*, resulted in a significant main effect in the sucrose experiment where phloroglucinol availability at 10 μM in the basic medium resulted in 38.9 % pollen germination (Table 1). Effect of phloroglucinol, as a simple phenol, was similar to those of other phenolic compound derivatives, specifically the flavonol aglycones of kaempferol and quercetin. Addition of quercetin at the concentrations of 0.1, 1.0 and 10 μM in the basic medium, in the similar manner used here, was effective both for the wild (normal) and the mutant pollen of *Lycopersicon esculentum*, which otherwise displayed lower fertility and reduced germination frequency (GROOT and DE RUITER 1993). In fact, in the study mentioned, the concentration of 1.0 μM resulted in the highest average germination percentages with 55.5 and 38.0 % in the wild and mutant genotypes, respectively, compared to 34.5 and 25.5 %, respectively, in the control treatments.

Promoting effects of kaempferol on the germination of mutant petunia pollen, has been well established (NAPOLI et al. 1999) where white pollen from white anthers of a petunia mutant (wha) and other petunia mutants lacking flavonols did not germinate or produce pollen tubes *in vitro* unless 0.4 M kaempferol was provided. The positive effect we demonstrated here by the use of phloroglucinol shows for the first time that not only the flavonols quercetin and kaempferol but also the simple phenol phloroglucinol is effective in the induction of pollen germination *in vitro*. In our study, despite the fact that the pollen colour of the cultivar was creamy white, to the best of our knowledge, a mutant characteristic in any form has not been detected in the cultivar 'Sakiz'. Together with the positive quercetin effect on non-mutant (wild) pollen of tomato (GROOT and DE RUITER 1993), it can be concluded that phenolic effect on pollen germination is not limited to the flavonol aglycones, hence simple phenols can also be effective on the germination of normal pollen *in vitro*.

Bursting of the pollen tube that is independent of the media may have been due to the imbalanced osmotic pressure between the pollen tube cytoplasm and the media. Lower osmotic pressure of the media in comparison to the osmoticum in the cytoplasm may have forced water enter the cytoplasm resulting in the pollen tube bursting only. The fact that bursting occurred only in the pollen tubes but not the pollen themselves may have been due to the possibility that the osmotic imbalance was not high enough to cause bursting in the pollen with thick walls whereas pollen tubes following germination may not have been able to withstand the difference in the osmotic pressures and got burst.

Also the fact that pollen tube bursting occurred in all the media tested may allow us to speculate that the common denominator of all the media tested in both the experiments was the osmotic pressure and consequently the other media factors may be relieved from the responsibility. The osmotic pressures of the media provided by 0.5 M and 1.0 M sucrose and maltose, even though stimulative of pollen germination, seem to have been too low resulting in bursting of the tubes and, therefore, an increase in the concentration of the sugar may cure the problem. Results of ADHIKARI and CAMPBELL (1998) are in line with ours, especially regarding the bursting of *Fagopyrum esculentum* pollen and tubes. In the study, not only substitution of PEG 20000 with a lower molecular weight PEG (3500 and 5000), but also both high and low sucrose levels, which they tested, resulted in bursting of the pollen and the tubes. Also, addition of considerably high levels of osmoticum providers such as 30 % sucrose as well as 15 % PEG in a salt medium resulted in severe pollen and pollen tube bursting, even though giving 45 % germination whereas increasing sucrose concentration to 37.5 % controlled the bursting substantially (JAYAPRAKASH and SARLA 2001). Experiments with higher concentrations of the osmoticum providers, which we tested may be helpful in further studies with the globe artichoke pollen. ZHANG et al. (1999) showed, that the manipulation of pH of the medium also was helpful in the reduction of pollen tube bursting in that increasing of pH from 4.5 to 6.0 resulted in 8-fold decrease in the Al^{3+}-induced bursting.

There may have been other reasons for the bursting of the pollen in our study, in that, even though not specifically recorded, the bursting may have been occurring in a higher frequency in the media containing PG. In the induction of Arabidopsis pollen germination *in vitro*, addition of a squashed stigma to the media, by which conditioning via phenolic release in to the media was aimed, although increasing pollen germination, resulted in "germinating pollen bursting" at a higher percentage (DANKERT and CONTANT 1968). Also in the same report, a low sucrose concentration of 10–15 %, although resulting in high frequency germination, caused bursting of the pollen and it was determined that 17.5 and 20 % was the suitable sucrose concentrations. In our study, despite the bursting effect, still when sucrose is used as the sugar and osmoticum provider, employment of 1.0 M concentration resulted in significantly higher germination levels, whereas from the main effect view point, maltose concentrations tested here did not appear with a significant difference.

The pH main effect was significant in the maltose experiment (Table 1) and it was determined that the germination was the highest at the pH level of 5 and the germination decreased with increasing pH levels of 6 and 7. Our results are in line with those of HOLDAWAY-CLARKE et al. (2003) working on the effects of extracellular calcium, pH, and borate on *Lilium* pollen germination and tube growth. They found that pH had a dramatic effect on pollen germination *in vitro* at the levels between 4.5 and 6 resulting in the optimum germination of 55–65 % whereas a strong inhibition was observed at pH 7 with a germination of only 12 %. On the other hand, in our study, pollen reacted indifferently to the pH levels in the sucrose media for which a plausible explanation, other than the possibility of an experimental error, is not available.

It was also noted that trinucleate pollen lost viability much faster than the binucleate pollen and were difficult to germinate *in vitro*. Some of the plant families, which carry trinucleate pollen are *Brassicaceae* (*Cruciferae)*, *Apiaceae* (*Umbelliferae)*, *Poaceae (Gramineae)* and *Asteraceae* (*Compositae*). It was further pointed out that the trinucleate pollen is more sensitive to hydration, radiation, and long storage because the second mitotic division deprives the pollen grain of adequate reserves for longevity and germination (BREWBAKER 1967; FRANKEL and GALUN 1977; DAFNI 1992). Pollen of *Kochia scoparia* is of the trinucleate type and difficulties

were encountered in the induction of germination *in vitro*. It was determined that pollen lost viability soon after dehiscence and less than 0.5 % pollen germinated in agar based media with various sucrose and other additions, but up to 17.8 % germination was achieved with a pre-treatment of 100 % relative humidity (MULUGETA et al. 1994). Pollen of the globe artichoke, being the same type, may be suffering the same problems (PECAUT 1993) and the pollen tested by KELES (1998) may have lost its viability before the initiation of the tests. The case is strengthened by the fact that KELES (1998) used almost all available germination methods covering wide range of media ingredients, in addition to the ones used in our experiments, including GA_3, $MgSO_4.7H_2O$, KNO_3, charcoal and sucrose concentrations up to 80 %, it seems, therefore, the only plausible explanation to the germination failure may be that the pollen must have lost its functionality prior to the tests. Also, exposure of globe artichoke pollen to high relative humidity, in further studies, may be helpful in the induction of pollen germination in addition to the positive effects of the factors we determined in our study. In fact, such a pre-treatment improved the reliability of the results considerably for pollen germination of the *Asteraceae* (HOEKSTRA 1979).

With regards to the different sugar sources, the germination of pollen in the sucrose experiment was almost twice as high of those of the maltose experiment, e.g., while 49.8 % germination was obtained from 1.0 M sucrose concentration only 22.9 % germination was obtained from the 1.0 M maltose (Table 1). Therefore, in the induction of globe artichoke pollen germination *in vitro*, sucrose can be preferred. In the maltose experiment, apart from the data for the pH 7, germination results varied little between main effects of the factors and, irrespective of the factors, germination ranged between 22.8 and 29.5 %, whereas the variation was much larger in the sucrose experiment ranging from 20.1 to 49.8 %. The low variation may be dependant on the source of sugar employed in the media. In a study on the effects of sucrose and maltose on the induction of pollen embryogenesis, it was determined that in the sucrose media due to sucrose hydrolysis osmotic pressure increased significantly, whereas in the maltose media changes in the osmotic pressure was much smaller and not significant (GÓRALSKI et al. 2002). Therefore, use of sucrose as the sugar source may be more favourable in the pollen germination in comparison to maltose.

Success in hybrid seed production may be limited by pollen quality of the pollen parent employed. Quality of the pollen designated in part as pollen's ability to germinate *in vitro* in a medium similar to the stigma exudate is important in the selection of paternal lines. However, performance of pollen *in vitro* may differ from that of *in vivo* in that pollen germination on the stigma and its further development under the two different conditions mentioned may be completely different. Therefore, *in vitro* pollen germination capability can be considered only as a provisional criterion and cultivars successful in the *in vitro* tests can then be further evaluated by hand pollination *in vivo*. The higher the rate of fruit and seed set, the better the line is as a pollinator. MORISON et al. (2000) pointed out that the pollen pool is limited in the hybrid seed production of artichokes, therefore, pollen quality and quantity are important factors.

In conclusion, results presented here, unlike those of KELES (1998), shows that pollen germination in the cultivar 'Sakiz' can be induced both in sucrose and maltose media. Availability of phloroglucinol in the sucrose medium was significantly effective in germination and pH 5 was more effective in comparison to the other levels. The highest germination obtained here (49.8 %) was higher than those of BERNAL et al. (2000) where the highest germination was 19.6 %. In general, sucrose as a source of sugar was more successful than maltose. In the pollen germination tests of globe artichoke, the basic medium employed here containing 1.0 M sucrose at a pH level of 5 in the presence of phloroglucinol at 10 μM, giving an average germination percentage of 68.5 %, can be recommended.

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