The relations between phenolic compounds and seed dormancy in *Pistacia* spp.

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SUMMARY – This investigation was carried out to study the germination percentage of seeds and relations between phenolics and seed dormancy of *Pistacia* spp. The seeds were collected from the trees of *Pistacia atlantica*, *P. terebinthus* and a hybrid, which has unknown parents, in Barbaros village of Çe_me region close to _zmir. They were sown in petri dishes for determining the germination percentage and mean time to complete germination (MTG) either without stratification or pretreated with 1000 ppm gibberellic acid (GA_3). The rest of the seeds were stratified in moist perlite at +4°C. The stratification was interrupted in days 15, 30 and 45, then seeds were sown. MTG of seeds pretreated with GA_3 and stratified were higher than the controls in all species. The germination percentage of *P. atlantica* was higher than in the other species. The phenolic compounds were analysed by using the Thin Layer Chromatography (TLC) method to determine their effects on seed dormancy. Generally, concentrations of phenolics varied with GA_3 and stratification treatments. As a result of examining two dimensional chromatograms, the highest number of phenolics were observed in *P. atlantica.*

Key words: *Pistacia* spp., germination, seed dormancy, phenolic compounds.

***RESUME –** "Les relations entre composés phénoliques et dormance des semences chez les espèces de* Pistacia*". Cette recherche a été menée pour étudier le pourcentage de germination des semences et les relations entre les composés phénoliques et la dormance des semences chez les espèces de* Pistacia. *Les semences ont été collectées à partir d'arbres de* Pistacia atlantica, P. terebinthus *et d'un hybride, dont les parents sont inconnus, au village de Barbaros de la région de Çesme près d'Izmir. Ils ont été semés dans des boîtes de Pétri pour déterminer le pourcentage de germination et le temps moyen pour achever la germination (TMG) soit sans stratification ou prétraités avec 1000 ppm d'acide gibbérellique (GA_3). Le reste des semences a été stratifié dans de la perlite humide à +4°C. Cette stratification a été interrompue aux jours 15, 30 et 45, puis les semences furent semées. Le TMG des semences prétraitées au GA_3 et stratifiées a été supérieur aux témoins chez toutes les espèces. Le pourcentage de germination de* P. atlantica *était supérieur aux autres espèces. Les composés phénoliques étaient analysés en utilisant la méthode de chromatographie en couche fine (TLC) pour déterminer leurs effets sur la dormance des semences. En général, les concentrations en composés phénoliques ont varié avec les traitements au GA_3 et de stratification. Comme résultats de l'examen de deux chromatogrammes dimensionnels, le plus grand nombre de composés phénoliques a été observé chez* P. atlantica.

***Mots-clés :** Espèces de* Pistacia*, germination, dormance des semences, composés phénoliques*

Introduction

It was reported that all of the *Pistacia* species and their hybrids may be used as rootstock in pistachio growing (Özbek and Ayfer, 1959).

Pistachio rootstocks are propagated exclusively by seed. But the germination of *Pistacia* seeds are generally low. For this reason, some treatments to improve germination are applied to the seeds before sowing. Dehulling or splitting, soaking in water, acid scarification, treating with gibberellic acid (GA_3) and stratification increase germination rate of *Pistacia* seeds (Ayfer and Serr, 1961; Crane and Forde, 1974; Ka_ka *et al*., 1992).

Dormancy is one of the efficient factors on seed germination. Seed dormancy is divided into two types: coat-imposed dormancy and embryo dormancy by some researches. Coat-imposed dormancy is derived from the tissues enclosing the embryo (endosperm, pericarp, basically or extra floral organs). In contrast, the other type of dormancy (embryo dormancy) is derived from the embryo itself (Bewley and Black, 1994).

Phenolic acids (caffeic, ferulic and cinnamic acids), phenolic substances such as polyphenols (tannins), flavonols (quercetin) in the fruit and seed inhibit the germination (Baskin and Baskin, 1998). The inhibitory effects of phenolic compound on seed germination are closely related with the regulation of endogenous auxin, seed coat permeability and oxygen supply to embryos (Willemsen and Rice, 1972; Bewley and Black, 1994).

The aim of this study is to investigate the relations between seeds germination of *P. atlantica*, *P. terebinthus*, a hybrid and their phenolic contents.

Materials and methods

The seeds were collected from the naturally growing trees of *P. atlantica*, *P. terebinthus* and a hybrid, in Barbaros village of Çe_me region, close to _zmir.

Fruits were soaked for 3 days to allow the pulp lightly fermented for slipping free from the seed (Pair and Khatamian, 1982). They were then rubbed in a screen to separate pulp from seed and finally washed. Viable seeds were selected by floating on tap water, only those seeds which sank were used for germination test. In order to facilitate germination, all the seeds were scarified with concentrated sulfuric acid (H_2SO_4). Scarification period was separately determined for the seeds of different species. Scarification period procedure performed at room temperature (21-22°C). Scarified seeds were washed for 24 hours under tap water (Crane and Forde, 1974).

Before sowing, 5 treatments were compared in an experimental random block design with 3 replications, each of 100 seeds: (i) untreated seeds (only scarified); (ii) soaked with 1000 ppm GA_3 for 24 hours; (iii) seeds stratified for 15 days; (iv) seeds stratified for 30 days; (v) seeds stratified for 45 days.

The stratification was performed in moist perlite at +4°C. The seeds were sown in sterile petri dishes, on the double layered filter paper. Germination tests were performed in incubator at 21°C and 90% RH, without light.

The germinated seeds were counted every day and seeds with protruding radicles were scored as germinated. The germination percentage and the mean time to complete germination (MTG) were measured (Bewley and Black, 1994). The latter parameter is an indication of speed, obtained by the formula:

$$MTG = \frac{\_ (t \times n)}{\_n}$$

where "t" is the time in days, starting from day 0, the day of the beginning of the test, and "n" is the number of seeds completing germination on day "t".

The phenolic contents of the seeds were analysed by Thin Layer Chromatography (TLC) method. After every treatment to the seeds, embryo and testa were taken out by crushing the endocarp. The seed samples of 1 g were extracted with 96% ethanol (25 ml). 10 x 10 cm sized cellulose coated plates (Merck 5577) were used chromatographic analysis of phenolic compounds. 100 µl of samples were spotted on the right corner of each plate and developed in two dimensions. The first development was carried out in a solvent of butan-1-ol: acetic acid: water (4:1:5 V/V) and the second dimension in acetic acid: water (5:95 V/V). Chromatographic plates were sprayed with Naturstoff (NS) reagent and examined under long wave (366 nm) ultraviolet lights (UV). After the second development, Rf value of each spot was calculated. The colour intensity of each spot was investigated as well (Özeker and Kara, 1999).

Results and discussion

The analysis of the variance of germination indicated significant differences ($p < 0.05$) among the species in relation to the germination percentage and MTG (Table 1).

Table 1. Comparison of germination percentage and MTG of the seeds of different *Pistacia* spp.

Species	Germination percentage (%)	Mean time to complete germination (day)
Hybrid	47.33 a	10.57 a
Pistacia atlantica	97.21 c	16.62 ab
Pistacia terebinthus	87.99 b	20.71 b

[a,b,c]Mean separation, within columns, by Duncan's multiple range test, 5%.

The highest germination percentages were obtained from the seeds of *P. atlantica*, *P. terebinthus* and hybrid, respectively. In contrary, the MTG of the hybrid was shorter than *P. atlantica* and *P. terebinthus*. Many researchers have reported that the seeds of *P. atlantica* has higher germination percentage and germinates quicker than *P. terebinthus* (Ayfer and Serr, 1961; Ka_ka *et al*., 1992). Considerable differences in coefficient of velocity of germination between the seeds collected from two different types of *Pistacia khinjuk* (Ak, 1992).

Effects of pretreatments on germination percentage have not been found significant statistically (Table 2). It was stated that there were not significant differences between the scarified and scarified + prechilled seeds of *P. lentiscus* (Piotto, 1995). Besides, in *P. atlantica* and *P. vera* seeds, soaking with 1000 ppm GA_3 for 24 hours did not increase the germination percentages (Ayfer and Serr, 1961; Ak *et al*., 1995). In this experiment, effect of stratification for 30 days on germination percentage was quite higher than control. Ak (1990), mentioned that the increases in stratification periods were found positively correlated with the germination percentages *P. vera* and *P. khinjuk* seeds. Non significant effects of pretreatments on germination percentages may be related with scarification of all the seeds (Table 2).

Table 2. Comparison of germination percentage and MTG relation to pretreatments applied to seeds

Pretreatment	Germination percentage (%)	Mean time to complete germination (day)
Control	67.93 ns[†]	31.00 a
GA_3	79.68 ns	17.29 b
Stratification for 15 days	75.68 ns	14.68 b
Stratification for 30 days	82.48 ns	8.84 c
Stratification for 45 days	81.61 ns	8.01 c

[†]ns = non significant.
[a,b,c]Mean separation, within columns, by Duncan's multiple range test, 5%.

It was determined that the effects of pretreatments on MTG were significant statistically ($p < 0.05$) (Table 2). All the treatments were shortened the MTG according to the control at the high level. The highest value was obtained from the control, while the lowest value was observed in stratification for 45 days (Table 2). The coefficient of velocity of germination in the seeds of different *P. khinjuk* types increased in parallel with the lengthen the scarification period. But differences among values of coefficient of velocity were not significant after a certain value (Ak, 1992). It is seen at Table 2, there were not significant differences between the MTG values of stratification for 30 and 45 days. Treatment with GA_3 was increased the MTG of the seeds significantly according to the control. Ka_ka *et al.* (1992), reported that GA_3 treatment followed by scarification to the seeds of *Pistacia* spp. excluding *P. vera* was increased the velocity of germination.

Effects of species and pretreatments interaction on the germination percentages was not found significant statistically. In contrary, the interaction effects on the MTG were significant ($p < 0.05$) (Table 3).

Table 3. Effects of different pretreatments on the MTG of different *Pistacia* spp. seeds

Species	Pretreatment				
	Control	GA_3	Stratification for 15 days	Stratification for 30 days	Stratification for 45 days
Hybrid	22.86 c	10.69 f	6.36 gh	7.05 fgh	5.88 h
P. atlantica	27.51 b	18.23 de	16.85 e	10.13 fg	10.39 f
P. terebinthus	42.65 a	22.94 c	20.83 cd	9.36 fgh	7.77 fgh

[a,b,c,d,e,f,g,h]Mean separation, within columns, by Duncan's multiple range test 5%.

The most rapid germination (5.88 days) was obtained from the hybrid seeds stratified for 45 days. Although the slowest germination was observed in control seeds of *P. terebinthus*, GA_3 and stratification treatments were significantly shortened the MTG in every 3 species according to the control (Table 3).

The lowest MTG values were obtained from stratification for 30 days in *P. atlantica* and for 45 days in *P. terebinthus* and hybrid. Stratification treatments were more shortened the MTG than GA_3 treatment in every three species (Table 3). This result agreed with findings obtained from *Pistacia* spp. seeds excluding *P. vera* (Ka_ka *et al*., 1992). Ak (1992), stated that the coefficient of velocity increased connected with increasing stratification periods in the seeds of different *P. khinjuk* types. The seeds of *P. chinensis* were more rapid and complete germinated within 15 days with stratification for 45 days (Pair and Khatamian, 1982).

Totally 10 phenolic substances possessing different Rf values and colours were identified in *P. atlantica*, *P. terebinthus* and hybrid seeds (embryo + testa). Spots belonged to these 10 phenolics were shown in the main chromatogram without regarding species and treatment (Fig. 1).

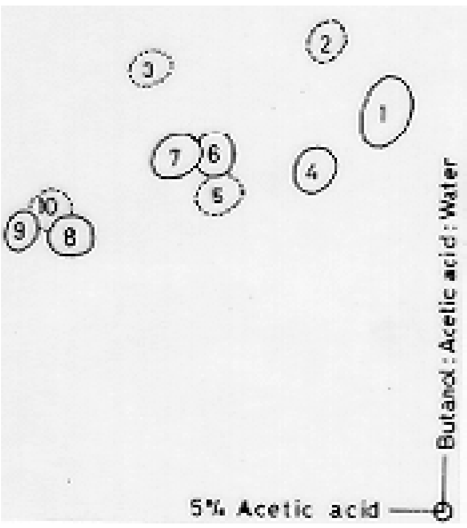

Fig. 1. The main chromatogram of the spots.

These substances were identified by means of Rf values, colours under UV light with NS reagent and colour reactions in literature (Table 4) (Tanrısever, 1982).

The investigation of the chromatograms under UV light revealed that light blue spots (numbered as 3, 8, 9) belonged to phenylpropane, light and dark orange spots (numbered as 1, 2, 4, 5, 6, 7, 10) to flavonoids.

Table 4. Rf values, colours and identification of the spots obtained for *P. atlantica*, *P. terebinthus* and hybrid seeds

Spot no.	Rf value†		Colour	Identification
	BAW	AA		
1	0.78	0.01	Light orange	Flavonoid
2	0.89	0.10	Dark orange	Flavonoid
3	0.84	0.69	Light blue	Phenylpropane
4	0.67	0.15	Light orange	Flavonoid
5	0.63	0.57	Light orange	Flavonoid
6	0.69	0.62	Dark orange	Flavonoid
7	0.70	0.66	Dark orange	Flavonoid
8	0.57	0.75	Light blue	Phenylpropane
9	0.58	0.80	Light blue	Phenylpropane
10	0.60	0.77	Light orange	Flavonoid

†BAW = Butan-1-ol: acetic acid: water (4:1:5); AA = 5% acetic acid.

The effects of different treatments on relative amount (spot size and colour intensity) of phenolics were shown in Table 5. Investigation of the control values belonged to species revealed that some phenolics were specific for species. Flavonoids numbered as 6 and 7 were only detected in *P. atlantica*. In addition, the highest number of spots belonged to phenolics were observed in *P. atlantica* as well (Table 5). Spot 4 belonged to flavonoids was at the highest level in the hybrid. But the same spot decreased with the treatments of GA_3 and stratification. Amount of flavonoid spot numbered as 6 and 7 specific for *P. atlantica* highly increased with GA_3 treatment. Besides, amount of the same substances also showed tendency to increase with the prolonged stratification period. Phenylpropane compound numbered as 3 revealed with GA_3 and stratification treatments in the same species (Table 5).

Table 5. Distribution of spots at different *Pistacia* spp. relation to different pretreatments

No.	Hybrid					*Pistacia atlantica*					*Pistacia terebinthus*				
	C	GA_3	15	30	45	C	GA_3	15	30	45	C	GA_3	15	30	45
1	++	–	–	–	–	–	–	–	–	–	+++	+++	++++	+++++	+++++
2	+	–	–	–	–	–	+	–	–	–	+	–	+	+	+
3	–	–	–	–	–	–	++	–	+	+	+	+	+	++	+
4	+++	+	+	+	++	+	+	–	–	–	–	–	–	–	–
5	+	+	+	+	+	+	++	+	+	+	–	–	–	–	–
6	–	–	–	–	–	+	+++	+	++	++	–	–	–	–	–
7	–	–	–	–	–	+	+++	+	++	++	–	–	–	–	–
8	++	+	+	+	++	++	++	++	++	++	+	++	++	++	+
9	++	+	+	+	++	++	++	++	++	++	+	++	++	++	+
10	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–

It appears that flavonoid glicosides, phenolic acids and tannins when present in the individual pulp fractions of *Liriope muscari* inhibit seed germination in a great degree and this inhibitory effect may be rather synergistic or at least additive (Hruska *et al*., 1982). Chow and Lin (1991), suggested that p-hydroxybenzoic acid in the sarcotesta has an inhibitory effect on germination of papaya (*Carica papaya*) seeds. Increasing amount of flavonoid spots 6, 7 and phenylpropane spot 3 may have indirect promotive effect on the seed germination of *Pistacia atlantica*. Similarly, Özgüven *et al*. (1995), reported that GA_3 and stratification were increased the germination rate considerably and germination inhibitors were disappeared and promoters increased in *P. vera*. Considerable decrease due to pretreatments of flavonoid spot 4 in the hybrid showed that this substance may be a germination inhibitor flavonoid. Thus, inhibitory effects on germination of phenolic substances are related with their amounts (Mayer and Poljakoff-Mayber, 1989).

Conclusions

Results showed that there are significant differences among the germination abilities of the scarified seeds of *P. atlantica*, *P. terebinthus* and hybrid. Pretreatments such as GA_3 and stratification were not effective on the germination percentages, but significantly decreased the MTG in the seeds of all three species above mentioned. All the stratification periods found more effective than GA_3 treatment to hasten seed germination. Thus, combined treatments (GA_3 + stratification) may be more beneficial than either treatment alone.

Investigations revealed that some phenolics taking place in seeds (embryo + testa) were specific for species. The amount of certain flavonoid and phenylpropane substances showed considerable variations relating to pretreatments. It was concluded that they may have important roles in seed dormancy by their inhibitory and promotive effects. More detailed investigations should be needed to definite identification of these substances.

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