SALT RESPONSE OF SEEDS AND POLLEN OF FIVE PISTACIA SPECIES

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Abstract
The salt response of five *Pistacia* species at the germination stage has been investigated and we have evaluated if the germination capability of seeds and pollen under salt stress parallel to each other. Seeds of *P. atlantica, P. terebinthus, P, palaestina, P. vera* and inter *P. atlantica et P. terebinthus* were germinated in six saline solutions with electrical conductivities (EC) ranging from 7 to 42 dS/m. Similarly, pollen was germinated in vitro in twelve saline solutions with ECs ranging from 0.5 to 11.5 dS/m. The EC_{50}, EC at which germination capacity was 50% of the control, was used as the salinity-tolerance parameter. Germination decreased with increasing salinities in the five species. The ranking in salinity tolerance for seed germination, as given by the EC_{50} (dS/m) in parenthesis, decreased in the order: *P. atlantica* (26.9) > inter *P. atlantica et P. terebinthus* (23.2) > *P. terebinthus* (22.5) > *P. palaestina* (19.8) > *P. vera* (18.4). Decreases of pollen germination with increasing salinities were much larger, indicating that the pollen is much more sensitive to salinity. The corresponding ranking for pollen germination was: *P: terebinthus* (7.3) > inter *P. atlantica et P. terebinthus* (7.0) > *P. atlantica* (6.2) > *P. vera* (3.1) > *P. palaestina* (2.9). The results suggest a certain parallelism between the salinity tolerance at the gametophytic (pollen) and sporophytic (seed) phases. If this conclusion is substantiated with a larger number of *Pistacia* species, it will offer a potential for selection at the gametophytic phase and therefore, for facilitating and speeding up the screening for salt tolerance.

Additional index words: Pistacia, seed, pollen, salt tolerance.

1. Introduction

Pistachio (*Pistacia vera*) is a salt-tolerant tree which could be grown as an alternative crop in salt-affected soils. The salinity tolerance of Pistacia vera has been well documented (Parsa and Karimian, 1975; Sepaskhan and Maftoun, 1981, 1988; Sepaskhan et al. 1985; Behboudian et al, 1986). While in some countries *Pistacia vera* is used as a rootstock, in others the crop is grafted in different species of *Pistacia* being the main rootstocks used *P. terebinthus, P. palaestina, P. atlantica, P. khinjuk, P. integerrima and P. vera* (Vargas, 1984). Rootstock choice has been shown to influence nutrient efficiency (Brown et al, 1994). Likewise, the salt tolerance of different rootstocks has been investigated (Walker et al.,1987; Picchioni and Miyamoto,1990)

The main limiting factor of this kind of trials is that they involve lengthy and extensive evaluations. Thus, searches have been made to find quicker and easier tests that may reduce the time and cost of these experiments (Picchioni and Miyamoto, 1991). In this direction, the existence of an overlap in the response to stress, between the whole plant and the pollen in a wide number of species, has lead to propose pollen selection as an early selection criteria (Hormaza and Herrero, 1992).

In this work we explore the possibility of using seeds and pollen germination response to salt as an early selection criteria. The aim of the work was to determine if there was a different response between the different species of *Pistacia* and if seeds and pollen behaviour parallel each other.

2. Material and Methods

Seeds and pollen originated from mature trees from *P. atlantica* Desf., *P. palaestina* Boiss., *P.terebinthus* L., *P. vera* L.and a hybrid inter *P. atlantica* et *P. terebinthus*. The seed germination experiment followed a protocol established by Martinez-Cob et al. (1987) for barley. Seeds were sown in plastic trays layered with a synthetic tissue (Spontex) to which 400 ml of the saline solution had been added. The seeds were separated between rows and from the tissue with filter paper. Covered trays were left at 21°C for four weeks. For each saline solution one tray was used a id in each tray 40 seeds per species were sown. Germination was recorded daily during the first 23 days and every three days during the third and fourth week.

Pollen germination was performed in a germination medium consisting of 20 % sucrose and 1% agar (Martinez-Pallé and Herrero, 1994a) diluted in a range of saline solutions. Pollen was left to germinate for 24 hours at 22° C. A pollen grain was considered to be germinated when the length of the pollen tube exceed the diameter of the pollen grain. Germination records were taken on full microscope fields, until over hundred pollen grains per repetition had been scored. Five repetitions per saline solution and species were performed.

To evaluate salinity response while preventing Na^+ toxicity, a mixture of NaCl and $CaCl_2$ was used (Wyn Jones and Lunt, 1967).The saline solutions were prepared from a stock, concentrated solution of NaCl:CaCl$_2$ (1:1 w/w) diluted with distilled water to get 7, 14, 21, 28, 35 and 42 dS/m for the seed germination experiment. For the pollen germination tests the stock solution was diluted to 0.5, 1.5, 2.5, 3.5, 4.5, 5.5, 6.5, 7.5, 8.5, 9.5, 10.5 and 11.5 dS/m. In both experiments a control with distilled water (CE< 0.01 dS/m) was also used.

3. Results

Seed germination in the control took place, depending on the species, between 5 and 15 days after sowing. While *P. palaestina* reached only 40% germination, all
 the other species tested had a good germination (95%) in these conditions. Saline conditions induced a delay and a decrease in seed germination. While at 42 dS/m none of the species were able to germinate, at lower salt concentrations a very different response was recorded between species (Table 1). Thus, at 21 dS/m, *P. atlantica* reduced its germination to 80%, as compared to a reduction to 27% in *P. vera*. The

different response between species is expressed by the EC_{50}, or electrical conductivity at which seed germination is reduced by 50%. Considering this parameter, the studied species show the following gradation in salt tolerance: *P.atlantica* (26.9 dS/m) > *inter P. atlantica et P. terebinthus* (23.2 dS/m) > *P. terebinthus* (22.5 dS/m) > *P. palaestina* (19.8 dS/m) > *P. vera* (18.4 dS/m).

Pollen germination in the control ranged from 44% for *P. palaestina* to 83% for *P. atlantica*. A previous experiment put forward that the saline conditions that pollen can support are much lower than those for seeds. Therefore,to test the salinity response of pollen a lower range of salt concentrations has been established. Table 2 indicates that pollen germination was also affected by salinity: While a slight increase was observed at low salinities, increasing salt concentrations induced a shortening of the pollen tubes and a decrease in germination capacity. As with seeds, the response of the different species was not uniform and some withstand salt better than others. Based on the calculated EC_{50} values, the species can be classified, on a relative basis, as more salt tolerant (*P. terebinthus*, 7.3 dS/m; inter *P.atlantica et P. terebinthus*, 7 dS/m; and *P. atlantica*, 6.2 dS/m) and salt tolerant (*P.vera*, 3.1 dS/m; and *P. palaestina*, 2.9 dS/m).

4. Discussion

Differences were recorded between species in the response to salt conditions. *P. atlantica, P. terebinthus* and inter *P. atlantica et P. terebinthus* have proven to be, in both trials, more tolerant than *P. vera* and *P. palaestina*. However, before further conclusions can be drawn it is necessary to correlate these results with those obtained under field conditions. Although various attempts have been made to evaluate salt tolerance at the field level, the results are not conclusive. While no effect was observed, for the saline conditions used, in *P. vera, P. atlantica* and *P. terebinthus* (Walker et al. 1987); little differences were recorded in the aerial and root development balance between *P.atlantica* and *P. terebinthus* (Picchioni and Miyamoto, 1990). This may be related to the practical difficulties encountered in this kind of experiment.

While the existence of an overlap in the response of seeds and pollen will have to be studied with a wider number of species, our results indicate a parallel response in seeds and pollen to salt stress. An overlap between the response of the pollen (gametophyte) and the plant (sporophyte) to external stress has been observed in other species (Ottaviano and Mulcahy, 1989) and is supported by the existence of a wide overlap in gene expression between the gametophytic and sporophytic phases (Willing and Mascarenhas, 1984). These two facts are fostered by the idea of gametophytic selection put forward by Mulcahy (1979) that suggest that selection pressure during the gametophytic phase may select for particular genotypes. For selection to occur a situation of pollen competition is necessary (Hormaza and Herrero, 1992). In pistachio, pollen competition occurs in nature and is manifested by a reduction in the number of pollen tubes that progress down the style. This situation appears to be exacerbated by pistil anatomy, since there is a gradual reduction in the space available for pollen tube growth (Martinez-Pallé and Herrero, 1994b). Pollen tolerance to salt is much lower than that of the seed. This is probably related to the fact that pollen is an isolated cell completely exposed to the medium, while a number of protective structures cover the

root cells. However, clear differences exist between species when testing pollen. Pollen has the advantage of permitting screening of a large population size. Thus, in the seed experiment, 40 individuals per treatment and species were used. However, working with pollen, this number exceeded 500. In addition, due to the haploid condition of pollen, the naked genotype is exposed. While, in experiments with the whole plant, it is only the response of the phenotype that can be observed. These two features of pollen, big population size and haploid condition are common to microorganisms (Mulcahy and Mulcahy, 1987) and make pollen a convenient tool for screening processes. While pollen screening trials cannot overtake testing the response of the whole plant. If the overlap in response to salt tolerance is substantiated with a larger number of *Pistacia* species, it will offer a potential for selection at the gametophytic phase and therefore, for facilitating and speeding up the screening for salt tolerance.

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Solution EC (dS/m)

	0	7	14	21	28	35	42
P. atlantica	95	95	90	80	35	12	0
P.terebinthus	95	82	87	62	12	2	2
inter P. atlantica et P.terebinthus	95	87	87	67	0	0	0
P. palaestina	40	30	17	32	5	2	0
P. vera	95	85	65	27	7	5	0

Table 1: Percentage of seed germination in a range of salt concentrations (dS/m).

Solution EC (dS/m)

	0	0.5	1.5	2.5	3.5	4.5	5.5	6.5	7.5	8.5	9.5	10.5	11.5
P. atlantica	83	82	88	77	-	71	53	26	38	24	17	0	0
P.terebinthus	51	59	52	44	48	52	38	42	17	23	6	6	3
inter P. atlantica et P.terebinthus	72	83	86	79	88	58	57	29	-	32	16	17	7
P. palaestina	44	44	60	21	-	13	11	0	-	-	-	-	-
P. vera	80	83	73	44	43	15	8	0	-	-	-	-	-

Table 2: Percentage of pollen germination in a range of salt concentrations (dS/m).