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SALINITY EFFECTS ON ARGAN (ARGANIA SPINOSA (L.) SKEELS) GERMINATION AND JUVENILE GROWTH

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SALINITY EFFECTS ON ARGAN (*ARGANIA SPINOSA* (L.) SKEELS) GERMINATION AND JUVENILE GROWTH

By

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ABSTRACT

SALINITY EFFECTS ON ARGAN (*ARGANIA SPINOSA* (L.) SKEELS) GERMINATION AND JUVENILE GROWTH

By

Janis Sipple Michmerhuizen

Soil salinization is increasing in Morocco as a result of irrigation of agricultural land. The argan forest in the Souss Valley region has been negatively impacted by urban expansion and the increase in agricultural land. Additionally, both artificial and natural regeneration efforts have had poor results. Seeds and seedlings of argan were exposed to 0, 2.5, 5.0, 7.5, and 10.0 g/l NaCl to determine the effect of salinity stress on germination and growth. Ten seed accessions were selected, five from a coastal site and five from a mountain site. Analysis of variance indicated survival, seedling growth, root length, root dry weight, and shoot dry weight significantly decreased with increasing NaCl treatments. There were some significant differences in salinity effects among the accessions; however, there was no significant difference between the coastal and mountain sites. In addition, root:shoot ratio for seedlings increased with increasing NaCl treatments. Argan growth is severely reduced at salinity levels greater then 2.5 g/l NaCl (3.9 dS/m) and the species is not likely to establish from seed on saline soils.

KEYWORDS: *Argania spinosa*, argan, salinity, germination, juvenile growth, Morocco

In memory of my father

Joseph Holland Sipple, Jr.

1925 – 1998

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CHAPTER 1

INTRODUCTION

Argan (*Argania spinosa* (L.) Skeels) is a medium-sized multipurpose evergreen tree. It is the only arid zone species of the Sapotaceae family north of the Sahara Desert. It is indigenous to Morocco and originates in the Souss Valley region. Although argan is primarily regarded as an edible oil fruit tree (Sasson, 1993), and even though its secondary products are of significant value to the rural community and the ecosystem, it has not yet been domesticated. Argan is also used for fuel, timber, cosmetics, and valued for its shade and role in stabilizing the soil. During summer drought, the leaves, fruits and pressed seed cake are the only fodder available for livestock (Prendergast & Walker, 1992).

The argan forest ecosystem, which originally covered 1.4 million hectares, has been reduced by over 40 % to approximately 800,000 hectares due to exploitation and human pressure on land resources. In the semi-arid regions of southern Morocco, the argan tree is of immense socio-economic importance. This native tree produces highly nutritious edible oil, fodder (leaves and fruits) for livestock, and fuel (charcoal). Historically, subsistence rural communities have relied on this forest ecosystem to maintain their livestock herds and provide argan oil for consumption and sale. Today, argan oil production continues to be a subsistence cottage industry managed by women (Prendergast & Walker, 1992).

It has been speculated that natural argan regeneration has not been significant for the past 50 years. Government sponsored reforestation projects are attempting to increase the argan population, though seedling survival has been low (Sasson, 1993).

Research into argan physiology has revealed many of the requirements and limitations that argan seeds must meet or overcome in order to germinate and survive (Arif, 1994; Nouaim & Chassoud, 1994; Nouaim *et al.*, 1994; Farradous *et al.*, 1996; Zahidi & Bani-Aameur, 1997; Zahidi & Bani-Aameur, 1998). However, more research is needed in this area to further define the tolerances of argan in both its natural habitat and in the nursery environment. Additionally, research may prove that schottenol, an anticancer agent, can be extracted from argan (Maurin, 1992; Sasson, 1993).

The cosmetic and the edible argan oil markets are strong within Morocco, and there is growing international interest in argan oil for industrial cosmetic uses. Edible oil also has international market potential because its nutritional value exceeds that of olive oil. However, with the argan forest in a degraded state, the current market cannot be expanded. Improved argan trees under cultivation could potentially produce as much oil as olive trees.

The Souss Valley, where argan populations are the greatest, has experienced urban expansion and intensification of agriculture since the 1970s (Benchekroun & Buttoud, 1989; Buttoud, 1990; Sasson, 1993). With the increase of agriculture and the use of irrigation, the soil is highly susceptible to salinization. In 1976, Morocco had an estimated 1,148 hectares of saline soils (Szabolcs, 1989). Current figures, while unavailable, are most certainly higher in these arid and semi-arid regions.

Argan has been documented to be tolerant of all types of soils and soil conditions with the exception of shifting sand (Gentil, 1906; Nouaim *et al.*, 1991; Prendergast & Walker, 1992). Nouaim *et al.* (1991) also stated that argan grows on saline soils; however, there has been no study to determine the salinity tolerance of argan. If argan is

found to be tolerant of high salinity levels, it could be used to reclaim abandoned agricultural land, prevent desertification, encourage ecosystem restoration, and improve the environment.

The goal of this research was to screen argan germplasm for salinity tolerance. Accessions that perform the best under salinity stress will be recommended for reforestation efforts on abandoned agricultural land. A salt tolerance range was investigated as well as the effects of salinity on germination and seedling growth.

CHAPTER 2

LITERATURE REVIEW

2.1. *Argania spinosa* (L.) Skeels

2.1.1. Botany

Argan is an arid zone evergreen tree in the Sapotaceae family. Within Sapotaceae, *Argania* is the only genus found north of the Sahara desert and the genus is monospecific. Argan can reach 10 m in height with a dense crown between 20 and 40 m in diameter. Many trees have a single stem, but since they coppice well, and are subject to heavy use, there are many with multiple stems. The leaves are small, lanceolate and leathery with a dark green upper surface and a paler underside. Spines (modified stipules) up to 1 cm long are at the base of the leaves. The flowers are greenish yellow, 5-6 mm in diameter and cluster in the leaf axils. The fruits are lime-green when developing and yellow when mature; shape varies from nearly spherical to ovoid, and size ranges from 20 to 50 mm. The drupe-like fruit consists of a tough pericarp and a smooth brown stone. The stone is extremely hard and may have up to five seed chambers, with one chamber dominant (Bani-Aameur *et al.*, 1998).

2.1.2. Ecology

Within the natural range, argan may be found at sea level up to the snow line of the High Atlas (approximately 900 m and minimum temperature 3.8°C). In the Anti-Atlas the upper limit is approximately 1300 m, as moisture becomes the limiting factor over temperature. In the hottest months (June, July, August, September), temperatures often exceed 50°C and lack of moisture becomes a limiting factor with temperature.

Average annual precipitation varies from 50 to 400 mm, and in the lower range, argan takes on a bushy appearance (Figures 1 and 2) (Monnier, 1965; Nouaim *et al.*, 1991; Prendergast & Walker, 1992).

Argan is a shade intolerant tree and has never been documented as an understory tree. The argan forest is described as a parkland, similar to the Spanish *dehesa* where the crowns of trees rarely meet (Le Houérou, 1981; Montoya, 1984; Mellado, 1989; Prendergast & Walker, 1992). Little is known about the root system, although it is speculated that the taproot may reach depths greater than 30 m (Nouaim *et al.*, 1991).

Argan achieves its best growth on the coastal plain where annual rainfall is 400 mm (Prendergast & Walker, 1992). It is found on marginal soils and has been assumed to be tolerant of all soil types other than shifting sands; however, no studies have been found which investigate soil and argan (Gentil, 1906; Nouaim *et al.*, 1991).

Argan is very drought tolerant. Contributing to its ability to survive in arid regions (50 mm of rainfall), the tree drops its foliage during prolonged droughts and remains in a state of dormancy, sometimes several years, until rainfall returns (Morton & Voss, 1987; Nouaim *et al.*, 1991).

Argan sprouts vigorously and coppicing is considered the most reliable method for the first regeneration. However, the sprouts that arise after a second coppice are not viable for maintaining a healthy tree. Seeds or seedlings are used to regenerate an area after this second harvest (Morton & Voss, 1989; Sasson, 1993).

Figure 1. Argan growing between Tiznit and Sidi Ifni where annual rainfall is approximately 250 mm.

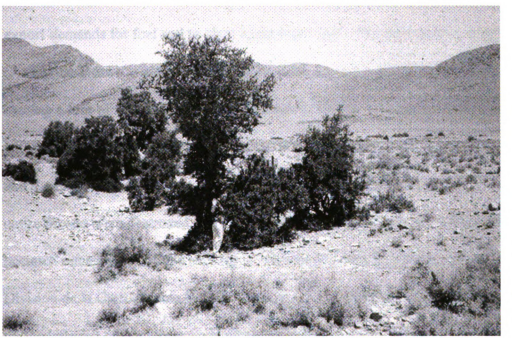

Figure 2. Argan tree growing southwest of Tiznit where annual rainfall is less than 100 mm.

2.1.3. Distribution

Argan was first documented by Leo Africanus, a traveler who visited Morocco in 1510, and according to Monnier (1965), the original natural range was 1.4 million hectares. The argan population experienced a decline in Morocco centuries ago when migration into this region began. The total area of the Mediterranean zone once covered by argan is still unknown; however, a recent find of silicified wood fragments on the Mediterranean island of Sardinia closely resemble argan and has been dated back to the Miocene period (roughly 25 million years ago). Because of this find, there is speculation that argan covered the entire Maghreb region (Morocco, Algeria and Tunisia) at one time (Prendergast & Walker, 1992). However, the origin of argan is in the Souss Valley of Morocco according to Mellado (1989).

During World War I the argan forest was harvested in large quantities to meet export demands for fuel and to clear agricultural land. The Moroccan Forest Service (during the French protectorate period 1912-1956) recognized that the harvests were causing a loss of forest area and imposed restrictions on the trees in 1924. There was little enforcement of this legislation, and during WWII the demand for fuel increased once again and the argan population was negatively impacted (Morton & Voss, 1987). In some areas entire forests were destroyed and the 1994 Moroccan forestry map indicated 828,300 hectares remaining, with the majority in the southwestern part of the country (Maroc Division de la Cartographie, 1994). There were two small forests outside this region, one on the hillsides of Beni Snassen in northeast Morocco near Oujda and another southeast of Rabat in Oued Grou (Nouaim *et al.*, 1991; Prendergast & Walker, 1992).

Even more significant than the estimated 40% decline in total area is that argan tree density had been reduced by two-thirds by the late 1980s (Benchekroun & Buttoud, 1989; Prendergast & Walker, 1992). This is attributed to the Moroccan government policy encouraging intensive agriculture in prime argan habitat in the 1970s, which resulted in an increase of cleared land for cultivation and more irrigation. In addition, many farmers removed argan near their fields because they attracted fruit flies, which damaged their crops (Sasson, 1993; Mazih & Debouzie, 1996). Recognizing the ecological value of argan to this region, an argan reforestation project was carried out in 1991, and about 3,000 hectares were regenerated. This was done mainly through coppicing combined with protection from grazing. Additionally, 4,700 hectares of sand dunes were stabilized through transplanting argan seedlings. The seedling survival has been low and more research, especially in biotechnology, is being conducted to improve survival (Sasson, 1993).

Unsuccessful attempts have been made to grow argan in the East Indies, Australia (including Tasmania), South Africa, Kenya, Cyprus, England, Florida, Curacao, and Haiti (Morton & Voss, 1987). Ten years ago, two argan plantations were established in the Negev Desert in Israel. Fruiting began at both sites during the third and fourth year, and this onset of fruit outside its natural range is very promising (Nerd *et al.*, 1994). Nerd *et al.* (1998) has found regular flowering and fruiting in cultivated argan. Israel is now investing in further research to explore the future of commercial argan oil production (Combating Desertification, 1995). Spain has also documented argan trees growing in the southern region (Montoya, 1984), though there are no reports of any commercial oil production. Southern California also has a climate similar to southern Morocco, however

it is not likely to give higher economic yields than the current citrus production[1]. At this point in time, it appears that the majority of argan plant resources will remain in Morocco.

2.1.4. Uses

Although argan is primarily regarded as a fruit tree (Sasson, 1993), its secondary products are significant to the local economy, livestock rearing, and the ecosystem. During annual summer drought, the leaves, fruits and pressed seed cake are the only fodder available for livestock (Prendergast & Walker, 1992). Argan is also used for fuel, timber, cosmetics (oil and seed residue), and valued for its shade and role in soil stabilization. Subsistence cultivation of barley is possible under the argan canopy, where the microclimate is greatly altered (Mellado, 1989).

Oil

A valuable edible oil is extracted from the seeds. The argan oil "industry" is based on wild trees, as cultivars do not yet exist. In Morocco, argan oil is preferred over olive oil; however, it represents only 1.6% of total annual edible oil consumption in Morocco. This is probably due to low supply and the labor intensive, rudimentary and incomplete extraction process (Sasson, 1993). Between seven and eight million working days are devoted annually to argan oil production on a national level (Prendergast & Walker, 1992). Approximately 100 kg of fruits are needed to produce 1-2 kg of oil, 2 kg of pressed seed cake, and 25 kg of dried "husk" (Ehrig, 1974; Nouaim *et al.*, 1991;

[1] These two regions have been compared numerous times. With enough water, Morocco had hoped to establish similar agricultural outputs as southern California. Although a large amount of land is under irrigation, the outputs have been disappointingly low for Morocco (Swearingen, 1987).

Sasson, 1993). The labor intensive extraction process, usually carried out by women, requires seed collection, breaking the seed husk, heating the seeds, then grinding the seeds with water between stones. In total, this process takes 8-10 hours for 1-2 kg of oil (Nouaim *et al.*, 1991; Sasson, 1993). The oil is highly nutritious because it contains 80 percent polyunsaturated fatty acids of which 30 percent is linoleic, one of the most important essential fatty acids in the human diet. Argan oil is a common part of the local diet. It is often mixed with crushed almonds and honey into a thin butter (locally called amalou) for eating with bread, mixed with barley and honey for a breakfast porridge, or bread is simply dipped in the oil (Morton & Voss, 1987).

The oil is also desirable for cosmetics. It is believed to be good in protecting the skin from aging by preventing wrinkles. This industry is quite small, partially due to the laborious extraction process (Nouaim *et al.*, 1991; Sasson, 1993). Locally, the residue from the oil extraction process is used as a skin "mask" for the same reason.

Agroforestry

Argan is the tree component of an agrosilvopastoral system. In the system, barley is planted in an entire plot of land, including the area underneath the tree canopy. Before the barley plants send up the reproductive shoot or flag leaf, livestock are permitted to graze the green swards. The livestock are then moved off of the field, and the barley is allowed to grow and set seed to produce grain. It is the second growth that is harvested for the cereal grain for human consumption. Other trees that may be a part of this system include almond, olive, or date palms depending on the location in the country and site characteristics. Literature sources frequently mention the cultivation of barley with argan (Monnier, 1965; Montoya, 1984; and Nouaim *et al.*, 1991); however, no details of this

agrosilvopastoral system has been written. This argan-barley type of farming remains common today along the slopes of the High and Anti-Atlas Mountains.

Argan leaves and fruits are fodder for livestock during the summer drought. Additionally, the pressed seed cake remaining after the oil has been extracted is fed to livestock (Montoya, 1984; Prendergast & Walker, 1992).

Wood

The wood is yellow, hard, durable and dense making it desirable for implements and utensils (Monnier, 1965; Morton & Voss, 1987). However, the wood is most often used for fuel (dried or charcoal). In 1993, Sasson (1993) estimated that the argan forests from the provinces of Agadir and Taroudant produced 20,000 tons of firewood.

2.1.5. Silviculture

Propagation

Two flowering periods have been observed; the first (or early) from October to November, and second (or late) flowering during February to March. However, trees have been noted to have some flowers throughout this period (October – March) (Ferradous *et al.*, 1995). Development of the fruit takes 9 to 16 months, so it is possible to see flowers, mature fruit and immature fruit on one tree at the same time (Bani-Aameur *et al.*, 1998; Nerd *et al.*, 1998). Since the flowering cycles are not the same for every tree, mature fruits can be found throughout the year. However, for the largest quantity, fruits are collected from May through June. It is important to note that some fruits will be aborted due to the fruit fly (*Ceratitis capitata*), which lay eggs in the pulp, or for other

unknown reasons. The largest fruits should be selected and collecting directly from the ground should be avoided.

Fruits should be placed in cool storage (4°C) to extend seed viability; however, exactly how long the seeds will remain viable is not known at this time. To remove the stone from the fruit, crack the dried pulp (mesocarp) with a hammer. Germination is higher if the seeds are not extracted from the stone. Also germination is higher and more rapid when the stones are mechanically scarified. Because of the presence of *Fusarium* spp. and other fungi in the mesocarp, the stones should be cleaned with a 2% chlorine solution for 15 minutes followed by three fresh water rinses. After scarification, additional treatment with a fungicide like Thiodan is recommended. Stones should be planted 1-2 cm below the soil surface, and if properly scarified and ambient temperatures are above 10°C, complete germination will occur within 21 days (Zahidi & Bani-Aameur, 1997; personal observation).

Establishment

Artificial regeneration continues to be difficult. Zahidi and Bani-Aameur (1997) recommend that container-grown seedlings should be at least 6 months old before out-planting due to *Fusarium* spp. They should be planted after the first rains to insure sufficient moisture during the establishment period. Seedlings should also be protected from grazing. More research is needed to determine the most effective establishment techniques.

Research into micropropagation is being conducted with the support of the French National Institute for Agricultural Research (INRA). At this time, in-vitro production is only supplying plantlets for research (Sasson, 1993).

Management

Little is known about management. Argan sprouts vigorously and coppicing is considered the most reliable method for the first regeneration. However, the sprouts that come after a second cut are not viable for maintaining a healthy tree (Morton & Voss, 1989; Sasson, 1993). To insure fruit production trees in flower should not be grazed.

2.1.6. Symbiosis

Argan tree roots contain endomycorrhizae, which enhance the water and nutrient (especially phosphorus) uptake ability of the trees (Sasson, 1993; Nouaim & Chaussod, 1994; Nouaim *et al.*, 1994).

2.1.7. Limitation

Argan is highly susceptible to *Fusarium* spp., a problem for germinating seeds and seedlings (Nerd *et al.*, 1994; Zahidi & Bani-Aameur, 1998). Overall seedling survival continues to be low and more research is needed (Sasson, 1993; Zahidi & Bani-Aameur, 1997).

2.1.8. Research

Physiology and artificial regeneration have been the primary focus of biological research on argan. Sasson (1993) reported that several thousand seedlings are being raised in nurseries. However, survival in both nurseries and after out-planting is low due to *Fusarium* spp. (Nerd *et al.*, 1994; Zahidi & Bani-Aameur, 1998). Zahidi and Bani-Aameur (1998) discovered that some genotypes are more tolerant to this fungus and that seedlings remain susceptible during the first five months after germinating. Seed handling before sowing has also been examined by Zahidi and Bani-Aameur (1997). They found

that a higher percentage of seeds germinated that had been stored for two years (60%) than those stored for three years (50%). They also developed a protocol (see Propagation) for reducing *Fusarium* infection on germinating seeds (Zahidi & Bani-Aameur, 1997).

Planting depth, scarification and temperature have all been found to affect argan germination. Arif (1994) determined the optimum planting depth to be 2 cm. He also found that surface sown seeds would not germinate. Scarification (cracking of stone) results in both higher percent and more rapid germination. Seedling germination has also been recorded to be affected by low daily ambient temperature. Seeds sown on January 15 were significantly slower in germinating and in attaining maximum germination in comparison to the other two seed sets, which were sown in November and December. It took longer for the seeds to begin germinating and to reach a maximum germination when ambient temperatures had a daily mean of 12°C (range 7.5°C and 20°C) compared to 22°C (range 15 – 28°C) in November and 18°C (range 12 – 25°C) in December (Zahidi & Bani-Aameur, 1997).

In its natural habitat, mature argan seeds that fall by the end of September achieve maximum germination (Metro 1952, Ferradous *et al.* 1996). This is because the seeds will benefit from the rains of October, and later months, and the relatively moderate high temperatures (> 25°C) (Ferradous *et al.* 1996). Seeds that germinate later are subject to longer periods of cooler temperatures and greater exposure to diseases. This may be a cause for poor natural regeneration of argan (Zahidi & Bani-Aameur, 1997).

Nouaim and Chaussod (1994) studied the effects of inoculating microprogagated seedlings with vesicular-arbuscular mycorrhizal (VAM) fungi, and found a significant

increase in above-ground seedling biomass after six months. A subsequent study of the mineral nutrient contents of the seedlings revealed greater mineral uptake in seedlings with VAM fungi (Nouaim *et al.*, 1994). This may explain the tree's ability to grow in low fertility soils.

2.2. Degraded Land In Morocco

Morocco is the northwest corner of Africa. The southern and southeastern portion of the country is part of the Sahara Desert. Morocco, excluding the Western Sahara, has 69.5 million hectares of land; however, only 12.7% (roughly 8.8 million hectares) is considered arable (Sasson, 1993). Eighty percent of this arable land is classified as arid or semi-arid (El Mourid & Karrou, 1996).

Land degradation, usually the result of adverse human impact, in arid and semi-arid regions generally results in desertification (Daily, 1995). Land degradation in the Mediterranean region is not a recent phenomenon. The over exploitation of the Mediterranean vegetation in the Near East began during the Neolithic period (10,000 to 2,000 BC) when human population increased greater than tenfold. Since that time, population has continued to grow and demands for building materials, fuel, and land for agricultural production have increased. Most recently, in the last fifty years, urban expansion has had the greatest impact on the fragile Mediterranean environment of Morocco, including the argan forests (Le Houérou, 1981; Melado, 1989). The Souss Valley where are argan populations are the greatest has experienced urban expansion and intensification of agriculture since the 1970s (Benchekroun & Buttoud, 1989; Buttoud, 1990; Sasson, 1993).

Additionally, drought affects more than 60% of Morocco's arable land and soil salinization is increasing (Janick, 1989; Sasson, 1993). In 1976, Morocco had 1,148 hectares of saline soils (Szabolcs, 1989). Current figures, while unavailable, are most certainly higher due to the increasing irrigation in these arid and semi-arid regions over the past 20-25 years.

2.2.1. Development of Salt-Affected Soils

Salt-affected soils can be found on the five major continents. While they can be found in most climatic conditions, they are more extensive in arid and semi-arid regions. These soils are an issue for agriculture as the accumulation of salts alters soil properties (physical, chemical and biological) and plant growth is inhibited in many species. Throughout the world there are approximately 955 million hectares (roughly 10% of total land surface) that are affected by salt and experience limited crop production (Szabolcs, 1989; Rhoades, 1990).

These soils are usually classified as saline, non-saline sodic, and saline-sodic. A combination of two factors primarily determines which salt-affected soil could develop in a given site: the type of salts present and climate. Salts naturally occur in all soils originating from mineral weathering. When water from rainfall, groundwater, or irrigation passes through a soil, the salts are dissolved and moved along a drainage path, which is ultimately to the sea. However, where drainage is limited, the water evaporates and salts become concentrated. Salts can even ascend through the soil in arid and semi-arid regions where evapotranspiration is very high. Over time, this results in saline lakes, brackish groundwater, salt deposits, or salinized soil (Rhoades, 1990; Singer & Munns, 1991).

Soil salinization caused by irrigation is considered to be a secondary cause. While an irrigation scheme provides a good growing environment for agricultural crops, the irrigation water contains salt, even if only trace amounts. If not leached out, soil productivity is reduced when salts accumulate in the root zone (Fereres, 1983; Rhoades, 1990).

2.2.2. Salinity Stress on Flora

Plants respond differently to salt-affected soils. The degree of response generally falls in three categories: salt-sensitive, salt-tolerant, and halophytic. Salt-sensitive plants quickly show a decline in growth soon after the soil electrical conductivity (EC) is greater than 4 deciSiemens per meter (dS/m)[2] and may die at 6 dS/m. Salt-tolerant plants begin to show decline around an EC of 10 dS/m; however, they may survive with poor growth in EC levels up to 20 dS/m. Halophytic plants actually show improved growth in low salt levels, but beyond EC levels of 25 dS/m, there is decline in growth (Figure 1) (BOSTID, 1990). Agricultural crops are rarely halophytic. The salt-tolerant crops may be further broken down into moderately and highly tolerant. Tolerance may also vary during the different stages of plant growth and development.

[2] deciSiemens per meter (dS/m) is the SI unit for electrical conductivity and is approximately equal to g/l divided by .64 (or EC x .64 = g/l).

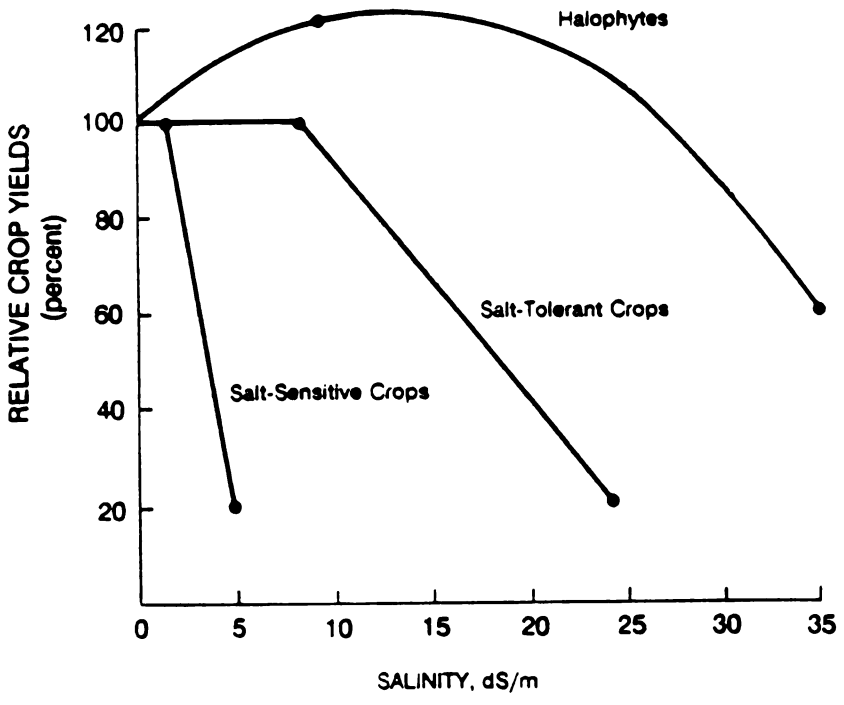

Figure 3. Growth response to salinity (from BOSTID, 1990).

Salt Effects on Germination

Most plants are far more sensitive during germination and seedling stages. In arid and semi-arid regions, seed germination and seedling establishment are the two most critical stages for survival (Kigel, 1995). When high concentrations of salt are present, most seeds do not survive, including halophytes. However, in some halophytes, germination of seeds may be inhibited by the presence of salt. This is caused by an osmotically induced or enforced dormancy. These species would be considered to be salt-sensitive or salt-tolerant at the germination level. Once the salt soil conditions reached a low level, these seeds would germinate and potentially establish themselves before the salt levels rose again (Ungar, 1995).

Salt Effects on Growth

High salt concentrations in soil affect the growth of most plants. The maximum amount of salt any plant can tolerate is variable among species. Primarily, moisture availability is reduced; nutrient availability is altered; physical condition of the soil limits root penetration; and/or specific ions become toxic.

Typically, soluble salts adversely affect plant growth as a result of osmotic effects on the availability of soil water. The osmotic effect actually creates a physiological drought for the plant (Abrol, 1986; Kafkafi & Bernstein, 1996).

Other salt damage has also been noted as increase in hydraulic resistance of roots and leaves; alteration of hormone levels that influence growth rates; direct damage, particularly to photosynethic mechanisms; and ion competition, thereby increasing energy to maintain the K:Na balance (Wild, 1973).

Salts may also induce toxic effects or deficiencies of specific ions (e.g. Cl, SO_4, B). Bañuls and Primo-Millo (1995) found that salt is most damaging to the leaves of *Citrus* spp. Their study determined that increasing concentrations of chloride in the foliage corresponded with increasing leaf injury and defoliation. Whereas high salt levels for *Prunus* spp. have shown leaf injury to be caused by boron toxicity (El Motaium *et al.*, 1994). High concentrations of NaCl may also interfere with uptake of other nutrients.

Osmotic and specific ion effects are the main factors that reduce root growth under salt stress. The osmotic effects of high salt concentrations may be observed in root cell size and rate of cell production, thus shorter and/or thicker roots. Also a higher root/shoot ratio is often observed for plants experiencing salt stress because shoot growth is inhibited before root growth (Kafkafi & Bernstein, 1996). Root dry weight has also

been documented to decrease with increasing salinity for salt-sensitive as well as salt-tolerant species (Tomar & Yadav, 1985; Gutierrez Boem *et al.*, 1994; Bañuls & Primo-Millo, 1995; Ungar, 1996).

Argan has been documented to be tolerant of all types of soils and soil conditions with the exception of shifting sand (Gentil, 1906; Nouaim *et al.*, 1991; Prendergast & Walker, 1992). Nouaim *et al.* (1991) also stated that argan grows on saline soils; however, there has been no study to determine the salinity tolerance of argan. The natural range of argan is being threatened by soil salinization and the germination and growth response to such conditions should be investigated further.

Two experiments were conducted to screen argan accessions for tolerance to salinity at both the germination level and seedling growth stage, and to observe the effects of salt on root growth.

CHAPTER 3

GERMINATION AND SEEDLING STUDIES

3.1. Introduction

The Souss Valley region, which has the greatest population of argan, has experienced urban expansion and an increase in intensive and irrigated agricultural land in the past 20 years. Irrigation of these arid to semi-arid soils can easily lead to salinization due to the low rainfall and high evapotranspiration if good drainage is not maintained (Janick, 1989).

Artificial regeneration has been poor and natural regeneration has been practically non-existent. While mature argan trees may be tolerant of saline soils (Nouaim *et al.*, 1991), seeds may not germinate and survive under high saline conditions. It is not known at this time if argan seeds have a salt-induced dormancy; however, gibberillic acid (GA) was used in this study to stimulate germination (Bewley & Black, 1994; Khan & Rizvi, 1994; Kahn & Ungar, 1998).

The objective of these studies was to screen argan accessions for salt tolerance at germination and seedling stage and to observe the effects of salt on early root growth. Questions regarding germination, seedling mortality, seedling height growth, root length and biomass, and root:shoot ratios among salt levels, genotypes and geographic seed origin relative to salt tolerance were investigated.

3.2. Site Descriptions

This experiment was carried out from November 1997 through May 1998 in the Laboratoire de Recherche sur la Variabilité Génétique at the Faculté des Sciences campus

of Université Ibnou Zohr in Agadir, Morocco. Accessions were selected based on their tolerance for *Fusarium* spp. (Zahidi & Bani-Aameur, 1998) and growing location. Two distinct geographic locations were chosen: a coastal site, Aït Melloul; and a mountain site, Argana.

Five accessions were selected from each location. Five argan trees were selected in Aït Melloul from those growing in the Horticulture Complex of the Hassan II Agricultural and Veterinary Institute along the route from Agadir to Taroudant. For this study, these accessions were called Aït Melloul (AM). This site was located approximately 12.5 km east of the Atlantic Ocean and was approximately 35 m above sea level. The bioclimate of this area is semi-arid and hot and is strongly influenced by the proximity of the ocean keeping temperature extremes to a minimum (Emberger, 1955). Over a thirty-two year period, the average seasonal temperatures were: fall 11.5°C, winter 9.0°C, spring 23.9°C, and summer 26.5°C. The majority of the annual rainfall occurs between October and April with less than 250 mm per year cumulative precipitation (Farradous *et al.*, 1996). The soil is a calcareous, dark brown entisol/aridisol with a thick calcium carbonate hardpan at a depth of 80 cm (Sols Maroc, 1997). Woody species associated with argan on this site are *Acacia gummifera, Ziziphus lotus, Rhus pentaphylla, Gymnosporia senegalensis* and on the calcium crust *Senecio antheuphorbium* (Farradous *et al.*, 1996).

Another five accessions were selected from five trees in Argana (AR), located 60 km inland from the ocean on the southern slope of the High Atlas Mountains between Marrakech and Agadir. The altitude of this interior mountain location is approximately 620 m and is semi-arid and cool; however, spring and summer temperatures are very hot

(Emberger, 1955). Over a seven-year period, the average seasonal temperatures were: fall 6.7°C, winter 5.8°C, spring 30°C, and summer 39°C. The majority of the annual rainfall occurs between October and April and totals approximately 400 mm per year (Farradous *et al.*, 1996). The soil is an iron-rich red entisol (Sols Maroc, 1997). The associated woody species on this site were *Genista tricuspidata*, *G. ferox*, *Chamaecytusus albidus* (rare except at the base of the slope), *Launaea arborescens*, *Periploca laevigata*, and *Salvia aegyptiaca*. Other herbaceous plants were *Cymbopogon schoenanthus*, *Hyparrhenia hirta*, and *Androcymbium gramineum* (Ferradous *et al.*, 1996).

3.3. Materials and Methods

Fruits were collected during May and June 1997 and stored in plastic bags at room temperature. Following a protocol for pretreatment of argan seeds set forth by Zahidi and Bani-Aameur (1997), one hundred twenty fruits (60 for germination study and 80 for seedling study) from each accession were hulled, stored in plastic bags, and placed in cold storage (4°C) for stratification on December 1, 1997. Then on April 20, 1998, sixty of these stones were soaked in 2% chlorine solution for 15 minutes and rinsed three times with distilled water. The stones were scarified by slightly cracking them between two rocks and were immediately treated with the fungicide Thiodan and placed in the refrigerator for overnight storage. Between scarification treatments for each accession, the work area and rocks were cleaned with 10% chlorine solution to prevent contamination. On April 21, 1999, the scarified stones were soaked in a gibberellic acid solution (GA_3 at 1000 ppm) for 24 hours. The following day, April 22, 1999, the stones were then rinsed twice with distilled water and placed in 9-cm glass petri dishes filled with

sterile vermiculite that had been moistened with the appropriate NaCl treatment solution. The petri dishes were placed on a laboratory bench according to the experimental design. The mean temperature in the laboratory was 15°C (with a range of 12 - 20°C) during the experiment.

This factorial experiment was arranged in a randomized complete block design with two geographic origins, five accessions, five salt treatment levels, and three blocks. Salt treatments were 0 g/l, 2.5 g/l (3.9 dS/m), 5.0 g/l (7.8 dS/m), 7.5 g/l (11.7 dS/m) and 10 g/l (15.6 dS/m) NaCl. Each petri dish contained four stones with a total of 150 petri dishes used (600 stones). Treatment solutions were added as necessary to maintain moist vermiculite during the experiment. The treatment solutions were made with 95% NaCl and distilled water.

Seed germination was recorded daily. After twenty-one days, the root of each stone (the longest root if multiple seeds germinated from one stone) was measured and its fresh weight recorded. The roots were then placed in a 65°C oven for 24 hours. Each stone in each dish was examined for number of seeds, condition of seeds (ungerminated, healthy, dead), and presence or absence of fungus. The following day, the dried roots were removed from the oven and dry weights were recorded.

For the seedling study, eighty stones were removed from cold storage February 28, 1998, and the same pretreatment protocol was followed. On March 2, 1998, these stones were then planted one to two centimeters below the surface (Arif, 1994) in five-liter pots which had been filled with a 1:1:1 (peat moss:sand:argan forest soil) planting mixture. Soil from the argan forest is rich in mycorrhizae, which upon inoculation, significantly increases initial growth of argan seedlings (Nouaim & Chaussod, 1994). Each pot

contained four stones with a total of 200 pots (800 stones) used. The experiment location was outside in an open area away from the university buildings as well as tall trees.

This factorial experiment was arranged in a randomized complete block design with two geographic origins, five accessions, five salt treatment levels, and three blocks. Salt treatments were 0 g/l, 2.5 g/l (3.9 dS/m), 5.0 g/l (7.8 dS/m), 7.5 g/l (11.7 dS/m) and 10 g/l (15.6 dS/m) NaCl. The amount of salt in the tap water was considered negligible.

From March 1 to May 1, 1998, the pots were irrigated with tap water to maintain field capacity (approximately 175 ml/pot/day). Care was taken to avoid through-flow of water to prevent leaching. Germination began on March 26, 1998 (day 25) and leveled off thirty-seven days later. On May 2, 1998, the oldest, healthy seedling in each pot was selected and all other seedlings were removed. Initial height measurements were taken for each seedling. Fourteen pots did not have seedlings that were healthy and at least 10-days old. These have been treated as missing plots. For eight weeks (53 days), salt treatments were then added to the irrigation water according to the experimental design.

Seedling height measurements were taken every ten days and any dead seedlings were harvested. Fifty-three days after treatments began, all remaining seedlings were harvested. Shoot and root length measurements were taken for each seedling. Next the root and shoot were separated and then placed in a 65°C oven to dry for 24 hours. Dry weights were recorded for each root and shoot the following day. Diameters were less than one centimeter for all seedlings and were not recorded.

On the first day of salt treatment, seedling height varied from 1 to 12 cm. In order to compare seedlings, growth was calculated after each measurement. The recorded data for both studies were analyzed by general factorial analysis of variance (ANOVA) using

SPSS for Windows release 8.0. Where significance was found, least significant difference (LSD) were determined among the means ($p<0.05$). Data summaries and figures were made using of Microsoft Excel 7.0.

3.4. Results

Seed germination

Accessions were significantly different for germination; however the geographic origin, salt treatments, and interactions were not significant (Table 1). Germination peaked after 19 days and overall, forty-seven percent of the seeds germinated. The highest percent germination was found in the control and decreased with higher salinity levels (Figure 4). One accession from Aït Melloul (AM 105) and two accessions from Argana (AR 17 and AR 22) had combined germination greater than 60 %. The lowest germination was less than 30 % from AM 97 (27 %) and AR 102 (23 %) with a total germination range of 23-68% (Figure 5).

Table 1. ANOVA table for germination by geographic origin, accession, and NaCl treatments.

Dependent Variable: Number of germinated seeds

Source	df	Mean Square	F	Sig.
Block	2	1.2	0.7	.479 ns
Origin	1	2.9	1.9	.175 ns
Accession	4	4.7	3.0	.022 *
Treatment	4	2.3	1.4	.212 ns
Origin * Accession	4	0.8	0.5	.750 ns
Origin * Treatment	4	0.8	0.5	.750 ns
Accession * Treatment	16	1.8	1.2	.303 ns
Origin * Accession * Treatment	16	0.8	0.5	.922 ns
Error	98	1.6		
Total	149			

CV = 24.4% * significance at 5%

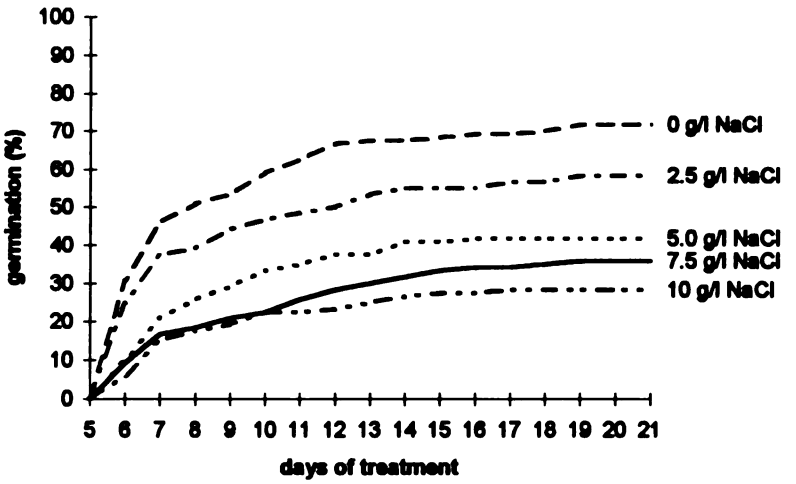

Figure 4. Percent germination by NaCl treatment levels, all accessions combined.

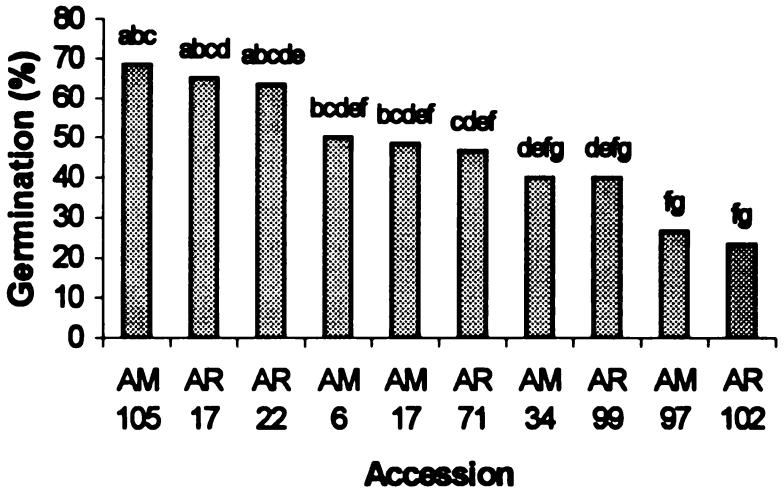

Figure 5. Percent germination of argan seeds by accession, all NaCl treatments combined. Bars with the same letters above are not significantly different (LSD 5%).

Seedling survival

Salt treatment significantly affected seedling survival (Table 2) and survival decreased with each increasing salt level (Figure 6). No significant differences were found for geographic origin of the seeds, accessions, interactions (Table 2).

Table 2. ANOVA table for seedling survival by geographic origin, accession and NaCl treatments.

Dependent Variable: Seedling survival

Source	df	Mean Square	F	Sig.
Block	3	0.1	1.6	.189 ns
Origin	1	0.0	0.7	.391 ns
Accession	4	0.1	1.2	.300 ns
Treatment	4	7.0	114.9	.000 **
Origin * Accession	4	0.1	1.6	.188 ns
Origin * Treatment	4	0.1	1.6	.188 ns
Accession * Treatment	16	0.0	0.7	.772 ns
Origin * Accession * Treatment	16	0.0	0.8	.637 ns
Error	147	0.1		
Total	199			

CV = 52.6% ** significance at 1%

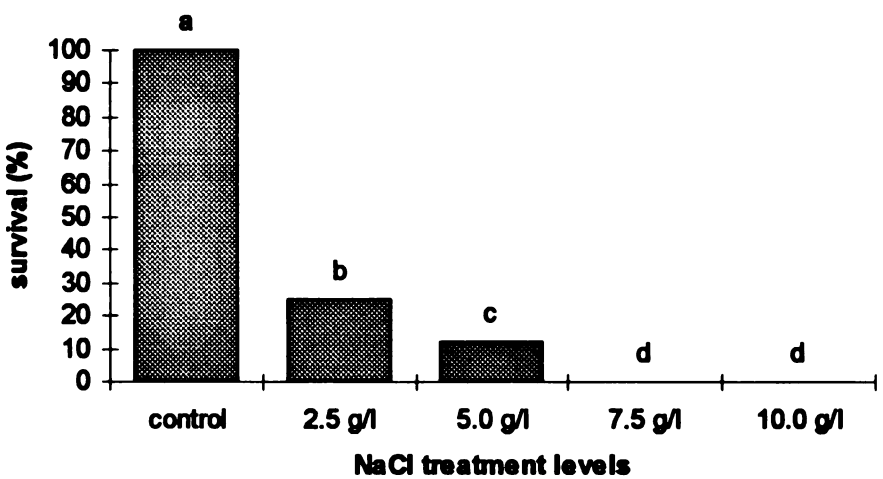

Figure 6. Percent of seedling survival by NaCl treatments, all accessions combined. Bars with the same letters above are not significantly different (LSD 5%).

Seedling height growth

ANOVA showed highly significant affects on height growth for accessions and treatments, but not for geographic location or interaction (Table 3). Growth decreased with increasing salt levels (Figure 7). All seedlings in the upper two salt levels (7.5 g/l and 10.0 g/l) died after 40 days of treatment (Figure 7). Height growth significantly decreased with each increasing salt treatment, with the two highest performing similarly (Figure 7). Additionally, no significant difference among treatments were noted until after 20 days of treatment (Tables 4 and 5). After eight days of treatment, salt became visable on pots receiving 5.0, 7.5, and 10.0 g/l NaCl. It is estimated that after10 days of treatment, salt accumulates had reached 0, 4.4, 8.8, 13.1, and 17.5 g NaCl in each respective treatment level. After 20 days, these accumulations were approximately 0, 8.8, 17.5, 26.2, and 35.0 g NaCl respectively.

Some accessions performed better under the salt treatments (Figure 8). An accession from Argana (AR 99) showed the greatest mean height growth under all the treatment levels. The next greatest height growth came from the site Aït Melloul (AM 97); the remaining accessions had similar growth.

Table 3. ANOVA table for total height growth by geographic origin, accession and NaCl treatments in seedling study.

Dependent Variable: Total growth (cm)

Source	df	Mean Square	F	Sig.
Block	3	7.1	2.2	.088 ns
Origin	1	2.5	0.8	.375 ns
Accession	4	27.2	8.5	.000 **
Treatment	4	166.1	52.0	.000 **
Origin * Accession	4	5.7	1.8	.135 ns
Origin * Treatment	4	5.5	1.7	.151 ns
Accession * Treatment	16	4.4	1.4	.162 ns
Origin * Accession * Treatment	16	2.3	0.7	.779 ns
Error	147	3.2		
Total	199			

CV = 7.5% ** significance at 1%

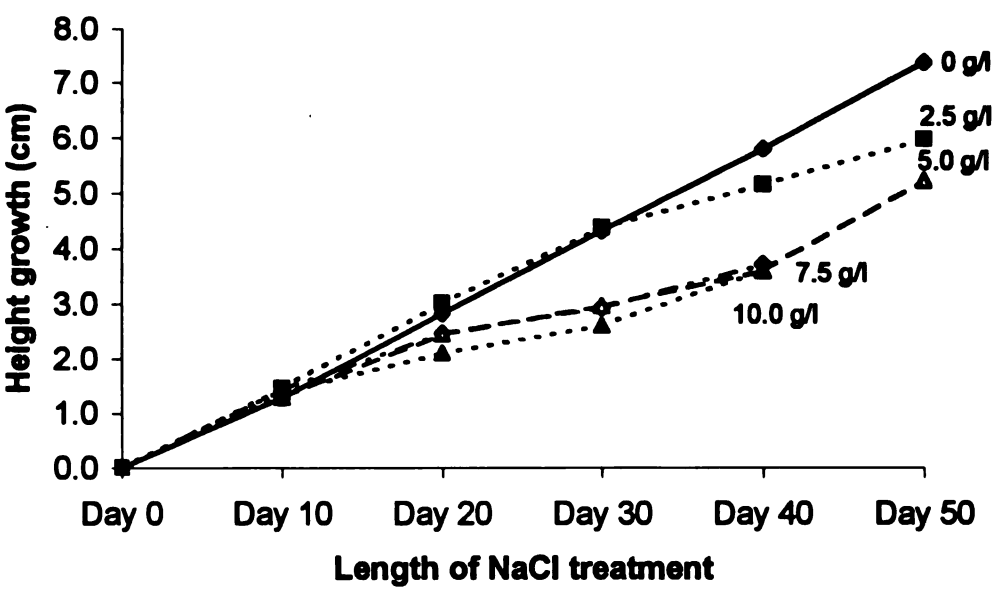

Figure 7. Cumulative height growth of surviving seedlings by NaCl treatment. Note: all seedlings receiving salt treatments 7.5 g/l (◊) and 10.0 g/l (□) died after 40 days.

Table 4. ANOVA table of seedling height growth after 10 days of NaCl treatments by accession and treatment.

Dependent Variable: Growth at day 10

Source	df	Mean Square	F	Sig.
Block	3	1.3	2.8	.043 *
Accession	9	1.3	2.9	.004 **
Treatment	4	0.5	1.0	.382 ns
Accession * Treatment	36	0.4	0.9	.630 ns
Error	147	0.4		
Total	199			

* significance at 5%; ** significance at 1%

Table 5. ANOVA table of seedling height growth after 20 days of NaCl treatments by accession and treatment.

Dependent Variable: Growth at day 20

Source	df	Mean Square	F	Sig.
Block	3	0.7	1.4	.243 ns
Accession	9	1.4	2.9	.004 **
Treatment	4	5.5	11.4	.000 **
Accession * Treatment	36	0.5	1.1	.322 ns
Error	147	0.5		
Total	199			

** significance at 1%

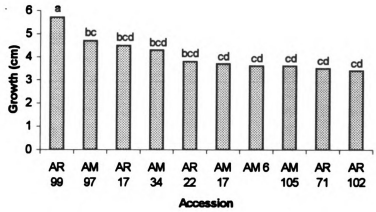

Figure 8. Mean seedling height growth by accessions (growth data summed over all NaCl treatment levels). Bars with the same letters above are not significantly different (LSD 5%).

Root length

Analysis of variance (ANOVA) for root length showed high significance within treatments and accessions for the germination study (Table 6); however, no significance was seen in the seedling study (Table 7). Neither study found significance for geographic origin or in the interactions (Table 6 and 7). In the germination study, salt treatments reduced root growth with increasing levels (Figure 9). The first three treatments were each significant when looking at least significant difference (LSD), while the two highest treatment levels were not significantly different (LSD 5%). As salt treatment levels increased beyond the 5.0 g/l treatment, root death significantly increased. Over 50% root mortality was evident at 7.5 g/l and that increased to over 80% at 10.0 g/l (Table 8). Accessions AM 34, AM 17 and AR 71 showed the longest root lengths while AR 102 and AR 99 showed the shortest (Figure 10).

Table 6. ANOVA table for root length by geographic origin, accession and NaCl treatments in germination study.

Dependent Variable: Root length (cm)

Source	df	Mean Square	F	Sig.
Block	2	32.0	2.1	.123 ns
Origin	1	39.6	2.6	.107 ns
Accession	4	52.5	3.5	.010 **
Treatment	4	504.5	33.7	.000 **
Origin * Accession	4	6.3	0.4	.794 ns
Origin * Treatment	4	4.7	0.3	.868 ns
Accession * Treatment	16	6.7	0.4	.965 ns
Origin * Accession * Treatment	16	3.2	0.2	.999 ns
Error	98	15.0		
Total	149			

CV = 5.6% ** significance at 1%

Table 7. ANOVA table for root length by geographic origin, accessions, and NaCl treatments in seedling study.

Dependent Variable: Root length (cm)

Source	df	Mean Square	F	Sig.
Block	3	50.6	0.4	.732 ns
Origin	1	25.4	0.2	.643 ns
Accession	4	273.5	2.3	.059 ns
Treatment	4	251.6	2.1	.079 ns
Origin * Accession	4	114.7	1.0	.423 ns
Origin * Treatment	4	280.3	2.4	.054 ns
Accession * Treatment	16	147.2	1.2	.236 ns
Origin * Accession * Treatment	16	173.8	1.5	.115 ns
Error	147	117.6		
Total	199			

CV = 1.0%

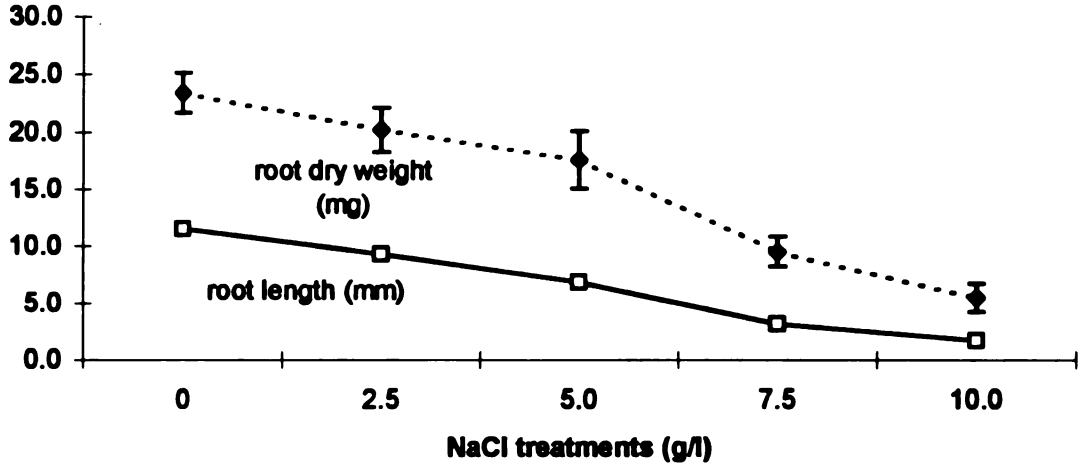

Figure 9. Root dry weight (---) (mean + standard error) and root length (—) (mean with standard error smaller than size of point) by NaCl treatments, all accessions combined, in germination study.

Table 8. Germinated stones (all accessions combined) by NaCl treatments and root conditions after 21 days in germination study.

NaCl	Germinated		Healthy roots		Dead roots	
Treatment	#	%	#	%	#	%
control	87	72.5	79	90.8	8	9.2
2.5 g/l	71	59.2	66	93.0	5	7.0
5.0 g/l	50	41.7	42	84.0	8	16.0
7.5 g/l	43	35.8	20	46.5	23	53.5
10.0 g/l	34	28.3	6	17.6	28	82.4

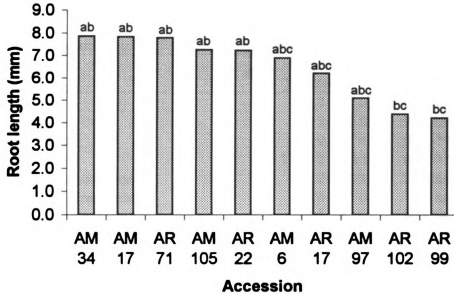

Figure 10. Mean root lengths by accessions, all NaCl treatments combined. **Bars with the same letters above are not significantly different (LSD 5%) in germination study.**

Root dry weight

The germination study found highly significant differences for root dry weight between salt treatments and accessions, however neither geographic origin nor interactions were significant (Table 9). Root dry weights declined as salt treatment levels increased (Figure 9). Two accessions from Argana (AR 22 and AR 71) performed the best, while AR 102 performed the worst (Figure 11).

Root dry weight in the seedling study was significant for salt treatments (Table 10) and decreased with increasing salt treatment levels (Figure 12). No significance was found for geographic origin, accession, or interactions (Table 10).

Table 9. ANOVA table for root dry weight by geographic origin, accession and NaCl treatments in germination study.

Dependent Variable: Root dry weight (mg)

Source	df	Mean Square	F	Sig.
Block	2	51.7	0.5	.621 ns
Origin	1	0.3	0.0	.956 ns
Accession	4	434.9	4.0	.005 **
Treatment	4	1689.2	15.7	.000 **
Origin * Accession	4	141.7	1.3	.270 ns
Origin * Treatment	4	51.4	0.5	.753 ns
Accession * Treatment	16	37.2	0.3	.991 ns
Origin * Accession * Treatment	16	35.5	0.3	.993 ns
Error	98	107.9		
Total	149			

CV = 2.8% ** significance at 1%

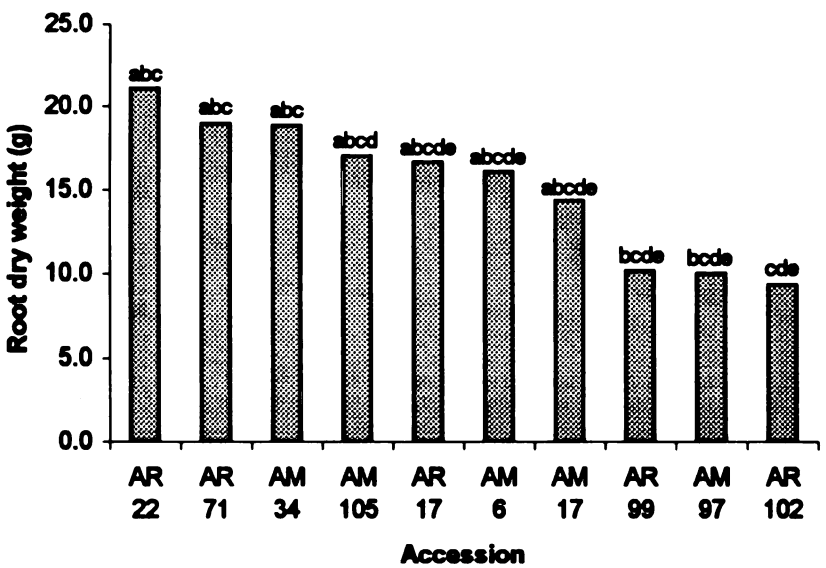

Figure 11. Mean root dry weights by accessions, all NaCl treatments combined. Bars with the same letters above are not significantly different (LSD 5%) in germination study.

Table 10. ANOVA table for root dry weight by geographic origin, accessions, and NaCl treatments in seedling study.

Dependent Variable: Root dry weight (mg)

Source	df	Mean Square	F	Sig.
Block	3	815.8	0.2	.913 ns
Origin	1	8713.8	1.9	.174 ns
Accession	4	9637.1	2.1	.089 ns
Treatment	4	210072.3	45.0	.000 **
Origin * Accession	4	8143.0	1.7	.144 ns
Origin * Treatment	4	6900.5	1.5	.212 ns
Accession * Treatment	16	2597.2	0.6	.912 ns
Origin * Accession * Treatment	16	5410.6	1.2	.309 ns
Error	147	4672.3		
Total	199			

CV = 0.2% ** significance for 1%

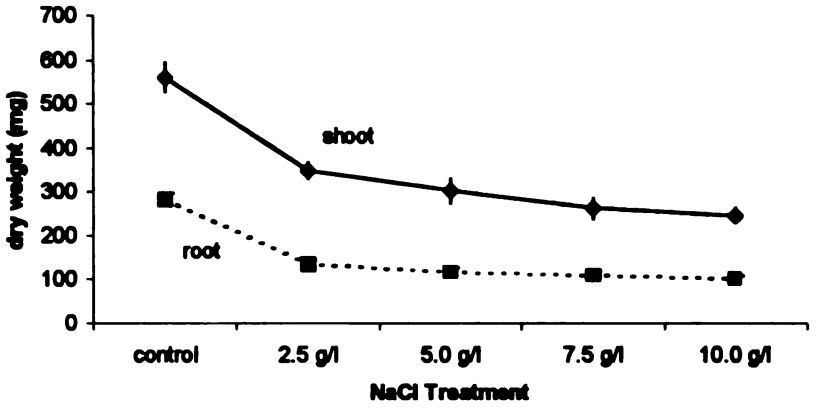

Figure 12. Root and shoot dry weights (mean + standard error, if no line then s.e. is smaller than data point) of dead seedlings by NaCl treatments, all accessions combined.

Seedling root:shoot ratio

Root:shoot ratio calculated on both length (Table 11) and dry weight (Table 12) basis was significant among the salt treatments. The ratio increased for each salt level except 10.0 g/l, which decreased in comparison to 5.0 g/l and 7.5 g/l; however, this was not significantly different from the other treatment levels, with the exception of the control (LSD 5%) (Figure 13). No significance was found for accessions or geographic seed origin (Tables 11 and 12).

Table 11. ANOVA table for root:shoot length ratio by geographic origin, accession and NaCl treatments in seedling study.

Dependent Variable: Root:shoot length ratio

Source	df	Mean Square	F	Sig.
Block	3	1.5	0.6	.642 ns
Origin	1	4.0	1.5	.225 ns
Accession	4	5.1	1.9	.113 ns
Treatment	4	14.4	5.4	.000 **
Origin * Accession	4	3.2	1.2	.322 ns
Origin * Treatment	4	3.6	1.3	.260 ns
Accession * Treatment	16	3.0	1.1	.328 ns
Origin * Accession * Treatment	16	3.0	1.1	.335 ns
Error	147	2.7		
Total	199			

CV = 11.4% ** significance at 1%

Table 12. ANOVA table for root:shoot dry weight ratio by geographic origin, accession and NaCl treatments in seedling study.

Dependent Variable: Root:shoot dry weight ratio

Source	df	Mean Square	F	Sig.
Block	3	0.0	0.2	.886 ns
Origin	1	0.1	4.6	.034 ns
Accession	4	0.0	1.4	.252 ns
Treatment	4	0.1	4.0	.004 **
Origin * Accession	4	0.0	1.0	.424 ns
Origin * Treatment	4	0.0	1.0	.385 ns
Accession * Treatment	16	0.0	0.6	.911 ns
Origin * Accession * Treatment	16	0.0	1.2	.239 ns
Error	147	0.0		
Total	199			

CV = 68.8% ** significance at 1%

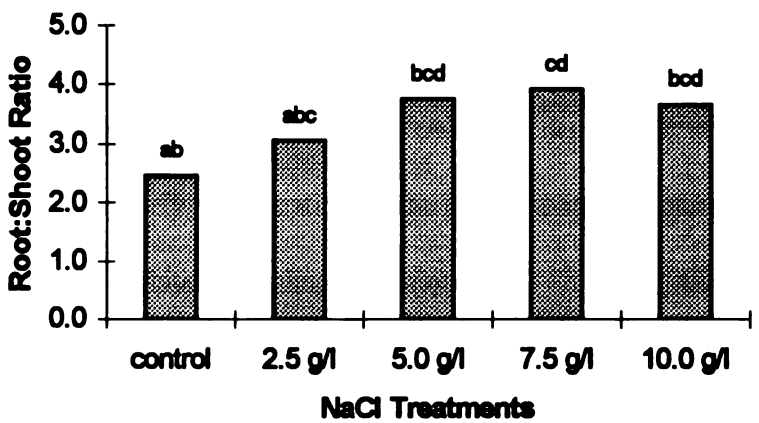

Figure 13. Average root:shoot length ratio by NaCl treatments, all accessions combined. Bars with the same letters above are not significantly different (LSD 5%) in seedling study.

3.5. Discussion

The salt treatment levels in these experiments ranged from slightly saline to strongly saline (Abrol, 1988). Salt sensitive plants will be adversely affected by salinity at 2-5 dS/m (1.3-3.2 g/l NaCl), while salt tolerant plants are not adversely affected until salinity reaches 10 dS/m (6.4 g/l NaCl). The growth of halophytes are stimulated between 5 and 15 dS/m (3.2-9.6 g/l NaCl) and may decline when salts exceed 25 dS/m (16 g/l NaCl) (BOSTID, 1990). The argan seedlings showed no increased growth and significant negative affects were recorded at the lowest salt level (2.5 g/l NaCl or 3.9 dS/m).

Root growth and root dry weight showed decreasing values with increasing salt treatments with the exception of germination. It is possible that the effects of GA negated saline effects on argan germination (Bewley & Black, 1994; Khan & Rizvi, 1994; Khan & Ungar, 1998). However, for those argan seeds that did germinate, growth was negatively impacted under saline conditions as most plants are in arid and semi-arid regions (Kigel, 1995). Tomar and Yadav (1985) found that *Acacia nilotica*, *Eucalyputs hybrida* and *Prosopis juliflora* all significantly decreased growth and dry weight when salinity levels reached 7 dS/m. Conversely, germination of the salt-tolerant companion crop of argan, barley, is not severely inhibited by saline conditions until 12 dS/m (Abrol, 1988).

Based on the results of these studies, there were no salt tolerance differences evident due to the two geographic origins of seeds. However, there was some variation in salt tolerance among individual accessions. The best germination and root growth was seen in accessions AM 105, AR 17 and AR 22. The seedling study did not reveal one or more accessions performing better than the others. Some accessions may be more tolerant

of saline conditions than others. However, more research is needed to determine a clear salt tolerance range for argan at each developmental stage (germination, establishment, and maturity). As this tree has yet to become domesticated, there may be other accessions that have a greater tolerance to salinity.

After 21 days, the end of the germination experiment, argan germination indicated a clear and consistent pattern. As salt treatment levels increased, percentage of germination declined. Arid and semi-arid plants have different germination strategies in establishing themselves in this harsh climate. There are many environmental factors (e.g. water availability, temperature, light, salinity) that interact and regulate seed germination (Kigel, 1995). Ungar (1995) found that some seeds have a salt-induced dormancy where seeds remain dormant until saline conditions cease to be present. In this study, the environmental factors were kept at optimal levels, with the exception of salinity. It is possible that argan seeds may have a salt-induced dormancy, however it is not clear from this study as gibberellic acid (GA) was used to stimulate germination (Bewley & Black, 1994; Khan & Rizvi, 1994; Khan & Ungar, 1998). It would be valuable to repeat this study without GA to determine possible seed dormancy.

The clear decline in dry weight, both root (for both studies) and shoot, with increasing salt treatment levels is consistent with other salinity studies. Gutierrez Boem *et al.* (1994) examined the effects of salinity on rapeseed (*Brassica napus*) and found the root dry weights decreased with increasing salinity. Ungar (1996) found the same results with the halophytic grass, *Atriplex patula*. Similarly, studies on trees (*Prosopis juliflora*, *Acacia nilotica*, *Eucalyptus hybrida*, and *Citrus* spp.) found the total plant dry weight

decreased with increasing salinity treatments (Tomar & Yadav, 1985; Bañuls & Primo-Millo, 1995).

Barley which is grown with mature argan trees is highly salt tolerant. At an EC level of 10 dS/m, barley germination is still greater than 60% (Abrol, 1988). More research is needed to determine if mature argan is more tolerant of salinity than at the establishment stage. It may be that mature argan trees have root systems which extend below the salt-affected soil. With irrigation, however, the salts may be leached to these lower levels and cause decline for even the established mature argan trees.

The response of growth and root:shoot ratio was indicative of a plant under salt stress. As Kafkafi & Bernstein (1996) noted for plants experiencing salt stress, argan shoot growth was affected while the roots were not. Only in the highest treatment level (10.0 g/l NaCl) was root length reduced, however, it was not statistically significant.

Kigel (1995) found that seed germination and seedling establishment are the two most critical stages for survival in arid and semi-arid regions. In the germination study, argan germination was negatively impacted by salinity. Results from this study demonstrated that argan seedlings were also salt sensitive, and the planting of argan in saline soils should be avoided. It is possible that older seedlings, which had hardened off or become lignified, may have yielded different results. Further study using lower salt treatments would be helpful to define argan salt tolerance limits.

Lastly, the data questions the common perception documented by Nouaim *et al.* (1991) that argan grows in saline soils. The results strongly indicate that argan is salt sensitive and should not be grown under saline conditions. While it is possible that

established argan trees continue to grow when surface soil has become saline, additional research is needed give a definitive answer.

CHAPTER 4

CONCLUSION

4.1. Limitations of Study and Future Research

Limitations

One limiting factor that pushed back the start date of both experiments was ambient temperature. Even though the argan fruits were available and ready for planting by January 1, 1999, it was too cold to plant the seeds (<10°C). By March, the temperature rose to above 10°C and the seedling study was initiated. It was not until mid-April that the temperatures inside the university buildings rose above 10°C allowing for better conditions for the germination study.

The greater limitation was time. This was partly due to the delay caused by cold temperature and also the time frame of the funding grant. Thus, experimental designs that could be adequately carried out with the remaining time of the grant had to be used.

Besides time and weather, argan seed viability is still very unreliable which resulted in fourteen missing plots for the seedling study. Working within this limitation, however, is not difficult. Pregermination of argan is highly recommended before transplanting for research purposes.

Future Research

In carrying out this research, it became clear that argan seed viability needs further investigation. Determining the maximum storage life at room temperature and in cold storage will help prevent using non-viable seeds. In addition, developing pretests that would remove immature or rotten seeds before planting would greatly improve

current practices. One possibility might be to develop a floatation test such as one used with acorns (Teclaw & Isebrands, 1986).

While the experiments carried out in this paper determined that argan is salt-sensitive, another study to determine seed dormancy caused by salt would enhance our understanding of argan's attempt in surviving in the arid and semi-arid regions. The salt treatments of the seedling study were cumulative. To better understand the effects on juvenile growth, a similar study with constant soil salinity levels may reveal accessions which could survive on moderately saline.

Additionally, a long-term study should be initiated to investigate the effects of salinity on mature trees. This study should attempt to model the current irrigation practices of the Souss valley where the tree is rapidly declining. Lastly, understanding the agrosilvopastoral system with argan, barley and livestock would also help to discover desirable traits of this wild tree and its interactions with crops and livestock.

4.2. Conclusions and Recommendations

Argan seeds and seedlings are negatively affected by salt. In slightly saline soil (2 dS/m) argan exhibited stress typical of other salt-sensitive plants. It is possible that argan seeds have a salt-induced dormancy that protect them from germinating during periods of high salinity; however, it also appears that seed viability is short allowing for a very narrow window of opportunity for establishment.

Both studies examined argan during the establishment phase, which is considered the most critical in arid and semi-arid regions. While these results cannot give a clear salt tolerance range for this phase, it is evident that argan cannot establish itself on salt-affected soils or with saline irrigation. It is also possible that if salt treatments were

begun after the seedlings had become lignified, the results would have been different. Many halophytes and salt-tolerant plants do not perform well in the establishment phase. These results can only identify salt as another limiting factor for argan establishment, but more research may reveal that established and mature trees can overcome saline soil conditions. If significant natural regeneration of argan is not occurring, surface soil salinization many be another barrier for argan to overcome. While argan may be able to bypass saline conditions with a deep taproot, neither seeds nor seedlings should be planted in saline soils.

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