Ministry of Agriculture, Water Resources and Fishing

National Institute of Research on Rural Engineering, Water and Forests.Tunis -Tunisia

Leaf anatomical and ultrastructural responses to salt stress of three *Populus alba* L. clones

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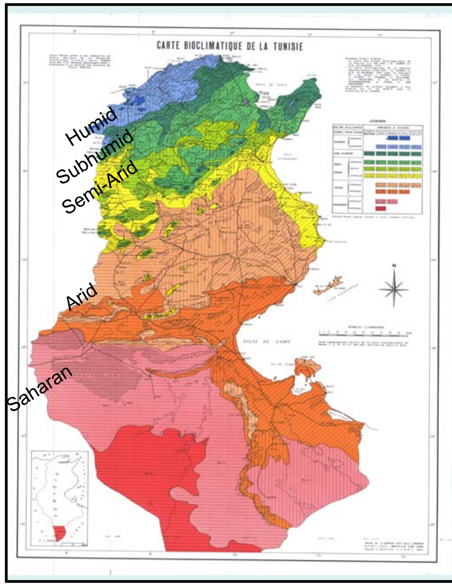

♦ Tunisia is characterised by a low rainfall and a wide variability of rainfall within the year and through the country

- o Humid to subhumid at the extreme North : 500 – 1000 mm / year
- oSemi – arid in the North and the Center : 400 – 600 mm/ year
- o Arid in the center : 200 – 400 mm / year
- oSaharan in the south : 100 – 200 mm / year

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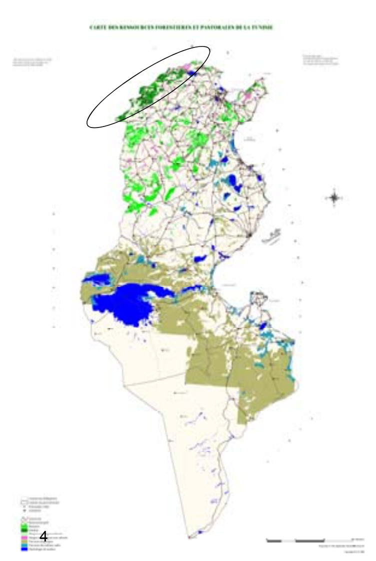

- There are three major agro – ecological zones :
- Forest areas located in Northwest Tunisia, where agro – forestry systems and agriculture are prominant.
- Agro-pastoral areas in north and Central Tunisia where livelihoods rely on agriculture, forest plantation and agro-pastoral systems.
- Arid areas in Southern Tunisia, where livelihoods rely on pastoral systems coupled with oasis cropping using deep groundwater resources.

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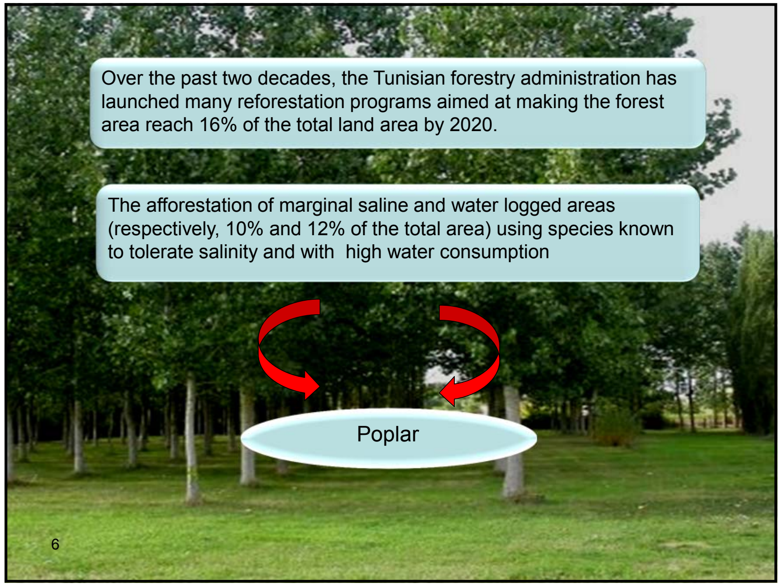
Over the past two decades, the Tunisian forestry administration has launched many reforestation programs aimed at making the forest area reach 16% of the total land area by 2020.
The afforestation of marginal saline and water logged areas (respectively, 10% and 12% of the total area) using species known to tolerate salinity and with high water consumption
Poplar
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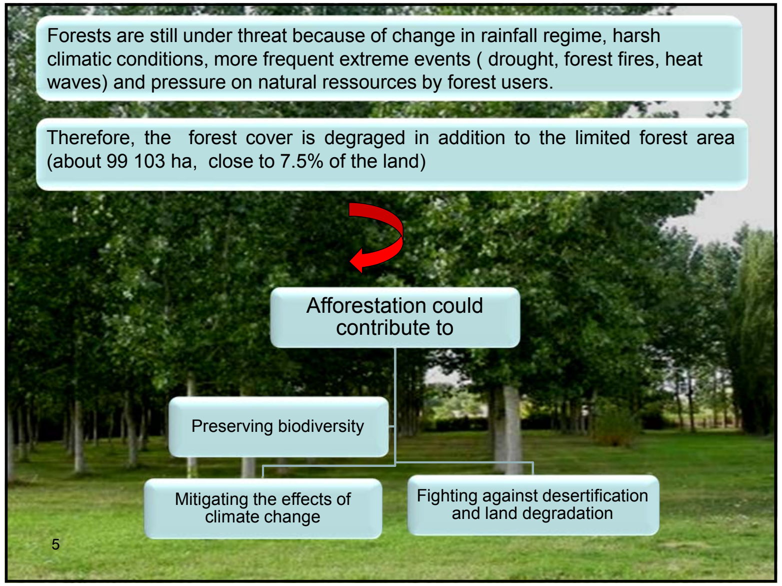
Forests are still under threat because of change in rainfall regime, harsh climatic conditions, more frequent extreme events (drought, forest fires, heat waves) and pressure on natural ressources by forest users.
Therefore, the forest cover is degraged in addition to the limited forest area (about 99 103 ha, close to 7.5% of the land)
Afforestation could contribute to
Preserving biodiversity
Mitigating the effects of climate change
Fighting against desertification and land degradation
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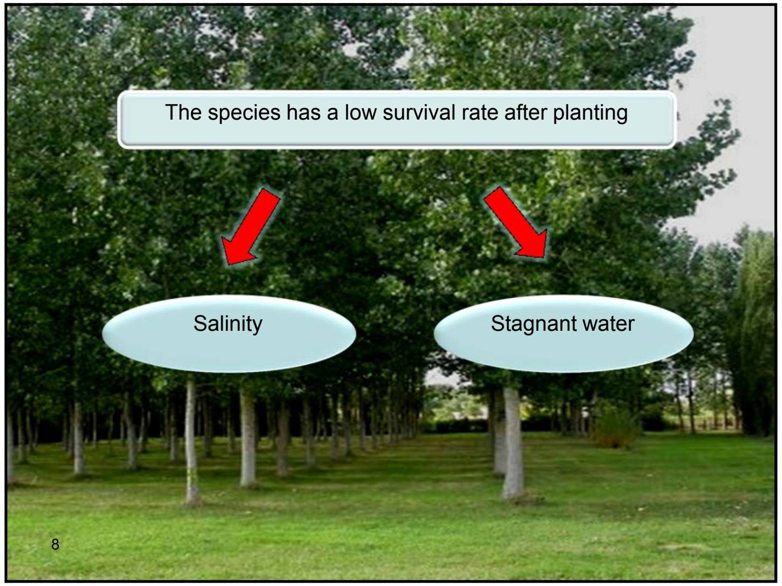
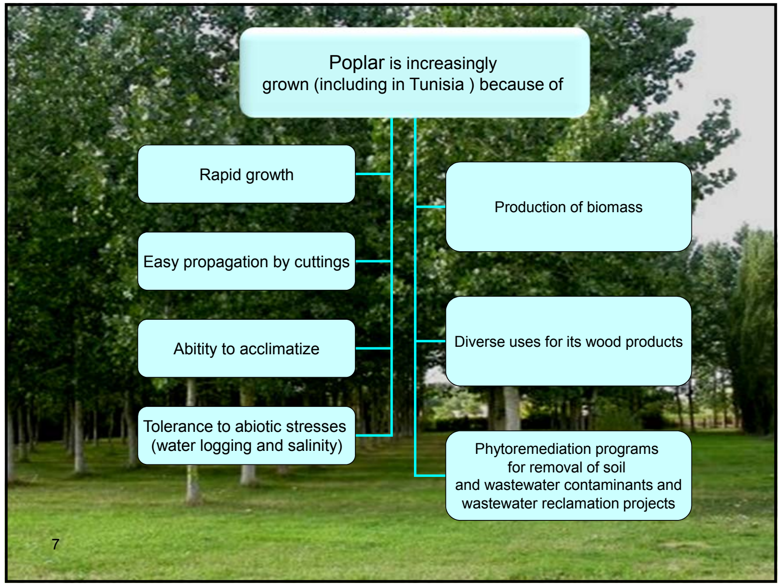

The species has a low survival rate after planting
Salinity
Stagnant water
8


Poplar is increasingly grown (including in Tunisia) because of
Rapid growth
Easy propagation by cuttings
Abitity to acclimatize
Tolerance to abiotic stresses (water logging and salinity)
Production of biomass
Diverse uses for its wood products
Phytoremediation programs for removal of soil and wastewater contaminants and wastewater reclamation projects
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Comparing the responses of different *P. alba* (18) and *P. nigra* (13) clones to increasing levels of salinity, results showed that *P.nigra* is sensitive to salinity, whereas there is an encouraging tolerance degree of *P. alba* but with great variability among clones (Abassi et *al*. 2004).

With regard to the high variability of *P. alba* clones, a knowledge of their

The overall goal of this study is to define the adaptive mechanisms developed by three Tunisian *P. alba* clones, different in terms of their tolerance, in response to a long term salt stress, through induced leaf micromorphological, leaf anatomical and chloroplasts modifications.

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Two research programs were conducted in the Laboratory of Forest Ecology

The resistance of poplar to salinity

The resistance of poplar to water logging

Plant material

One year-old *Populus alba* L.var. *Hichkeliana* (Dode) Marie clones. MA-104 (salt-tolerant), MA-195 (moderately salt-tolerant) and OG (salt-sensitive) (Abassi et al. 2004) .

Growth conditions

- Semi-controlled
-.- Container:free-draining plastic pots (45 depth × 30 cm diameter)
- Substratum : coarse river sand
- Fertigation : Hoagland solution
- Treatments : 0, and 6g/l NaCl were carried out during two successive years.

Experimental Design

A split-plot experimental design with twelve replicates was used with clones as the main plot and water regime (control and stress) as subplot in a factorial experiment.

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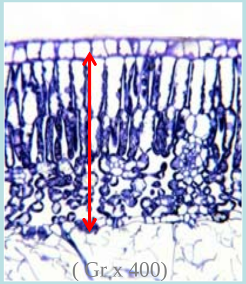
MA – 104 clone (salt - tolerant)

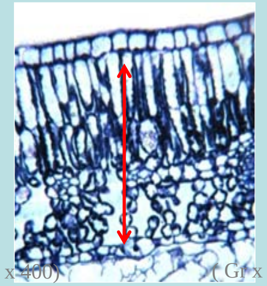
Thickening the mesophyll

Increase in the cell size of the palisade parenchyma cells

Spongy parenchyma became larger and looser



(Gr x 400)



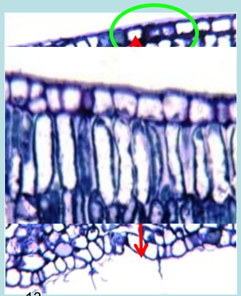
(Gr x 400) (Gr x 400)

12 **Control** **Salt treatment (6g/l NaCl)**

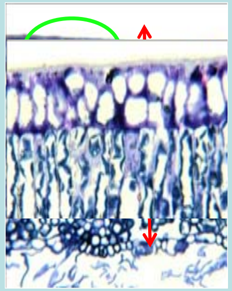
MA-195 clone
(Intermediate salt - tolerant)

Narrowing of the thickness of the mesophyll which has a compact appearance.

Leaf cuticle and upper epidermis became thicker



Control



Salt treatment 6g/l NaCl

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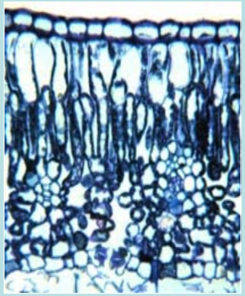
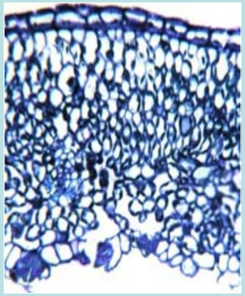
OG clone (salt - sensitive)

Salt stress reduced both epidermal and parenchyma cell size showing an atypical structure in mesophyll in comparison with control.



Control



Salt treatment 6g/l NaCl

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Leaf succulence resulting from increase in leaf thickness as a response to salinity, was noted in the case of MA-104 clone (salt – tolerant).

In the case of MA-195 (Intermediate salt – tolerant), the mesophyll thickness was reduced but leaf cuticle and upper epidermis became thicker.

In the case of OG (salt – sensitive), salinity reduced both epidermal and parenchyma cell size resulting in an atypical structure in mesophyll.

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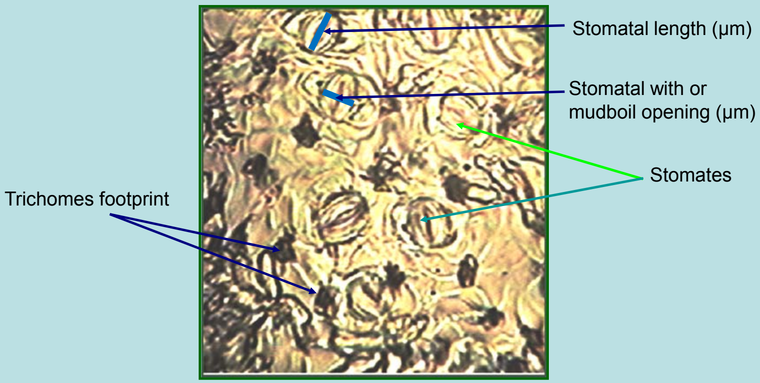
Leaf micromorphological parameters



Epidermal footprint

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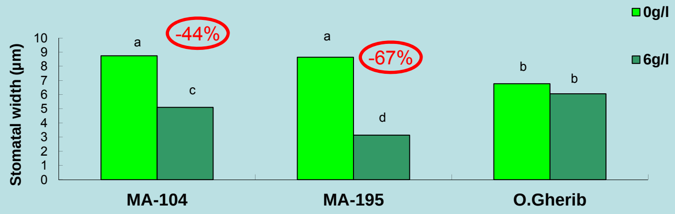

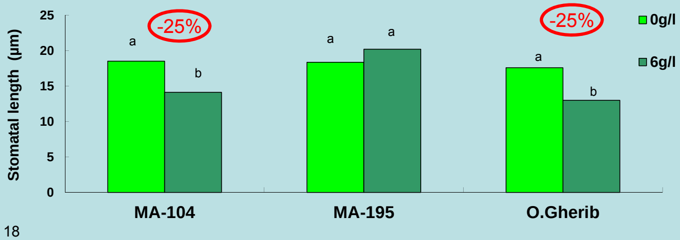

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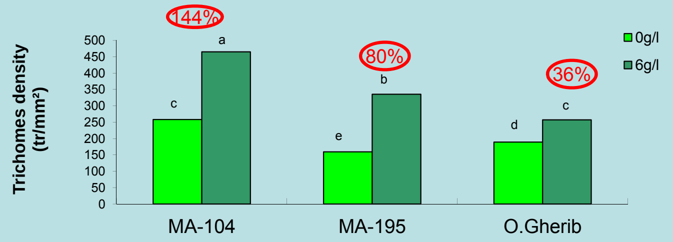

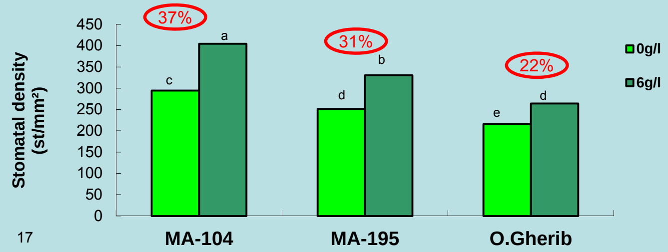

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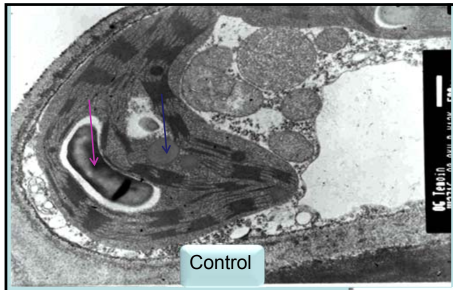
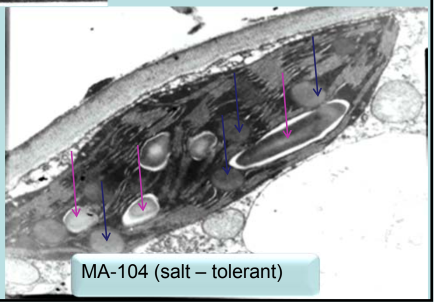


Control

Chloroplast of control *P.alba* plant exhibiting well - developped grana and stromal thylakoids, starch granules and plastoglobules.

Under 6g/l NaCl: The arrangement of thylakoids was slightly disturbed.

The number of plastoglobules and the number of strarch granules increases.



MA-104 (salt – tolerant)

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The measure of the Leaf micromorphological parameters has shown a **high degree of variation** between clones

It was observed that the clone **MA-104 (salt – tolerant)** has developed the **highest density of trichomes and stomata.**

It showed a **plasticity of it's stomata** as evidenced by a **reduction** in **their length** and their **width** or **opening of the mudboils**.

The clone **MA-195 (intermediate – salt tolerant)** showed a smaller **increase in the number of stomata** compared to MA-104, **but the opening of its mudboils is greatly reduced**.

The clone **OG (salt - sensitive)** developed the **lower stomatal density** and no **reduction of the opening** of mudboils related to **low tolerance** to salinity.

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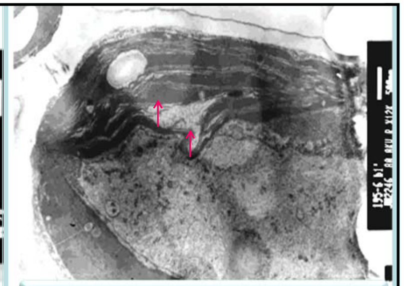
OG (salt – sensitive)



MA-195 (intermediate salt – tolerant)

Salt stress induced damages on chloroplast ultrastructure: Swelling of thylakoids, increase in number and cell size of plastoglobules, disruption of chlorpoplast membrane.

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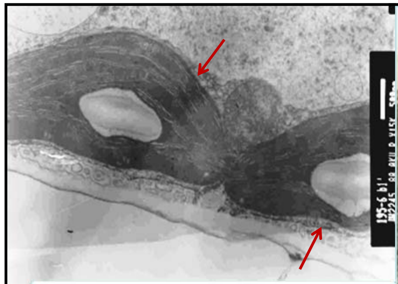
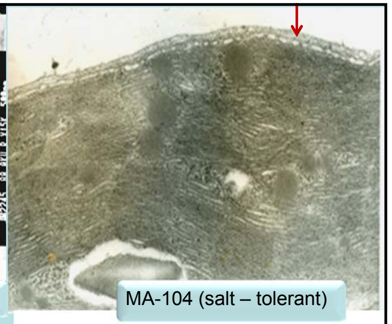


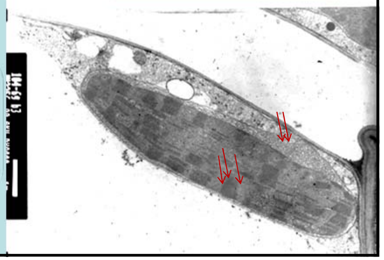
MA-195 (intermediate salt – tolerant)



MA-104 (salt – tolerant)

A peripheral reticulum is present in chloroplasts of MA-195 and

The MA-104 clone (salt – tolerant) showed a well organized thylakoids and grana (triple arrow) and a well developped peripheral reticulum (double arrow)



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The electron microscopy showed an increase in starch accumulation and in number or size of plastoglobules, a disorganization on the thylakoid structure and a disruption in chloroplast membrane of NaCl treated leaves.

However, the clones displayed differences in their responses to salinity

A swelling of thylakoid, an increase in the number and size of plastoglobules and disruption of chloroplast membrane were produced by NaCl in chloroplasts of the most stressed clone.

Whereas, in the tolerant one, thylakoid structure organization was maintained, increases in the number of plastoglobules as well as a decrease on their size were produced.

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The **occurrence of a chloroplast peripheral reticulum** has been noted in MA-104 and MA-195 clones (**C_3 photosynthesis pathway**).

Although the presence of peripheral reticulum has been proposed as an important criterion **unique to C4** plants (Laestch 1974 a), this structure has been described in Wheat (C3 plant).

The peripheral reticulum is contiguous with the inner membrane and the lamellar membranes in the grana and stroma of the chloroplasts

It has been suggested that its function is one of **facilitating the transport of photosynthate** to the peripheral region of the chloroplast.

Peripheral reticulum, can be the cause of the highest tolerance to salinity of MA-104 clone.

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Perspectives

Populus alba clones like MA – 195 and especially **MA-104** can be used as windbreaks in croplands, marginal areas, river banks and contribute to reduce the wood deficit in our country

Reclamation of marginal areas through the creation of irrigated schemes using treated waste water by planting rapid growth forest species such as Poplar and Salix.

Use of such fast species with short-term exploitations in phytoremediation programs for removal of soil and wastewater contaminants and production of bio - energy.

Saline zones could be managed by introducing rapid growth species, tolerant to salinity such as ***Populus Euphratica***.