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Seed production in *Atriplex halimus*: Effect of ploidy on seed size, germination capacity and initial plant vigour

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SUMMARY – Mediterranean saltbush (*Atriplex halimus*) has been evaluated by ICARDA and other Mediterranean research institutions as a potential alley crop with cereals to provide protein and mineral supplement to cereal straw and stubbles, and in regard to its other environmental effects such as control of soil erosion in cultivated steep slopes, and as a refuge and feed for wild fauna. Recent results have shown the existence of diploid and tetraploid saltbush populations in the Mediterranean basin, and that saltbush ploidy has an effect on the plant tolerance to environmental stresses, the quantity of its edible biomass, and also the quantity and quality of seed it produces and the vigour of young seedlings. Tetraploid saltbush populations produce more seed per plant, larger seeds and more vigorous seedlings than diploid saltbush populations; however, diploid saltbush fruits contain a larger proportion of seed and seeds germinate better; beside these ploidy differences, there is also a large inter population variability. All together, these factors justify the need to evaluate the quality of the seed before any large saltbush plantation programme is started.

Key words: *Atriplex halimus*, ploidy, seed production, germination.

RESUME – *"Production de graines chez Atriplex halimus : effet de la ploïdie sur la taille des semences, la capacité germinative et la vigueur initiale de la plante". Le Pourpier de mer (*Atriplex halimus*) a été évalué par ICARDA et d'autres établissements méditerranéens de recherches comme une récolte potentielle cultivée en couloirs avec des céréales pour fournir la protéine et le supplément minéral à la paille et aux chaumes de céréale, et en vue de ses autres effets sur l'environnement tels que le contrôle de l'érosion du sol dans les pentes raides cultivées, et comme refuge et alimentation pour la faune sauvage. Les résultats récents ont montré l'existence de populations diploïdes et tétraploïdes de* A. halimus *dans le bassin méditerranéen, et cette ploïdie chez A. halimus a un effet sur la tolérance de la plante aux effets adverses environnementaux, la quantité de sa biomasse comestible, et également la quantité et la qualité de la graine qu'elle produit et la vigueur des jeunes plantes. Les populations tétraploïdes de* A. halimus *produisent plus de graines par plante, de plus grandes graines et des jeunes plantes plus vigoureuses que les populations diploïdes de* A. halimus *; cependant, les fruits diploïdes de* A. halimus *contiennent une plus grande proportion de graines et les graines germent mieux ; outre ces différences de ploïdie, il y a également une grande variabilité inter populations. Tous ensemble, ces facteurs justifient la nécessité d'évaluer la qualité de la graine avant de commencer un quelconque grand programme de plantation de* A. halimus*.*

Mots-clés : Atriplex halimus*, ploïdie, production de semences, germination.*

Introduction

Mediterranean saltbush (*Atriplex halimus*) has been evaluated by ICARDA and other Mediterranean research institutions as a potential alley crop with cereals to provide protein and mineral supplement to cereal straw and stubbles (Jones and Arous, 2000; Correal and Sotomayor, 1999; Sotomayor and Correal, 2000), and in regard to its other environmental effects such as control of soil erosion in cultivated steep slopes, and as a refuge and feed for wild fauna. Recent results have shown the existence of diploid and tetraploid saltbush populations in the Mediterranean basin (Ortiz-Dorda *et al*., 2005; Walker *et al*., 2005) and differences in flower architecture and phenotypes, describing *A. halimus* as trimonoecious (with male, female and bisexual flowers) (Talamani *et al.,* 2001, 2003).

Materials and methods

Fruit size and seed production. Fruits of 19 *Atriplex halimus* populations (11 spp. *schweinfurthii,* 8 spp. *halimus)* cultivated in Mazarrón (Murcia, SE-Spain) were sampled in December 2000 and analysed; 3 shrubs per population, 2 replicates/shrub, 50 fruits/replicate: 300 fruits/population.

Fruit production. Fruits of 4 populations (Riff, Sousse, Figueretes, Cieza) were sampled in February 2002 at Mazarrón; total production/plant of fruits, leaves and young stems was measured.

Seedling growth rate. Fruits used in the experiment were harvested at Mazarrón on 22 December 2000; 7 accessions were collected; 3 of spp. *schweinfurthii* (Heraclion, Ziz, Beer Sheva); 4 of spp. *halimus* (Almudevar, Marsella, Cala Tarida, Cordoba); 3 shrubs/population and 12 seedlings/shrub. Fruits were sown in PVC trays maintained in a growth cabinet (12h light at 20ºC; 12h darkness at 15ºC); a mixture of 1:1 soil/peat was used as substrate.

The following parameters were measured: (i) seedling height at 16, 23, 30 and 38 days from sowing; (ii) internodes length between the 2nd and 3rd seedling leaves at 30 and 38 days from sowing. The same plants were always measured (those in the diagonal of trays); 2 seedlings per shrub, 3 shrubs/population, and 2 dates, making a total of 12 measurements/population.

Germination of fruits. Fruits from *A. halimus* collection at Mazarron were collected on 22 December 2000; 19 populations, 11 spp. *schweinfurthii,* 8 spp. *halimus*; 3 shrubs per population, 2 replicates/shrub, 50 fruits/replicate: 300 fruits/population. Germination percentages were measured after 20 days in growth cabinet (12 h light at 20ºC, 12 h without light at 15ºC). Germinated fruits were removed every day; at day 20, ungerminated fruits were opened to find out which fruits contained seeds and which were empty.

Results and discussion

Fruit size and seed production. There was a significant difference between the weight of fruits and seeds of the two *Atriplex halimus* subspecies (Table 1); fruits and seeds of spp. *schweinfurthii* were about twice as heavy as those of spp. *halimus*; but fruits of spp. *halimus* contained a higher percentage of seeds. The range of variability for any parameter (fruit and seed weight, and the percentage of fruits containing seed) was larger in spp. *schweinfurthii* than in spp. *halimus*.

Table 1. Mean fruit weight, seed weight and percentage of fruits containing seed in *Atriplex halimus*

Subspecies	Fruit weight (mg)	Seed weight (mg)	Fruits with seeds (%)
schweinfurthii	3.5 b	1.3 b	56.7 a
halimus	1.7 a	0.7 a	90.4 b
Ratio schw/hali	2.0	1.8	0.6

Different letters indicate significant differences (95% LSD, Fisher test).

Fruit weight ranged from 1.2-6.8mg/fruit in spp. *schweinfurthii* and from 1.1-2.7 mg/fruit in spp. *halimus*; Beer Sheva and Wady El Azib, both from eastern Mediterranean countries, presented the largest fruits (mean of 5.7 and 5.2 mg/fruit respectively).

Seed weight ranged from 0.5-2.3 in spp. *schweinfurthii* (CV = 30%) and from 0.5-0.9 in spp. *halimus* (CV = 15%); accessions from eastern Mediterranean countries, such as ICARDA (Syria), Wadi el Azib (Syria), Heraclion (Crete) and Beer Sheva (Israel), had the largest seeds (mean values of 1.8-1.5 mg/seed). This may be related to geographic differences, like the rainfall distribution regime; in eastern Mediterranean countries, winter rainfalls are more frequent, whereas in other countries of the Mediterranean basin, autumn rainfalls are more common.

The percentage of fruits containing seed ranged from 10-100% in spp. *schweinfurthii* (CV = 48%) and from 75-99% in spp. *halimus* (CV = 9%); fruits of spp. *halimus* presented about 2 times higher seed content (90.4%) than fruits of spp. *schwainfurthii* (56.7%); there were significant differences between populations (95% LSD, Fisher test)*;* values ranged between 86.6% for Cala Tarida and 10% for Hoggar and Riff. Analysis of variance for the factors analysed (subspecies, population, shrub and replicate) indicates that 74% of the variance was due to the factor subspecies.

The larger weight of fruits and seeds of spp., *schweinfurthii* populations could be explained by its larger ploidy level (4x) compared to spp. *halimus* (2x), because tetraploids usually produced larger fruits and seeds than diploids. The lower seed content of fruits from spp. *schweinfurthii* populations could be explained by differences in flower phenotypes; thus, Talamani *et al.* (2001 and 2003) described five-six flower phenotypes (male, female and hermaphroditic or sterile) on individual plants of North African *A. halimus* populations; hermaphroditic or sterile flowers do not produce viable seed, which could explain the large amount of empty fruits (without seed) found in North African *A. halimus* populations, all of them tetraploids (Ortiz-Dorda *et al*., 2005; Walker *et al*., 2005).

Fruit production. Accessions of spp. *schweinfurthii* produce more fruits per shrub than those of spp. *halimus* (Table 2); hence, spp. *schweinfurthii* accessions spend more energy (photosynthetic carbohydrates) producing fruits than spp. *halimus* accessions; on the contrary, fruits of spp. *schweinfurthii* contain a smaller percentage of seeds and have lower germination rates than those of ssp. *halimus*, what for reproduction purposes can be a serious problem; further analysis of these fruits revealed that in accession from Riff, less than 1% of fruits contained seed, whereas in the other three accessions, 81-91% of fruits contained seeds; this results emphasize again the need to control the fruit quality of shrubs which are going to be used for the specie's propagation.

Table 2. Fruit production (g dry matter/shrub) and its ratio to browsing biomass (BB = leaves + young stems)

Subspecies	Population	Fruit (F) yield (g)	BB yield (g)	Leaf yield (g)	Ratio F/BB
schweinfurthii	Riff	500	600	280	0.83
schweinfurthii	Sousse	640	1610	310	0.40
halimus	Figueretes	150	1350	600	0.11
halimus	Cieza	90	1180	650	0.08

Germination capacity. Fruits of spp. *halimus* gave higher germination rate (80%) than spp. *schweinfurthii* (55%) (Table 3). There were significant differences between populations (95% LSD, Fisher test). Values ranged between 97.6% for Figueretes and 35.2% for Maraga; analysis of variance for the factors analysed (subspecies, population, shrub and replicate) indicate that 37% of the variance was due to factor replication, 33% to subspecies and 19.8% to shrub, what means that germination is controlled by several factors, but mostly by the shrub and branch from which fruits were harvested.

Table 3. Effect of subspecies on the germination capacity of *A.halimus* fruits (expressed as %)

subspecies	% fruits germinated
schweinfurthii	55.1 a
halimus	80.1 b
Ratio halim/schw	1.5

Different letters indicate significant differences (95% LSD, Fisher test).

If direct fruit sowing is going to be used to propagate *Atriplex halimus*, attention should be paid to the proportion of fruits containing seed and the germination capacity of seeds; both traits are controlled in part by genetic factors (subspecies, population, shrub), but also by environmental factors (soil moisture, air temperature, wind, etc) during the fructification period (autumn months).

Seedling growth rate. Populations of spp. *schweinfurthii* presented higher height values (80.9 mm) than those of spp. *halimus* (34.7 mm), differences being significant at the 95% level (Fisher test, LSD intervals). At population level, seedling height ranged from a minimum of 26.1 mm for Marsella to a maximum of 87.5 mm for Heraclion. There was an excellent correlation between seedling height and internodes length: seedling eight = 15.5349 + 3.1636 internodes length; the model fitted explained 89.7% of the variability in seedling height.

The higher seedling growth rate of spp. *schweinfuthii* accessions is a relevant trait for seedling establishment under sub-optimal field conditions (e.g., short rainy periods followed by drought). We think the differences obtained at subspecies level are genetically controlled and related to differences in ploidy between both subspecies.

Conclusions

Tetraploid saltbush populations produce more seed per plant, larger seeds and more vigorous seedlings than diploid saltbush populations; however, diploid saltbush fruits contain a larger proportion of seed and seeds germinate better. The greater variability of spp. *schweinfurthii* could be partly explained by its higher ploidy level (4x) and its greater geographical dispersion area, what implies more environmental diversity. All together, these factors justify the need to evaluate the quality of the seed before any large saltbush plantation programme is started.

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