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SALT-TOLERANCE IN WILD *HORDEUM* (POACEAE) SPECIES: DIFFERENCES BETWEEN *H. MARITIMUM* WITH. AND *H. HYSTRIX* ROTH.

Abstract - The paper describes the results of experimental tests carried out on seeds of *Hordeum maritimum* With. and *Hordeum hystrix* Roth., two Mediterranean wild grasses typical of saline coastal or inland meadows and marshes, whose distribution in nature is always distinct. The aim of this study was to show the differences in germination and initial growth pattern between the two species, with relation to the level and the type of saline substrate (NaCl or Na_2SO_4). The study tries also to contribute to the knowledge of ecophysiology and natural distribution of *H. hystrix*, a species which has not been studied in-depth under this respect. The study shows also that the distribution of *H. hystrix* is always different from that of *H. maritimum* and often wider than what is reported in literature, especially in some Italian regions. The results of the germination tests have not highlighted significant differences between the two species, nor between NaCl and Na_2SO_4. On the contrary, the early growth of the seedlings was affected mainly by the presence of the sulfates, whose treatment shows important differences between the two species.

Key words - *Hordeum*, salinity, seed germination, early growth.

Riassunto - *Tolleranza ai sali in specie spontanee di* Hordeum *(Poaceae): differenze tra* H. maritimum *With. e* H. hystrix *Roth.* Sono riportati i risultati delle indagini sperimentali condotte su popolazioni di *H. maritimum* L. e *H. hystrix* Roth., due specie spontanee della flora mediterranea tipiche di prati e paludi salmastre di aree litoranee o interne, la cui distribuzione in natura risulta sempre distinta. Scopo principale della ricerca è stato quello di individuare eventuali differenze di germinazione e di crescita iniziale tra le due specie, in relazione al livello e al tipo di substrato salino (NaCl o Na_2SO_4) al fine di spiegare la loro separazione di areale. Mentre le prove di germinazione non hanno mostrato differenze significative tra le due specie, lo sviluppo iniziale delle plantule dimostra una diversa sensibilità nei confronti dei due sali, permettendo alcune importanti considerazioni sulla loro diversa capacità di colonizzazione degli ambienti salini.

Parole chiave - *Hordeum*, salinità, germinazione, crescita iniziale.

Soil salinity is one of the major factors influencing the growth and yield of crop over large areas of the world (Rhoades, 1990). No continent and no climatic zone is free from salt affected soils of primary or secondary origin. Today 25% of irrigated lands are affected by this problem, this phenomenon being particularly relevant in the arid and semiarid regions of the tropic, subtropic and Mediterranean zones, and in those areas affected by salinity due to human activity (Levigneron *et al.*, 1995). The use of halophytes in breeding programs to produce salt tolerant crops and the growing of these plants directly as crops have been advocated as means of solving, at least in part, several agricultural problems associated with soil salinization (Glenn *et al.*, 1991). At the same time many researchers considered very important the studies on the tolerance of crop wild relatives to identify more tolerant genotypes to use in breeding programs. In this context, wild *Hordeum* species that have not been thoroughly explored for their potential to contribute to the genetic improvement in salinity tolerance, could provide the necessary genetic means to develop tolerant barley cultivars (Subbarao & Johansen, 1994). The present report describes the results of a set of experiments in controlled conditions using seeds of *H. maritimum* With. and *H. hystrix* Roth., two Mediterranean wild grasses usually found in saline coastal or inland meadows and marshes, sometimes in riverbeds. In spite of their common predilection for soils with average or high salinity (testified also by the contemporary presence in the sites where halophytes have their greatest diffusion), the two species are never found together in the same area.
The evidence of their separation in the natural distribution has lead to study their behaviour in relation with the levels and types of salinity, from the germination to the early growth. In this way the study tries also to contribute to the knowledge on biology and ecophysiology of *Hordeum hystrix* that, from what emerges from bibliographical references, does not seem to be studied in-depth from this point of view.

MATERIALS AND METHODS

For the experimental tests seeds of *H. maritimum* With. (= *H. marinum* Huds. subsp. *marinum*) (2n = 14) and *H. hystrix* Roth. (= *H. gussoneanum* Parl.; *H. marinum* Huds. subsp. *gussoneanum* (Parl.) Thell.) (2n = 14, 28) were collected at horn maturity in June 2000 in two Italian sites (Tuscany region): Patanella in the eastern part of Orbetello Lagoon (Grosseto) for *H. maritimum* and Luciana (Pisa) for *H. hystrix*. Seed samples were stored in paper bags at room temperature (20 ± 2°C) in darkness until they were used. As far as morphology is concerned the two species differ mainly for the lower lateral glumes that are setaceous or slightly compressed in *H. hystrix*, with a more or less broad wing in *H. maritimum* (Bothmer *et al.*, 1991) (Fig. 1).

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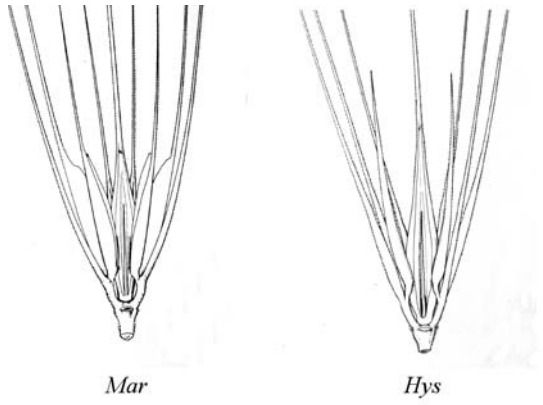

Fig. 1 - Particular of spikelets of *H. hystrix* (Hys) and *H. maritimum* (Mar).

Seeds of each species were placed to germinate in Petri dishes (9 cm inner diameter) on Whatman n. 2 filter paper moistened with 9 ml of NaCl or Na_2SO_4 solutions (in deionized water) of 0, 50, 100 and 200 mM (corresponding to about 0, 10, 20 and 40% of seawater). The Petri dishes were cellotaped to prevent evaporation. Germination tests were carried out in a germination chamber in darkness at a constant temperature of 20°C. Four replicates of 15 seeds for each species and treatment were used. Seeds were considered germinated when the radicle reached 1 mm in length. Percentage germination was recorded every 24 h for 10 days. After 3 days of germination, seedlings of uniform size of two *Hordeum* were placed in an Eppendorf tube (1.5 ml) filled with vermiculite and perforated at the bottom. 10 Eppendorf tubes per species were placed on a plastic net put on the border of a 500 mL pot containing one-fourth Heller solutions (Heller, 1953) and NaCl or Na_2SO_4 50, 100 or 200 mM (for salt treatments).
The nutrient solutions were continuously aerated and renewed every 4 days to avoid ion depletion. The water loss was compensated daily for 21 days. For each assay five replicates were placed in a growth chamber supplied with four Philips T2 40W/33 lamps (70 µmol photons $m^{-2}s^{-1}$); temperature was 10°C during the night (14 h) and 20°C during the day (10 h) and relative humidity was about 70%.
At the start and at the end of the experiment root (r) and shoot (s) length (l) were measured. The two-way analysis of variance and Duncan's multiple range test for all data were performed.

RESULTS

No significant differences were detected between the species for the germination percentage. This is true above all after 3 days of culture (Fig. 2) when the presence of salt – independently from type and concentration – permits values of germination capacity always close to 100%. A slight delay into 3 days was shown in presence of highest NaCl concentration.
Relative root and shoot length data showed that the increase of salinity in the medium culture induced a reduction of growth both for roots and for shoots in the seedlings of both species (Fig. 3). Nevertheless, some differences were detectable in relation to salt type (NaCl or Na_2SO_4).
The decreasing of growth values was higher with Na_2SO_4 treatment with respect to the control and NaCl treatment.
This is shown above all in *H. maritimum* that turns out much more sensitive to sulfates than to chloride also at the lowest concentration. In fact, in this species, a decrease of 75% for root and 37% for shoot (48 and 28% in *H. hystrix*) is recorded and partial necrosis of roots was evidenced.
On the contrary *H. hystrix* reveals a greater sensibility to chlorides; in fact even with the minimal concentration of NaCl (50 mM), it is evident in seedlings a more relevant reduction of the root growth with respect to what is found in sulfate treatment (Fig. 4).

DISCUSSION

The results of our study can represent a further contribution to the knowledge of the ecophysiology of *Hordeum* wild species.
The research supplies the first information about the diffusion in Italy and the behaviour at germination and initial growth of *H. hystrix*, not thoroughly studied under these respects. Moreover our study brings to light some of the binding factors of the distribution of *H. hystrix* in nature; distribution that, at least in Italy, turns out to be definitely more limited, with respect to other species that are taxonomically analogous.
As reported, the two species turned out to be interesting for their tendency, in spite of the remarkable similarities from the morpho-taxonomic point of view, to colonize structurally and geographically different lands. Investigations conducted in field showed that in Italy *H. maritimum* occupies coastal areas where salinity is strictly related to the presence of NaCl as dominant salt (Onnis & Lombardi, 1995; Lombardi *et al.*, 2000); *H. hystrix* indeed seems to prefer sometimes ruderal inner or calanchive zones where other ions like sulfates or carbonate seem to prevail regarding chlorides. Based on these considerations a study of the effects of chlorides or sulfates on the initial phases of the life cycle of the two species was conducted. Analyzing the data of the germination tests, it has been shown that the seeds of the two species were not influenced by the presence either of NaCl or of Na_2SO_4, allowing values of germination capacity close to 100% already after 3 days of cultivation, at all the concentrations.
The observation of a similar behaviour, already found in *H. maritimum* in some periods of the year and also in other spontaneous species of *Hordeum* such as *H. murinum*, does not allow to understand the real sensibility to the salt of the two species, since germination cannot be considered a valid index of halotolerance.

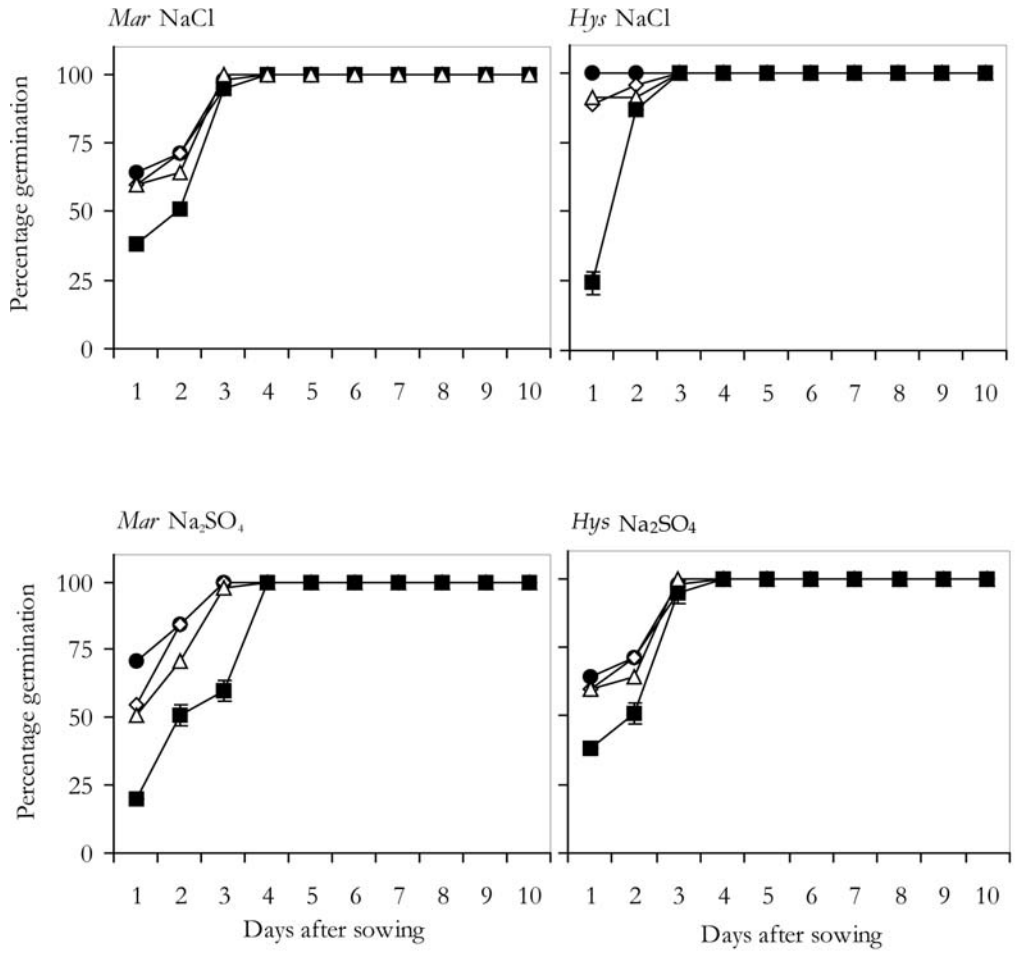

Fig. 2 - Germination percentage of *H. maritimum* (Mar) and *H. hystrix* (Hys) seeds during 10 days of culture in NaCl or Na_2SO_4 at different concentrations (mM). Vertical bars (when visible) indicate standard errors.

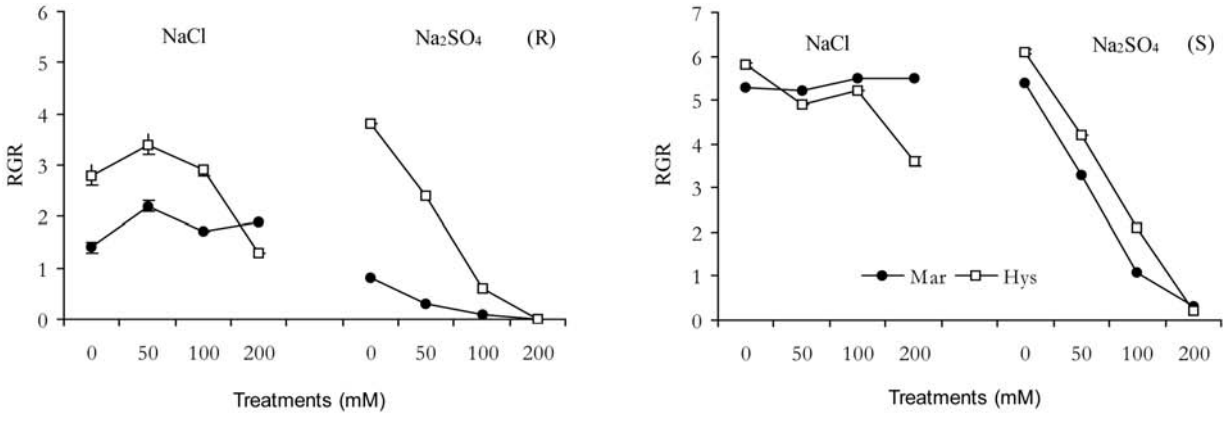

Fig. 3 - Relative growth rate (4-21 days) (± S.E. when visible) of root (R) and shoot (S) of seedlings of *H. maritimum* (Mar) and *H. hystrix* (Hys) treated with NaCl or Na_2SO_4 at different concentrations (mM).

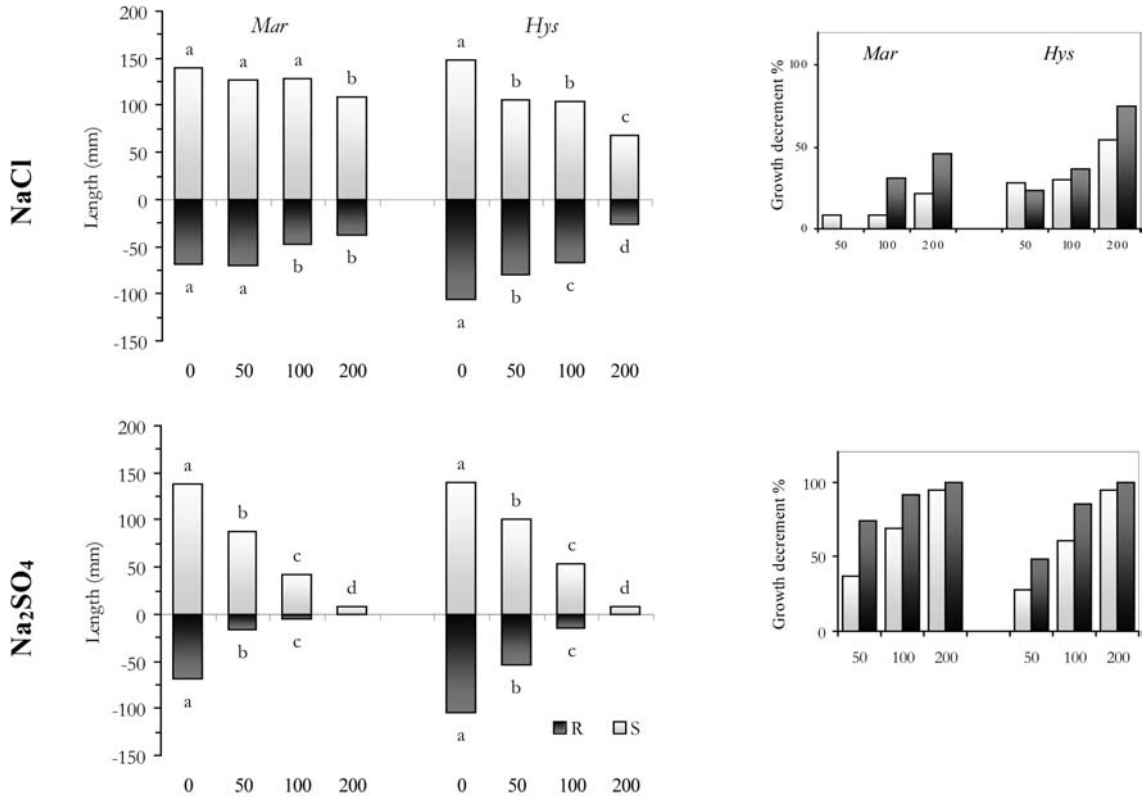

Fig. 4 - Lenght (mm) and growth decrement percentage respect to control in plants (R = root; S = shoot) of *H. maritimum* (Mar) and *H. hystrix* (Hys) treated with different concentrations of NaCl or Na_2SO_4.

If a species shows high germination values also with high salinity, it is dangerously exposed, in natural environments, to the harmful effects of salt, with serious consequences for the survival of seedlings and of the entire population.

Moreover, in this context the influence of the time of seed post-maturation must not be forgotten: as demonstrated in many species, this can favour a scalar germination in time with the maximum percentages in the less critical seasons for temperature and salinity. The investigations carried out on the growth rate allow to confirm the concepts previously expressed. The negative effect of the salt not found at seed germination is in fact evidenced by the growth of root and shoot and proportionally to the concentration used.

The seedling growth tests show also some interesting differences between the two species which are not experienced at the germination phase, above all regarding the type of salts. A greater sensibility to sulfates was found in both species, which, also at an intermediate concentration, show equal or advanced decrements to 50% regarding the control. In presence of NaCl the seedlings of *H. maritimum*, after a short slowing down to the 4 days, equal the increase of the controls or however never exceed after 20 days 25% of decrement for the shoot and 45% for the roots.

On the other hand, these values go up respectively to 54 and 75% for the seedling of *H. hystrix*, that therefore shows a greater sensibility to this type of treatment. It is then meaningful to analyze the behaviour of the two *Hordeum* in sulfate presence. Unlike NaCl treatment, the use of Na_2SO_4 seems to influence mainly the seedlings of *H. maritimum* that, after 20 days of culture, show obvious signs of suffering such as necrosis in the radical apexes or decrements that, at least at the lowest concentration, result close to 75% (against 45% of *H. hystrix*). However, also in this case, *H. hystrix* shows an important and greater slowing down in the time of increase of root growth. This is shown by the analysis of the rate of growth between 4 and 20 days (RGR) that, as represented in Figure 3, decreases significantly, as the concentration is increasing, above all for the root and respect to *H. maritimum*.

In conclusion, it can be assumed that the separation between the two species is linked to the quality of the salt content in the soil, depending dissemination of *H. maritimum* from substrates rich of NaCl. Its tolerance to chlorides in fact, contrarily to what observed in *H. hys-*

trix, is greater with respect to sulfates. And just for this species the present study has allowed to deepen also the knowledge of its distribution, which has resulted wider in some Italian regions such as Tuscany, where it is found again in dense populations in many localities.

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