Atti Soc. tosc. Sci. nat., Mem., Serie B, 113 (2006)
pagg. 37-42, figg. 5

T. Lombardi (*), B. Lupi (**)

EFFECT OF SALINITY ON THE GERMINATION AND GROWTH OF *HORDEUM SECALINUM* SCHREBER (POACEAE) IN RELATION TO THE SEEDS AFTER-RIPENING TIME

Abstract - Effects of salinity on dormancy, germination and early growth in *Hordeum secalinum* Schreber seeds were studied. Observations were carried out as a function of seed age (after-ripening time). The caryopses, collected in the costal line of Presidential Estate of San Rossore (Pisa), whose localization in proximity of the sea produced continuous modifications in the degree of soil salinity, were placed to germinate with 100, 200, 300, 400, 500 mM NaCl solutions. The highest germination was obtained under non-saline conditions, and an increase in NaCl concentration progressively retards and decreases (even though does not prevent) germination: in fact, in presence of 400 and 500 mM NaCl concentration at the tenth day, germination is between the values of 62,2 and 60% respectively. Evolution of seed germination was studied in relation to dormancy and after-ripening time in the first year of seeds: from 30 days after the harvest to 270 days of after-ripening, the germination energy of caryopses was very high, only in the months of April, June and July a persistence of relative dormancy was detected. This spontaneous species shows a biorhythm that reflects the plant cycle, with high values of germination in the months following the seeds maturation, and low percentages of germination after 240 days of conservation.

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

Riassunto - *Effetto della salinità sulla germinazione di* Hordeum secalinum *Schreber (Poaceae) in relazione al tempo di post-maturazione dei semi*. Sono riportati i risultati delle indagini sperimentali condotte su popolazioni di *Hordeum secalinum* Schreber, che si sviluppano in alcune aree costiere di San Rossore (Pisa) ove la vicinanza al mare determina continue modificazioni a livello della salinità dei substrati. Le germinazione delle cariossidi è stata studiata in funzione del tempo di post-maturazione dei semi, e della salinità del mezzo di coltura; in quest'ultimo caso sono state utilizzate concentrazioni di NaCl pari a 100, 200, 300, 400 e 500mM. I valori più elevati di germinazione si osservano nel controllo; l'aumento di NaCl determina invece un progressivo ritardo e decremento della capacità germinativa. L'evoluzione della germinazione è stata valutata anche in relazione alla dormienza nel corso del primo anno di vita dei semi. A partire da 30gg dalla maturazione fino a 270gg, i valori in percentuale sono risultati sempre molto elevati tranne nei mesi di Aprile, Giugno e Luglio successivi alla raccolta, quando si rileva uno stato di dormienza relativa. La specie mostra quindi un bioritmo che sembra riflettere il ciclo vitale, con valori di geminazione elevati nei mesi successivi e prossimi alla maturazione dei semi e inferiori dopo 240gg di post-maturazione.

Parole chiave - *Hordeum*, salinità, germinazione, post-maturazione.

Hordeum secalinum Schreber (= *Hordeum nodosum* Auct. non L.; = *Hordeum pratense* Hudson; = *H. maritimum* O.F. Müller; = *Criteison secalinum* (Schreb.) A. Löve) is a perennial species with a poorly developed root system, mainly outbreeding, and naturally found in closed, perennial grasslands. The plants, up to 85 cm tall, have erect and usually slender culms, the basal leaf sheaths are densely hairy, whereas the nodes are glabrous. The leaves are flat, densely hairy with long hair, and sometimes with involute margins. The spike has a thin and lengthened shape (30-70 mm long), with pale green or rarely greenish violet, and is made of ternate spikelets, among which the lateral ones are peduncolate and sterile, while the central spikelet is sessile and fertile (Pignatti, 1982; Baum & Bailey, 1989). It grows along the seashore as well as in inland meadows, under saline or rarely fresh water conditions. *H. secalinum* was formerly abundant in inland localities in Europe, but is now rare due to urbanization and expansion of cultivation. Thus, the common use of fertilizers and drainage has changed formerly rather poor grassland into lusher pastures. For similar reasons the coastal population is decreasing (Bothmer *et al.*, 1991).

The population considered in our study, colonizes with some halophytes species like *Hordeum maritimum* With., and other less halo-tolerant ones like *Carex distans* L., *Carex otrubae* Podp., *Inula viscosa* L., a moderately salty zone at the «Lame» of the presidential Estate of San Rossore (PI) (Lombardi *et al.*, 2000). This area presents continuous modifications of hydro system with variations in water level, and in the quality of groundwater and superficial water, because of its nearness to the coast.

The knowledge of this species has been little expanded; the Authors have studied mostly the phylogenetic aspects (De Bustos *et al.*, 1996, 2002; Nishikawa *et al.*, 2002; Shcherban & Vershinin, 1997), while biology or ecophysiology of *H. secalinum* is less known.

Coastal regions represent desirable habitats for the study of plant salt tolerance. Salinity is known to affect many aspects of plants; salinity may either induce adaptations, which increase the chance of plants to endure stress imposed by salinity, or damage and disrupt the normal equilibrium of life processes (Poljakoff-Mayber & Gale, 1975). In saline soils, the most common adverse features are delayed germination, high mortality of seedlings and poor growth of crops.

(*) Dipartimento di Agronomia e Gestione dell'Agroecosistema, via S. Michele degli Scalzi 2, 56124 Pisa, E-mail: tlomb@agr.unipi.it
(**) Laboratorio MES, SSSUP via San Zeno, 56100 Pisa.

Numerous studies have focused on these topics, showing that both halophyte and glycophyte plants respond in a similar manner to increased salinity stress. It has also been shown that many halophyte plants – similarly to other species growing in extreme environments (Baskin *et al.*, 1993a, 1993b) – are endowed with as yet poorly understood dormancy mechanisms, allowing alternation of germination and dormancy depending on soil environmental conditions (Ungar, 1996). The study of germination strategies have a high ecological importance, above all it is related to investigation of the effects of the external conditions on the seed imbibition.

In this study, the effects of salinity on dormancy and germination were analyzed in *H. secalinum* seeds. Observations were carried out as a function of seed age (after-ripening time), which is known to exert a marked influence on germination rate and consequently on the response of seeds exposed to stress (Poljakoff-Mayber *et al.*, 1992).

Germination and seedling growth responses were tested because early stages in the life cycle are most sensitive to stress factors (Harper, 1977), and tolerance to stress in these stages is a critical for plant fitness (Grime, 1979).

MATERIALS AND METHODS

Mature caryopses of *Hordeum secalinum* Schreber were harvested in June 2003 in the costal line of Presidential Estate of San Rossore (Pisa, Italy), and in particular between two ancient drainage ditches.

The soil analysis showed low electrical conductivity values, ranging between 0.3 in winter and 0.7 dS/m in summer. The first set of germination experiments were initiated after 150 days from the collection; in this period caryopses (H1) were used to characterize the salt tolerance of the seeds. Caryopses harvested in 2004 (H2) and 30 days old, were used in subsequent tests, carried out to evaluate the relation between after-ripening time and salinity during seeds germination. In all cases caryopses were stored in a dark room at 20 ± 2°C until they were used in germination and growth tests.

Salt tolerance

H1 dehulled caryopses (150 days-old) were placed in 9 cm diameter Petri dishes with filter paper (Whatman n.2) containing 9 ml of deionized water. The Petri dishes were placed in growth chamber at constant temperature (20°C), in light with a 12/12 h photoperiod (irradiance of 70 mol photons m^{-2} s^{-1}, Philips T2 40W/33 lamp) with NaCl solutions at different concentrations (0 100, 200, 300, 400 and 500 mM). For each trial four replications were made, each replication consisting of 15 fruits. The percentage of germinated caryopses was recorded every 24 h for a total of 10 days. Caryopses were considered germinated upon rupture of the pericarp by the root.

After-ripening time and salt stress

H2 caryopses (30 days-old) were tested in different solutions of NaCl (0, 100, 200 and 300 mM). Trials were set up as described above, every 30 days over the first year of seeds life. After 10 days of culture, young seedlings were evaluated for determination of shoot and root length, and for possible presence of chlorosis and necrosis.

Germination data were transformed (arcsine) before a statistical analysis was performed. A Bonferroni test was used ($P < 0.05$) to determine significant differences between means of percent germination, and germination energy among salinity treatments.

RESULTS

Salt tolerance

High germination percentages were obtained also in presence of 400 and 500 mM NaCl with values of 62 and 60% at tenth day respectively (Fig. 1). The germination energy detected in the control and with 100 and 200 mM NaCl, showed a remarkable delay with respect to 300 mM treatment (Fig. 2); caryopses grown on this concentration reached 50% of germination at fourth day, contrarily to the control and to the lower saline concentrations, where the 50% was already exceeded after 48 h.

After-ripening time and salt stress

Already at 30 days after the harvest, and until 270 days of after-ripening, both in the control and in the saline treatments (except 300 mM NaCl), germination energy of caryopses was very high, because the threshold of 50% seed germination was always reached on the second days. In spring-summer (after 270 days of after-ripening) germination was much slower (Fig. 3).

From the germination pathway of *H. secalinum* seeds, a higher germination capacity is observed, for the first seven months, at tenth day of cultivation in deionized

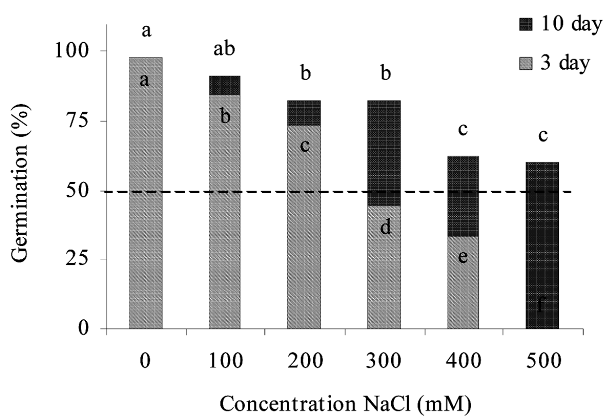

Fig. 1 - Germination percentage of *H. secalinum* seeds after three and ten days of culture (after-ripening 150 days), at 20°C temperature and 12/12 h photoperiod, with different NaCl concentrations. Different letters within the same day of the culture, indicate differences (P > 0.05) between the levels of NaCl.

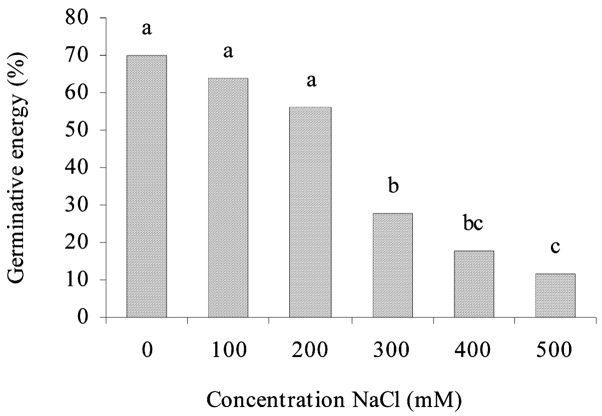

Fig. 2 - Germination energy at tenth day of culture (after-ripening 150 days), 20°C temperature and 12/12 h photoperiod, with different NaCl concentrations. Different letters within the same day of the culture, indicate differences (P > 0.05) between the levels of NaCl.

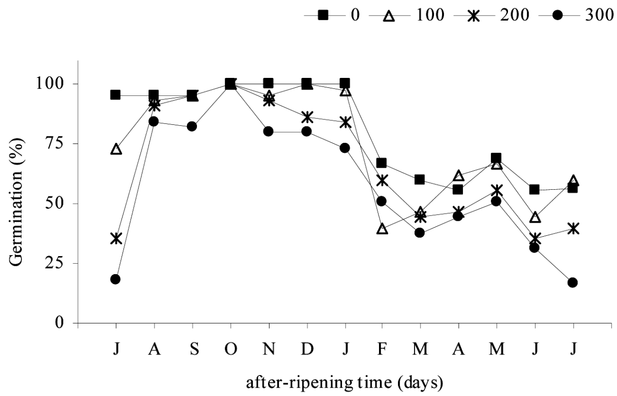

Fig. 4 - Germination percentage during the first year of the seeds at 10^{th} days of culture at 20°C temperature and 12/12 h of photoperiod, and in the presence of different NaCl concentration (mM).

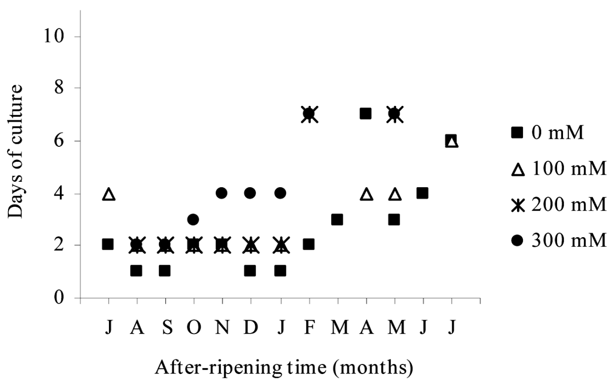

Fig. 3 - Days necessary for reaching 50% of germination during the first year of caryopses placed to germinate with different concentration of NaCl.

water, with values ranging from 95,5 and 100% (Fig. 4). The lower germination was shown between April and July following the harvest. In presence of salt solution in the culture medium the maximum values were registered between end of summer and the end of winter, when germination was between 73.3 and 100%, even with the higher NaCl concentration.

Starting from February, a rapid decrease was observed for all treatments; this decrease, for 300 mM NaCl concentration, brought the values always below 51%.

The lengths of root and shoot of ten-days-old seedlings showed the maximum growth for all treatments in autumn, and in particular in the case of control the maximum values were reached in September with 36,3 mm for root and 36,2 mm for shoot (Fig. 5).

At 100mM NaCl concentration the development of seedlings was modestly affected, but with the higher concentration the growth was retarded, and specifically the root was the most damaged, being its growth completely inhibited in some cases.

Discussion

The research contributed to deepen the knowledge about the ecology of *H. secalinum* and confirmed the preliminary observations carried out on the early stage of its growth (Lombardi & Lupi, 2005).

Germination and the seedling stage are the most critical periods for the establishment of plant species (Grime & Campbell, 1991), and salt concentration, especially NaCl, is one of the factors controlling germination, seedling survival and growth (Mayer & Poljakoff, 1975). Plants may show low or high variation for salt tolerance, defined as sustained growth in a saline environment (Brady & Weil, 1996). In general, Authors have concluded that salinity is inhibitory to the germination in two ways: either causing a complete inhibition of the germination process at salinities beyond the tolerance limits of a species, or delaying the germination of seeds, at salinities that cause some stress to seeds but do not prevent germination (Ungar, 1995). For caryopses of *H. secalinum* the germination capacity was always high with all treatments, although NaCl had a negative effect on germination rate and energy.

This effect was proportionate to NaCl concentration in the culture medium; in presence of > 200 mM concentrations the germination percentage did not reach 50% on the third day, but on the tenth day, such threshold is exceeded also at 500 mM of NaCl.

As far as germination energy is concerned, the values were significantly reduced at 300 mM NaCl with respect to control; therefore, this concentration must be considered a threshold for the caryopses germination, although *H. secalinum* shows the ability to germinate at salinity levels of up to 500 mM NaCl, which

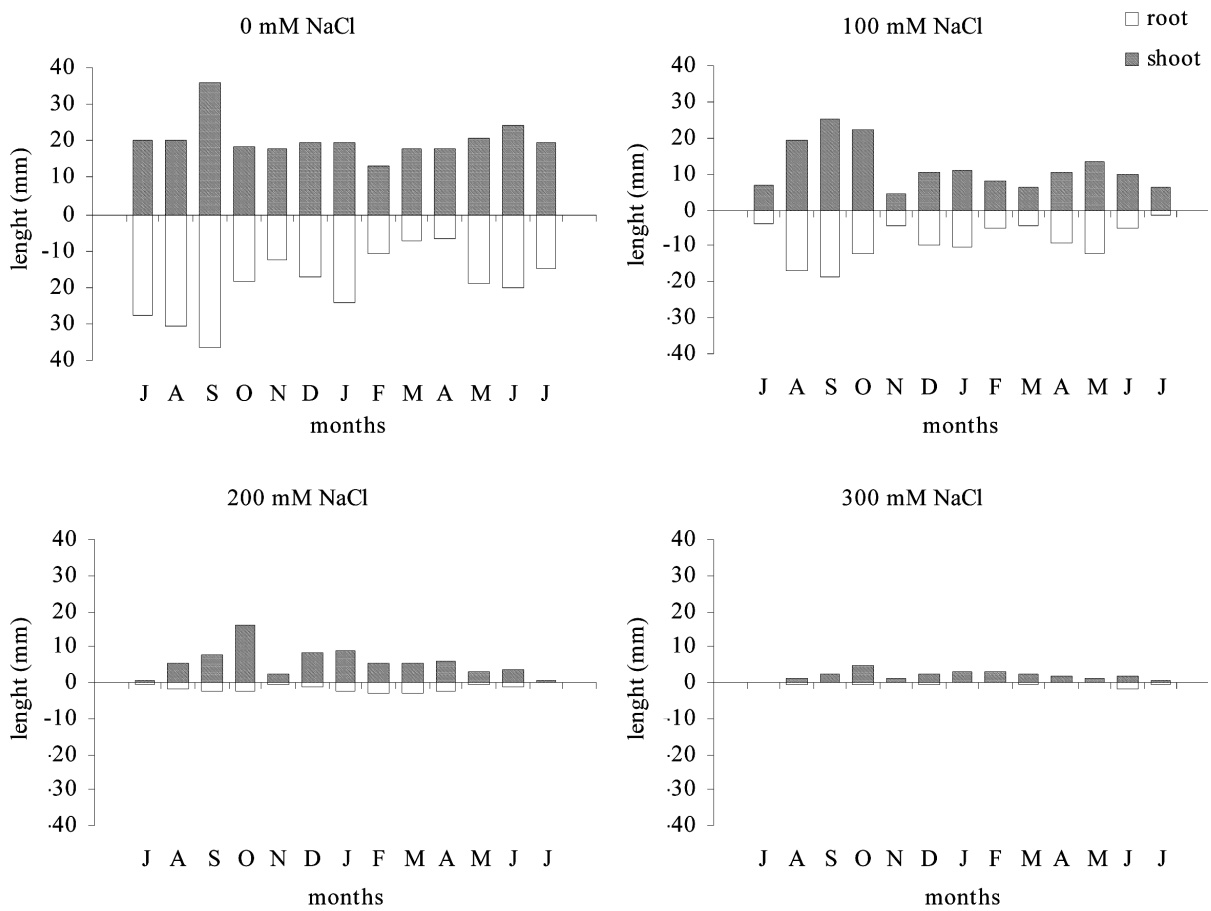

Fig. 5 - Root and shoot growth (mm) in seedlings ten days-old of *H. secalinum* germinated with different NaCl concentration and tested during the first year of caryopses.

is close to seawater salinity (600 mM NaCl). Seeds of halophytes often germinate best under non-saline conditions and their germination decreases with increases in salinity (Khan & Ungar, 1998), germination is substantially inhibited at 500 mM NaCl in *Limonium stocksii* and *Sporobolus ioclados* (Zia & Khan, 2004; Khan & Gulzar, 2003), at 430 mM NaCl in *Diplachne fusca* (Myers & Morgan, 1989), at 300 mM NaCl in *Halopyrum mucronatum* and *Briza maxima* (Noor and Khan, 1995; Lombardi *et al.*, 1998). 60% of the caryopses of *H. secalinum* could germinate in 500 mM NaCl, at an appropriate temperature regime, indicating that this grass species is more salt tolerant than most other ones.

The first year of life of *H. secalinum* seeds was characterized by a significant germinative capacity, with values that exceed, more often than not, 50% of germination already at the third day of cultivation. A persistence of relative dormancy (Meletti, 1964, 1968) was present, only for a short period, in the months of April, June and July: the values of germinative percentage after 72 h of cultivation, in fact, are lowered than 50%. The restoration of the dormancy in summer reduces the risk of an early germination that could be induced by short rains. The type of dormancy and the consequent modality of germination, if correlated to the quality and the intensity of the main environmental factors allowed to draw interesting considerations on the ecology of different species. It appears also obvious that the different levels of dormancy that characterizes the various taxa, are the result of long periods of adaptation; therefore, in some cases, they can be the indication of particular ecological conditions (Onnis, 1984). With salt solution (100 and 200 mM NaCl) the percentage of caryopses germinated reached 50% within the third day in the months between August and January, in successive months seeds showed a relative dormancy. This trend of germination follow the natural germination course of *H. secalinum* seeds, which germinate generally in November and tiller out in February: therefore in the spring and summer the caryopses do not germinate.

Hordeum secalinum, moreover shows a biorhythm that seems to reflect the plant cycle, with high values of germination in the months following the seeds maturation, and low percentages of germination after 240 days of conservation: however it presents a clear lowering of the germinative energy in all the spring and summer period. The presence of a biorhythm is well-known for many species, and in particular for those typical of environments characterized by high salinity, high temperature or water scarcity, at least in a period of the year, as for example, *Hordeum maritimum* (Lombardi, 1991), *Ammophila arenaria* (De Martis *et al.*, 1976), *Salicornia* spp. (Langlois, 1966), and the hydrophytes *Althenia filiformis* (Onnis & Pelosini, 1978) and *Zannichellia palustris* L. (Lombardi *et al.*, 1996).

The sinusoidal trend of the germinative capacity, found in these and other species, has been interpreted with the presence of an endogenous biorhythm probably correlated with the seasonal cycle and with the environmental variations of light, temperature and precipitations (Gellini, 1969; Onnis *et al.*, 1979; Onnis, 1984; Lombardi, 1991). It can therefore be assumed that the right moment for germination is characterized by the combined action of two distinct mechanisms: the germination is postponed both by environmental physical parameters and by the dormancy.

The seedlings development with increase of salinity, was always remarkable above all as regards the shoot; in fact, after ten days of culture, the shoot showed a reduction in the length only at concentration in excess of 200 mM NaCl.

The root was markedly affected by salt presence regarding the shoot; its growth was strongly reduced in presence of 200 mM and nearly inhibited in its development at 300 mM NaCl.

The general slowing down of growth, already demonstrated in literature for many halophytes like *Puccinellia festucaeformis* (Onnis *et al.*, 1981), *Hordeum maritimum* (Lombardi, 1991), *Kolchia indica*, *Atriplex crassifolia* e *Sporobolus arabicus* (Mahmood *et al.*, 1996), can be interpreted as a confirmation of a moderate degree of salt tolerance in *H. secalinum*. In the halotolerant species, in fact – contrarily to the glycophyte – it has been demonstrated that the root is the organ mainly affected by the presence of salt.

Salinity is a key determinant of the organization of plant communities in Mediterranean coastal marshes (Callaway *et al.*, 1990), which are subject to strong oscillations within and between years, associated with seasonal flooding-drought cycles.

The influence of salinity is decisive during the reproductive period, both for the germination of seeds (Ungar, 1998) and the establishment of seedlings (Noe & Zedler, 2001).On the basis of the above considerations on germination behaviour it is possible to confirm a fair degree of halotolerance of *H. secalinum*, and to assume for this species the tendency to increase its coverage in the study area, and in the adjacent zones, as a result of an eventual increase of substrate salinity. Moreover, it could be very interesting, in the future, to measure the soil moisture requirements that may limit the distribution of *H. secalinum* in the study area.

Acknowledgements

The author wishes to thank Dr. E. Mattei for his contribution to the English translation.

References

Baskin C.C., Baskin J.M., Chester E.W., 1993a. Germination ecology of *Leptochloa panicoides*, a summer annual grass of seasonally dewatered mudflats. *Acta Oecologica* 14 (5): 693-704.

Baskin C.C., Baskin J.M., Meyer S.E., 1993b. Seed dormancy in the Colorado plateau shrub *Mahonia fremontii (Berberidaceae)* and its ecological and evolutionary implications. *The Southwestern Naturalist* 38 (2): 91-99.

Baum B.R., Bailey G., 1989. An investigation on the taxonomy of *Hordeum capense* and *H. secalinum* (Poaceae: Triticeae). *Can. J. Bot.* 67: 594-599.

Bothmer R. Von, Jacobsen N., Baden C., Jorgensen R.B., Linde-Laursen I., 1991. An ecogeographical study of the genus *Hordeum*. IBPGR, Roma.

Brady N.C., Weil R.R., 1996. The Nature and Properties of Soils. Eleventh ed. Prentice-Hall, New Jersey, 740 pp.

Callaway R.M., Jones S., Ferren J.R., Parikh A., 1990. Ecology of a Mediterranean climate estuarine wetland t carpenteria, California: plant distribution and soil salinità in the upper marsh. *Can. J. Bot.* 69: 1139-1146.

De Bustos A., Cuadrado A., Soler C., Jouve N., 1996. Physical mapping of repetitive DNA sequences and 5S and 18S-26S rDNA in five wild species of the genus *Hordeum*. *Chromosome Res*. 4 (7): 491-499.

De Bustos A., Loarce Y., Jouve N., 2002. Nuclear rDNA (internal transcribed spacer) sequences in the genus *Hordeum*. *Genome* 45 (2): 339-347.

Gellini R., 1969. Periodicità annuale della capacità di germinazione di semi di *Pinus pinaster* Ait. *Italia Forestale e Montana* 24: 1-8.

Grime J.P., 1979. Plant strategies and vegetation processes. John Wiley & Sons, Chichester.

Grime J.P., Campbell B.D., 1991. Growth rate, habitat productivity, plant strategy as predictors of stress response. In: Mooney H.A., Winner W.E., Pell E.J., Chu E. (eds.), Response of Plants to Multiple Stresses: 143-159. Academic Press, Inc., San Diego, London 422 pp.

Harper J.L., 1977. Population biology of plants. Academic Press, San Diego.

Khan M.A., Gulzar S., 2003. Germination responses of *Sporobolus ioclados*: a potential forage grass. *J. Arid Environ.* 53: 387-394.

Khan M.A., Ungar I.A., 1998. Seed germination, dormancy of *Polygonum aviculare* L. as influenced by salinity, temperature, giberellic acid. *Seed Science and Technolog* 26: 107-117.

Lombardi T., 1991. Alotolleranza in specie selvatiche di *Hordeum: Hordeum murinum* L. e *H. maritimum* With. Ph. D. Thesis, Pisa University, Italy.

Lombardi T., Lupi B., 2005. Effetto della salinità su germinazione e crescita iniziale di *Hordeum secalinum*. Atti 100° Congresso della Soc. Bot. Ital. Inf. Bot.It. 37: 798-799.

Lombardi T., Bedini S., Onnis A., 1996. The germination characteristics of a population of *Zannichellia palustris* subsp. *pedicellata*. *Aquat. Bot.* 54: 287-296.

Lombardi T., Bertacchi A., del Zoppo M., Tomei P.E., 2000. Le fitocenosi delle Lame costiere della tenuta di S. Rossore: prime indagini sulle caratteristiche floristico-vegetazionali in relazione alla salinità del substrato. 10° Congresso Nazionale della Società Italiana di Ecologia, vol. 1, pp. 63, Pisa.

Lombardi T., Fochetti T., Onnis A., 1998. Germination of *Briza maxima* L. seeds: effects of temperature, light, salinity, seed harvesting time. *Seed Sci. & Technol.* 26: 463-470.

Mahmood K., Malik K.A., Lodhi M.A.K., Sheikh K.H., 1996. Seed germination, salinity tolerance in plant species growing on saline wasteland. *Biologia Plantarum* 38 (2): 309-315.

Meletti P., 1964. Nuove prospettive nello studio dei fattori che controllano la germinazione dei semi. *Giorn. Bot. Ital.* 71: 372-384.

Meletti P., 1968. Conseguenze e significato del prolungamento nella durata della dormienza in *Triticum durum* Desf. *Giorn. Bot. Ital.* 102: 515-520.
Myers B.A., Morgan W.C., 1989. Germination characteristics of the salt tolerant grass *Diplachne fusca*. I. Dormancy, temperature responses. *Australian Journal of Botany* 37: 239-251.
Nishikawa T., Salomon B., Komatsuda T., Von Bothmer R., Kadowaki K., 2002. Molecular phylogeny of the genus *Hordeum* using three chloroplast DNA sequences. *Genome* 45 (6): 1157-1166.
Noe G.B., Zedler J.B., 2001. Spatio-temporal variation of salt marsh seedling establishment in relation to the abiotic, abiotic environment. *J. Veg. Sci.* 12: 61-74.
Noor M., Khan M.A., 1995. Factors affecting germination of summer, winter seeds of *Halopyrum mucronatum* under salt stress. In: Khan M.A., Ungar I.A. (eds.), Biology of Salt Tolerant Plants: 51-58. Department of Botany, University of Karachi, Karachi.
Onnis A., 1984. Il significato ecologico della germinazione. *Informatore Botanico Italiano* 16: 58-68.
Onnis A., Pelosini F., 1978. *Juncus acutus* L.: suo possibile uso quale indicatore biologico della qualità dell'ambiente. *Giorn. Bot. Ital*. 112: 318-319.
Onnis A., Pelosini F., Stefani A., 1981. *Puccinellia festucaeformis* (Host) Parl.: germinazione e crescita iniziale in funzione della salinità del substrato. *Giorn. Bot. Ital*. 115: 103-116.
Onnis A., Stefani A., Bisalia L., 1979. *Ampelodesmos tenax* Link (Gramineae): effetti della temperatura sulla germinazione in relazione alle condizioni dell'habitat. *Atti Soc. Tosc. Sci. Nat. Mem. Ser. B* 86: 133-147.
Pignatti A., 1982. La Flora d'Italia. Edagricole. Bologna.
Poljakoff-Mayber A., Gale J., 1975. Plants in Saline Environment: 73. Springer-Verlag, New York.
Poljakoff-Mayber A., Somers G.F., Werker E., Gallagher J.L., 1992. Seeds of *Kosteletzkya virginica* (Malvaceae): Their structure, germination, salt tolerance. I. seed structure, germination. *Am. J. Bot.* 79: 249-256.
Shcherban A.B., Vershinin A.V., 1997. BARE-ID, a representative of a family of BARE-like elements of the barley genome. *Genetica* 100 (1-3): 231-240.
Ungar I.A., 1995. Seed germination, seed-bank ecology in halophytes. In: Kigel J., Galili G. (eds.), Seed development, germination: 529-544. Marcel Dekker, New York.
Ungar I.A., 1996. Effect of salinity on seed germination, growth, ion accumulation of *Atriplex patula* (*Chenopodiaceae*). *Am. J. Bot.* 83 (5): 604-607.
Ungar I.A., 1998. Are biotic factors significant in influencing the distribution of halophytes in saline habitats? *Bot. Rev.* 64: 176-179.
Zia S., Khan M.A., 2004. Effect of light, salinity,, temperature on seed germination of *Limonium stocksii*. *Can. J. Bot.* 82: 151-157.

(ms. pres. il 15 settembre; ult. bozze il 28 febbraio 2007)