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Seed Germination of a Halophytic Grass *Aeluropus lagopoides*

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Aeluropus lagopoides (Linn.) Trin. Ex Thw. (Poaceae) is a perennial grass distributed from coastal Sindh and Balochistan to saline flats of Punjab, Pakistan. Seeds collected from an inland population of *A. lagopoides* located on the University of Karachi campus were germinated under various levels of salinity (0, 100, 200, 300, 400 and 500 mM NaCl) and temperature regimes (10/20, 15/25, 20/30 and 25/35 °C) in a 12 h dark/12 h light photoperiod. Highest germination was obtained under non-saline conditions, and an increase in NaCl concentration progressively inhibited germination. Inhibition of germination was greater at cooler temperatures (10/20 °C) when no seed germinated above a concentration of 300 mM NaCl. The germination response at moderate temperatures (20/30 °C) was optimal, with 30 % of seeds germinating in 500 mM NaCl. The rate of germination decreased as salinity increased. Germination rate was highest at 20/30 °C and lowest at 10/20 °C. Seeds were transferred from salt solutions to distilled water after 20 d and those from high salinities recovered quickly at warmer temperatures with an optimal response at 20/30 °C.

Key words: *Aeluropus lagopoides*, germination, halophyte, Karachi, salinity, temperature.

INTRODUCTION

Halophytic grasses found on the Arabian Sea coast are relatively intolerant of high NaCl concentrations during germination. *Halopyrum mucronatum* and *Sporobolus arabicus* were found to germinate in up to 200 mM NaCl (Noor and Khan, 1995; Khan and Ungar, 2001) while *Urochondra setulosa* seeds germinated at 500 mM NaCl (Gulzar *et al.*, 2001). Perennial halophytic grasses from other regions showed restricted germination at NaCl salinities approaching half strength seawater: *Puccinellia nuttalliana* at 344 mM (Macke and Ungar, 1971); *Diplachne fusca* at 400 mM (Morgan and Myers, 1989); *Hordeum vulgare* at 344 mM (Badger and Ungar, 1989); *Briza maxima* at 310 mM (Lombardi *et al.*, 1998) and *Typha latifolia* at 310 mM (Lombardi *et al.*, 1997), while some grasses like *Spartina alterniflora* showed some germination in 1027 mM NaCl (Mooring *et al.*, 1971).

Salinity and temperature interact in their control of seed germination (Khan and Ungar, 1999), with the greatest inhibition due to salinity usually found at the minimum or maximum limits of tolerance to temperature (Badger and Ungar, 1989). The dependence of the response to salinity on temperature may be due to an initial inhibition of germination at higher salt concentrations at some temperatures rather than others (De Villiers *et al.*, 1994; Khan and Ungar, 1997, 1998, 1999; Khan and Gul, 1998), while alternating day and night temperatures have been shown to promote germination in a number of halophytes (Okusanya, 1977; Ungar, 1995; Khan and Ungar, 1997, 1998, 1999; Gul and Weber, 1999).

Seeds of halophytes have been found to germinate even after prolonged exposure to hyper-saline conditions (Macke and Ungar, 1971; Woodell, 1985; Keiffer and Ungar, 1995, 1997). However, they differ in their capacity to recover from exposure to salinity (Keiffer and Ungar, 1995), a variation in recovery that could be due to differences in the temperature regimes to which seeds are exposed (Khan and Ungar, 1996, 1997, 1998, 1999). Gulzar *et al.* (2001) found that seeds of *U. setulosa* had 85 % recovery when pretreated with 500 mM NaCl for 20 d at moderate temperatures (20/30 °C). Macke and Ungar (1971) found 87 % recovery in distilled water as compared to 5·2 % germination after 45 d of exposure to 344 mM NaCl in seeds of *Puccinellia nuttalliana*.

Aeluropus lagopoides (Linn.) Trin. Ex Thw. (Poaceae) is a salt-secreting rhizomatous perennial grass ranging in distribution from Northern Africa (Morocco to Somalia), Sicily and Cyprus, through the Middle East to Central Asia, Pakistan and India (Cope, 1982). The plant propagates vegetatively by rhizome growth after monsoon rains and it also produces numerous flowers and seeds from April to October (Gulzar and Khan, unpubl. res.). It is often found in association with *Cressa cretica* in inland communities and with *Cyperus arenarius*, *Cressa cretica* and *Halopyrum mucronatum* in coastal communities in the backwaters of the Manora creek, Pakistan. *Aeluropus lagopoides* is used throughout the year as a forage plant in parts of India. Its foliage shows little variation in salt content despite a three-fold increase in soil salinity in

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summer (Joshi and Bhoite, 1988); this is possibly due to salt secretion through leaf glands. The low salt content of *A. lagopoides* shoots is advantageous in its use as a fodder crop. Currently, little information is available regarding the relative salt tolerance of halophytic grasses from subtropical regions of the world. The primary objective of this investigation was to determine the response of *A. lagopoides* to saline conditions, to ascertain if *A. lagopoides* could germinate at NaCl concentrations approaching seawater (500 mM NaCl) and how this response might be governed by prevailing temperature conditions.

MATERIALS AND METHODS

Spikelets of *Aeluropus lagopoides* were harvested in winter 1997 from a population on the Karachi University campus. Seeds were separated from each inflorescence, cleaned, and dry-stored in a refrigerator at 4 °C after surface sterilization with 0·85 % clorox for 1 min. Germination experiments were initiated in September 1998. Six salinity concentrations (0, 100, 200, 300, 400 and 500 mM NaCl) were used, based on a preliminary test of salt tolerance. Germination was tested in a programmed incubator (Percival, Boone, USA) at (dark : light) 10/20, 15/25, 20/30 and 25/35 °C temperature regimes with a 12 h photoperiod (25 µmol m^{-2} s^{-1}, 400–700 nm Sylvania cool white fluorescent lamps). In the inland community where *A. lagopoides* germinates, salinity is usually around 300 mM, decreasing to about 100 mM depending on the extent and duration of rainfall. Average day and night-time temperatures during the monsoon period are around 30 and 20 °C, respectively. Seeds were germinated in two folds of Whatman No. 1 filter paper placed in 2·5 cm × 18 cm glass test tubes with 5 ml of test solution. The tubes were sealed using parafilm. A seed was considered to have germinated at the emergence of the radicle. Germination was noted on alternate days. After 20 d, all non-germinated seeds were placed in distilled water at their initial temperature regimes for another 20 d. Rate of germination was calculated with a modified Timson's germination velocity index: $\Sigma G/t$, where G is the percentage of seed germinated after 2 d intervals, and t is the total time of germination (Khan and Ungar, 1998). Rate of recovery of germination was calculated using the relation: $[(a\text{-}b)/(c\text{-}b)]$ 100, where a is the total number of seeds germinated after being transferred to distilled water, b is the total number of seeds germinated in saline solution and c is the total number of seeds. Germination data (20 d) and recovery germination data (20 d) were arcsine transformed before statistical analysis to ensure homogeneity of variance. Data were analysed using SPSS, version 9.0 (SPSS, 1999). The effect of salinity and temperature on germination, rate of germination and recovery of germination were examined using two-way ANOVA and regression analysis.

RESULTS

Two-way ANOVA indicated that germination of *A. lagopoides* seeds was significantly affected by temperature, salinity and their interaction (Table 1). Seed germination was highest in distilled water in the 20/30 °C temperature regime (Fig. 1). Thirty percent of the seeds germinated in 500 mM NaCl at 20/30 °C while no seed germinated at 10/20 °C (Figs 1 and 2). Germination was affected by temperature and was reduced from 100 % at 20/30 °C to only 60 % at 10/20 °C in the non-saline control. Seeds showed higher germination in all treatments at 15/25 °C and 20/30 °C, with 100 % germination in the

TABLE 1. *Effect of salinity (S), temperature (T) and their interaction on generation of* A. lagopoides *seeds*

Source of variation	S	T	SXT
Germination	131·7	129·1	3·4
Rate of germination	141·2	156·4	8·0
Recovery germination	39·3	82·6	14·3

Data are F values (two-way ANOVA) significant at $P < 0{\cdot}001$.

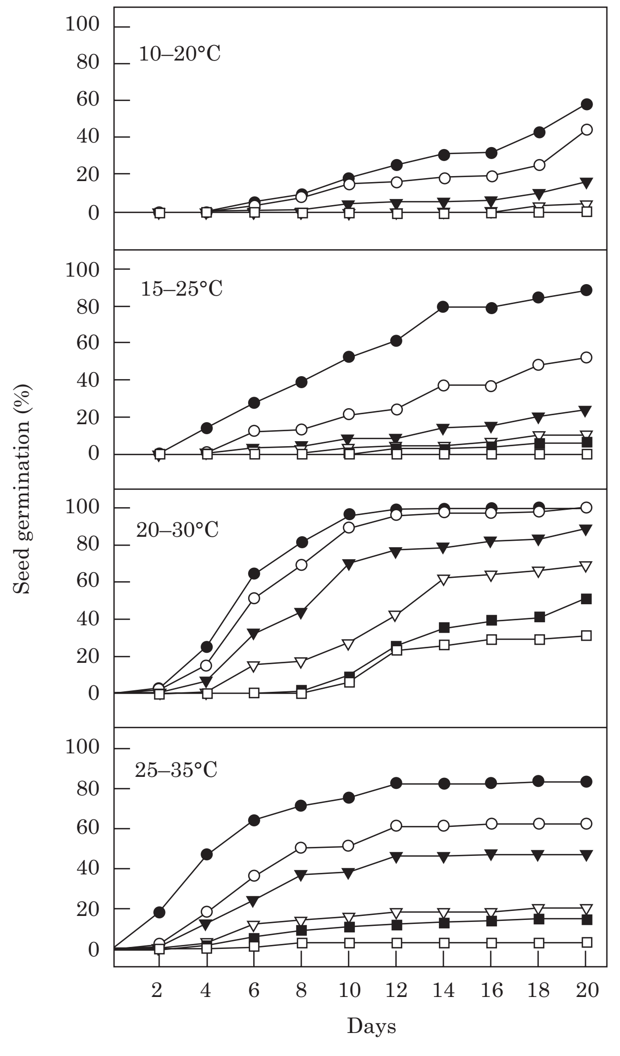

FIG. 1. The effect of temperature regime and salinity on the percentage germination over time of *Aeluropus lagopoides* seeds. NaCl concentration (mM): ●, 0; ○, 100; ▼, 200; ■, 400; □, 500.

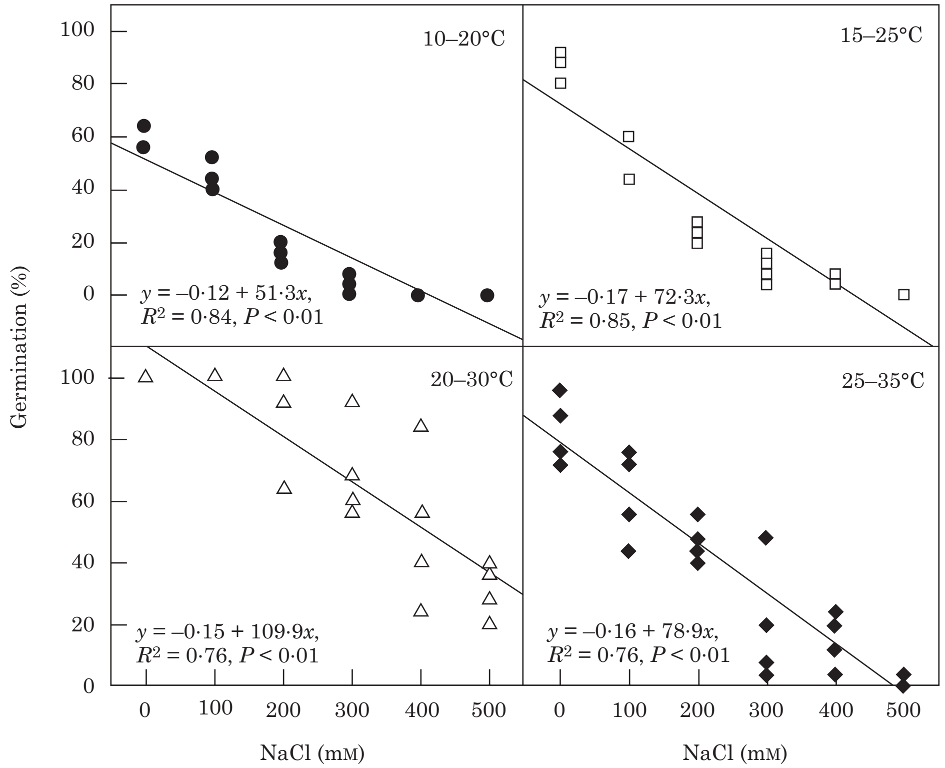

FIG. 2. Regression plots for mean final germination percentage of *Aeluropus lagopoides* seeds in different salinity and temperature regimes.

non-saline control; however, a further increase in temperature (25/35 °C) inhibited germination.

The rate of germination (calculated using a modified Timson's index) was highest at 20/30 °C and lowest at 10/20 °C (Fig. 3). Addition of NaCl to the medium adversely affected the germination rate at all temperatures, with a less marked response at 10/20 °C and 15/25 °C where non-saline control values were lower (Fig. 3). Two-way ANOVA indicated a significant effect of salinity and temperature on rate of germination (Table 1).

When seeds were transferred to distilled water after 20 d of salinity treatment, the recovery of germination percentages increased with an increase in pre-transfer salinity treatments except for 10/20 °C (Fig. 4). Seeds subjected to high temperature had higher recovery percentages (Fig. 4). Seed germination recovered completely in distilled water at higher temperatures and at all salinity treatments, but recovery was lower (25 %) at 10/20 °C. A two-way ANOVA indicated significant ($P < 0{\cdot}001$) effects of both salinity and temperature on total germination (Table 1).

DISCUSSION

Halophyte seeds germinate best under non-saline conditions (Ungar, 1995). Salinity inhibits germination of halophyte seeds in one of two ways: (1) preventing germination without loss of viability at higher salinities; and (2) delaying germination of seeds at salinities that cause some stress to seeds but do not prevent germination. The threshold salinity for a significant reduction in germination varies between grass species from 100–500 mM NaCl (Lombardi *et al.*, 1998; Gulzar *et al.*, 2001; Khan and Ungar, 2001). The germination tolerance of plant species to salinity under laboratory conditions does not necessarily correlate with their response to salinity under field conditions, and may be many times lower. Seeds usually germinate after leaching of salt due to the monsoon rainfall in the vicinity of Karachi (Khan and Gul, 1998). Mahmood and Malik (1996) found that the grasses *Sporobolus arabicus*, *Cynodon dactylon*, *Polypogon monspeliensis* and *Desmostachya bipinnata* showed a decrease in germination with an increase in salinity from 3–20 dS m^{-1}. Only *D. bipinnata* exhibited salt stimulation at low salinity (5 dS m^{-1} or 50·16 mM NaCl) and had the highest (40 %) germination at 20 dS m^{-1} (206 mM NaCl) with no seeds germinating at higher salinities. Hyder and Yasmin (1972) found that the germination of *Sporobolus airoides* seeds was inhibited by a specific ion effect. They exhibited 82 % germination in distilled water and 2 % in 325 mM NaCl. Gulzar *et al.* (2001) reported that seeds of *U. setulosa* could germinate in up to 500 mM NaCl while those of *H. mucronatum* failed to germinate at 300 mM NaCl (Noor and Khan, 1995). *Aeluropus lagopoides* seeds showed no dormancy under non-saline conditions. An increase in salinity inhibited germination; however, about 30 % of the seeds germinated at 500 mM NaCl. These results indicate that *A. lagopoides* is a highly salt tolerant grass in comparison to other grasses found at inland and coastal sites near Karachi.

Sensitivity to periodic temperature and salinity fluctuations constitutes an important system which enables plants to respond to daily variations in the soil surface conditions.

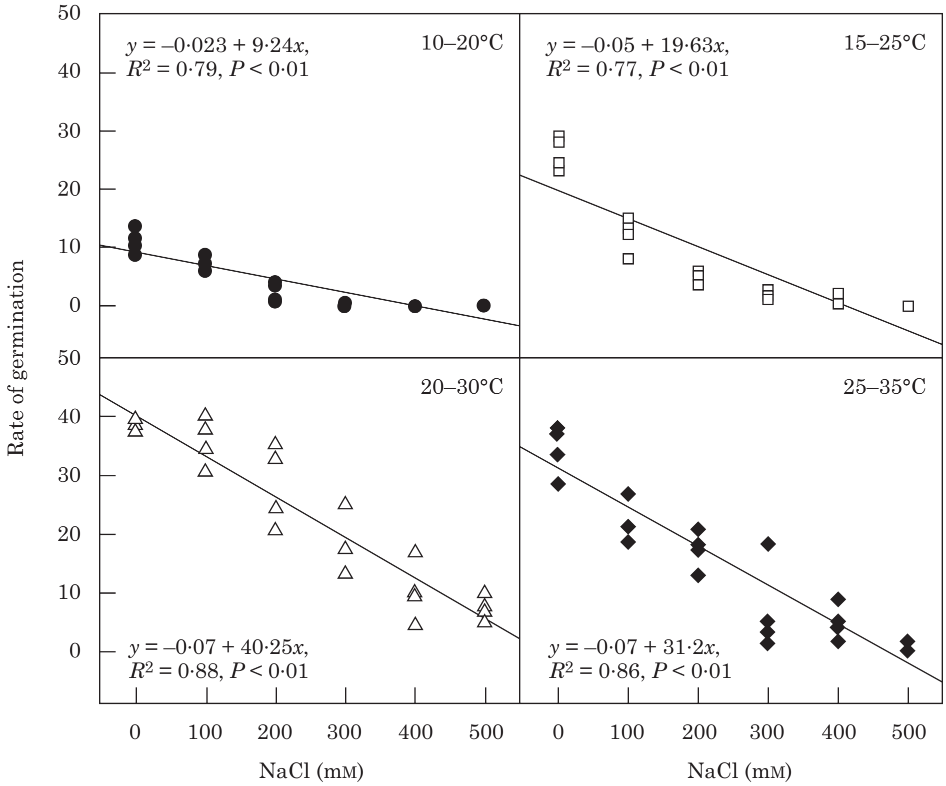

FIG. 3. Regression plots for the rate of germination (maximum = 50) of *Aeluropus lagopoides* seeds in different salinity and temperature regimes.

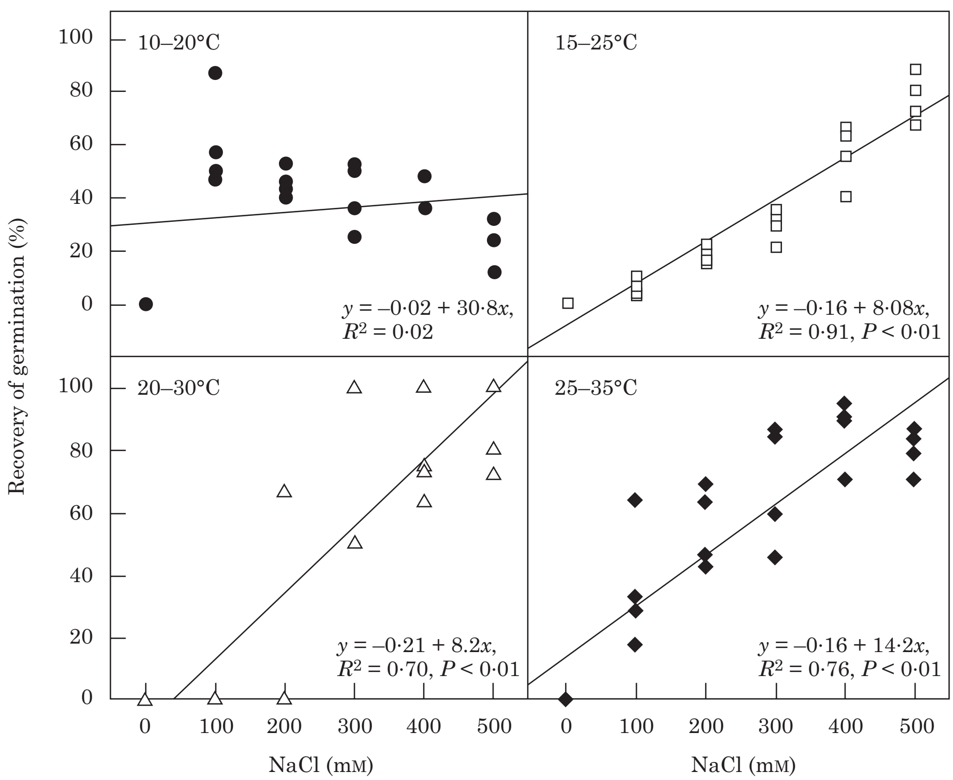

FIG. 4. Regression plots for percentage recovery germination of *Aeluropus lagopoides* seeds in different salinity and temperature regimes.

Temperature shifts may affect a number of processes determining the germinability of seeds, including membrane permeability, activity of membrane-bound proteins, and cytosol enzymes (Bewley and Black, 1994). The response of halophytic seeds to alternating temperatures and soil salinity levels is of ecological significance. Germination of halophyte seeds in subtropical coastal and inland salt marshes usually occurs after monsoon rains when there is a reduction in temperature and soil salinity (Khan and Ungar, 1996, 1997, 1998, 1999; Khan and Gul, 1998). Salinity and temperature interact in their control of seed germination and the greatest inhibition is usually found at the maximum and minimum limits of tolerance of these two environmental variables, as reported in *Hordeum jubatum* (Badger and Ungar, 1989), *Crambe abyssinica* (Fowler, 1991), *Puccinellia ciliata* (Myers and Couper, 1989), *Halopyrum mucronatum* (Noor and Khan, 1995), and *Urochondra setulosa* (Gulzar *et al.,* 2001). The effect of salinity on germination varies considerably with temperature (Fowler, 1986; Badger and Ungar, 1989; Morgan and Myers, 1989; Myers and Morgan, 1989; Romo, 1990; Gutterman, 1993; Noor and Khan, 1995; Gulzar *et al.,* 2001). Cluff *et al*. (1983) reported that a temperature regime of 10/40 °C (16/8 h) is optimal for seed germination of *Distichlis spicata*, whereas −5 and 50 °C were the lower and upper threshold temperatures for seed germination. Only 1 % germination was observed in −15 bars NaCl concentration at the optimum temperature of 10/40 °C. *Aeluropus lagopoides* showed similar results with 31 % germination at the optimal temperature under the highest salinity treatment (500 mM NaCl), but an increase or decrease in temperature regime significantly inhibited germination at all salinities. This is in contrast with *Arthrocnemum macrostachyum* (Khan and Gul, 1998) and *Suaeda fruticosa* (Khan and Ungar, 1998), where temperature variations were not significant at lower salinities.

The response of seeds of *A. lagopoides* transferred to distilled water after 20 d at various salinities varied depending on the temperature regime. Seeds exposed to higher salinity recovered quickly at warmer temperatures. Under hyper-saline conditions, seed survival rather than germinability may be an appropriate criterion for success, since recovery germination does occur in seeds of *A. lagopoides* and other halophytes when hyper-saline conditions are alleviated (Ungar, 1995; Khan and Ungar, 1996, 1997, 1998, 1999). Recovery germination responses were also dependent on temperature, ranging from 29 % recovery at 10/20 °C to 85 % at 20/30 °C.

Aeluropus lagopoides is a perennial grass with an extensive rhizome system that can tolerate high salinities (Bodla *et al*., 1995). Coastal populations are exposed to seasonal tidal inundation and a highly saline shallow water table. Inland populations grow in highly saline wet soils. *Aeluropus lagopoides* propagates primarily through rhizomes, although it produces a large number of seeds and maintains a transient seed bank (Khan and Gul, 1999). The present study demonstrates that seeds are not dormant and showed 100 % germination at the optimal temperature. Seeds of *A. lagopoides* should be considered among the most salt tolerant of local grasses found in saline soils in the Karachi region. Thirty percent of seeds germinated at 500 mM NaCl, a salinity concentration approaching seawater (600 mM NaCl). These data showed that *A. lagopoides* could be recruited easily in highly saline inland and coastal conditions under warm temperatures. It seems that the proximate strategy of *A. lagopoides* is to use vegetative methods for recruitment of new individuals—a less costly way of recruitment in a highly unpredictable, harsh environment. Production of a large number of seeds is probably an ultimate strategy to introduce new genotypes into *A. lagopoides* populations at an appropriate time to maximize fitness. Further investigations are necessary to understand the ecophysiological strategies of plants for survival under natural environmental conditions.

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