

Photosynthesis of natural cocksfoot populations under water and salt stresses

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Abstract

Sampling of natural cocksfoot (*Dactylis glomerata* L.) populations was carried out on the O Morrazo peninsula in NW Spain, characterized by a strong moisture gradient. The plants were kept in greenhouse under standard conditions. Nevertheless, they differ in height of plants, length and width of flag leaves, panicle size, stomatal density and size as well as in flowering period. The effects of two levels of soil water deficit and two levels of salinity on photosynthetic rate were tested. One population was exceptionally well adapted to its original environment with great tolerance to water deficit and salinity.

Additional key words: *Dactylis glomerata* L., *Gramineae* ecophysiology.

Introduction

To understand the distributional limits of plants it is proposed to uncover the physiological responses to suboptimal environmental conditions, that is, when absorption of water or nutrients, the process of photosynthesis, respiration, *etc.*, are limited during some part of the life cycle of the plant (Levitt 1980, Salisbury and Ross 1992).

Our interest is centered on the study of a grass species, *Dactylis glomerata* L., whose great adaptive potential allows it to colonize very diverse ecosystems. Numerous subspecies have been described with different cytotypes: diploid, tetraploid and hexaploid (Stebbins and Zohary 1959, Borril 1961, Lumaret *et al.* 1987, Ortiz and Rodriguez-Oubiña 1993). In Galicia there is sympatry between

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various Galician diploid and tetraploid autochthonous cultivars which are morphologically indistinguishable but possess different adaptive capacities (Chorlton and Young 1980). In this study an ecophysiological characterization of various *D. glomerata* populations is given, with special emphasis on photosynthetic responses under two environmental stresses, *i.e.* water stress and salinity. Therefore we have chosen a restricted geographic area in which we find a strong thermopluviometric gradient and a different marine influence in the distinct locations.

Materials and methods

Adult plants were collected from natural cocksfoot (*Dactylis glomerata* L.) populations at eight locations on the O Morrazo peninsula (Pontevedra, Spain), during November and December 1991 (Fig. 1). The choice of locations was determined by topographic and climatic criteria. Considering the topography, five populations were sampled at sea level, all being in the splash zone and influenced by sea mist: Home (H), Nerga (N), Punta Fanequeira (PFa), Punta do Crego (PC) and Punta de Moa (PM). Three inland populations high above the splash zone and marine influence were chosen: Alto Carballiño (AC), Monte Ermelo (ME) and Faro Domaio (FD) at 300, 400 and 600 m, respectively. A marked gradient of mean annual precipitation (Fig. 1.) as well as dryness in summer characterize the peninsula (Carballeira *et al.* 1983).

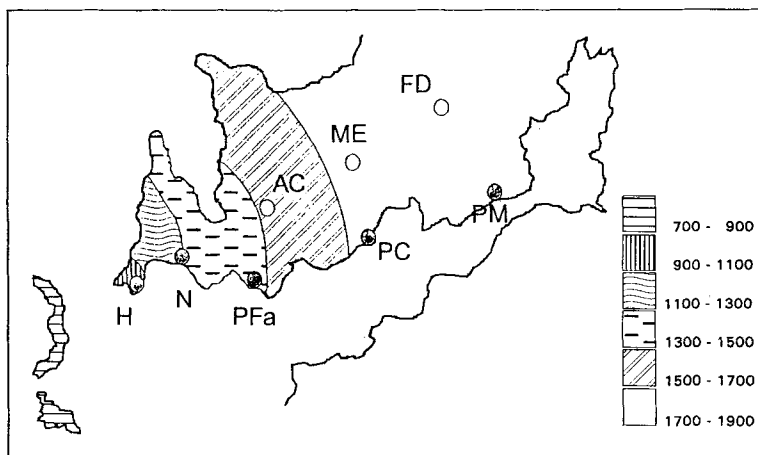


Fig. 1. Location of the eight sampling sites for *Dactylis glomerata* L. on the O Morrazo peninsula (NW Spain) and their distribution along the mean annual precipitation gradient [mm]. Scale 1:225 000.

At least 30 plants per population were collected and planted in plastic pots with commercial substrate. Vegetative reproduction was performed on the samples to obtain enough plants for later tests. Samples were kept in a greenhouse under identical conditions of watering and temperature.

Leaf epidermal tissues were studied by Laroche's method (Laroche 1982). In the replicas of the leaf surface the stomatal densities were estimated under microscope as well as the length of 30 stomata per plant for at least 10 plants per population. The stomata/leaf ratio (S/L) was calculated as:

$$(\text{average stomatal density} \times \text{stomatal length}) / (\text{average length} \times \text{width of flag leaf})$$

The six characters which showed the most variation in the Galician *Dactylis glomerata* L. populations and were the most useful in the discrimination of the groups, were selected according to previous studies (Borriol 1961, Lumaret 1985, De Ramon and Reigosa, unpublished). Thus, when the plants were at anthesis, the height of plant, length and width of flag leaf, ligule length, panicle length and the length of the first branch of the panicle were measured.

Simultaneously with the measurement of morphological characters, the starting date of flowering and anthesis were recorded as well as the total duration of anthesis in each population.

The ploidy level of the plants was determined by laser cytometry in the laboratory of the Institut des Sciences Végétales (CNRS), Gif-sur-Yvette (France), showing that most samples were tetraploid except for two hexaploids (Horjales *et al.* 1996).

Two salinity stress treatments were applied via watering with NaCl solution at two different concentrations: 0.25 and 1 % (m/v). Two water deficit treatments were set up by maintaining two soil water contents: 35 and 45 % (m/m). A control treatment was kept watered at field capacity. These tests were carried out by treatment in a greenhouse for four months and each of the five treatments was applied to nine randomly chosen plants from each population.

At the end of the tests the net photosynthetic rate was measured for the 36 plants that underwent the treatments using the Portable Photosynthetic System *LI-COR 6200* (Lincoln, USA). Simultaneously the leaf and air temperatures, air flow, relative humidity, carbon dioxide concentration and the irradiance were measured.

The statistical analyses were carried out using the statistical program *SPSS/PC+*. An *ANOVA* and *LSD* tests (multiple comparison of mean minimum significant difference) were carried out for the photosynthetic and biometric data. A cluster analysis was performed on the data for six morphological characters. To clarify which part of the variation in the photosynthetic rate was due to the distinct treatments, an *ANCOVA* analysis was carried out for the variables: photosynthetic rate, treatments and populations, introducing as covariables the environmental parameters: irradiance, air and leaf temperature, carbon dioxide, air flow and relative humidity. After that, a multiple regression analysis on these photosynthetic data was carried out.

Results and discussion

The analysis of variance showed highly significant differences ($P < 0.001$) in the stomatal length among the eight populations and significant differences ($P < 0.01$) in

the stomatal density. Population N had the longest stomata and H the highest stomatal density. These two populations had the largest stomatal/leaf ratio (Fig. 2). Considering that these populations withstand the hardest climatic conditions on the peninsula, with the lowest annual rainfall and most severe summer drought, a larger stomatal/leaf ratio might allow an effective regulation of water loss in the dry seasons. Furthermore these populations show a reduction in leaf size.

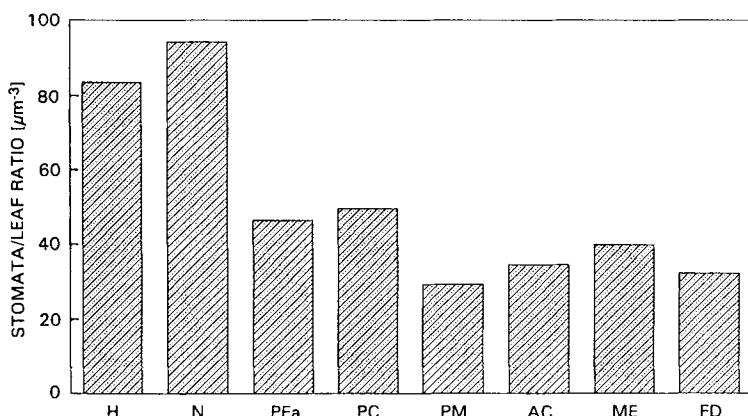


Fig. 2. Average stomatal leaf rate for each population of *Dactylis glomerata* L.

One way ANOVA carried out for each of the six morphological variables showed significant differences between populations, with different significance levels for each variable (Fig. 3). The result of the LSD test showed the existence of many significant differences between pairs of populations for each variable. The lowest values are found in the outermost populations on the peninsula, both on the coast and in the mountains, coinciding with the lowest mean annual rainfall, while the highest values were reached in the easternmost areas, which has more favorable climatic conditions. A clear positive correlation between rainfall and the growth is thus confirmed.

The cluster analysis carried out according to the data for the six characteristics (Fig. 4), clearly shows the existence of two morphologically different groups: one made up by the two outermost populations on the peninsula, H and N, and the other which covers all the other populations. On a second level of grouping the separation of the easternmost populations from the coastal ones (PM) results, while a group with intermediate morphological characters formed populations in an area intermediate in the climatic gradient. Plants from ME and FD, see Fig. 3, showed variable morphological characteristics and are not included in defined groups.

The start of flowering in the population H withstanding the hardest climatic regime, occurred some weeks before that of the other populations. Earlier flowering in populations which withstand strong summer drying conditions is a useful strategy to assure maximum seed production before the reproductive period in the season of low water availability in the soil.

A multiple regression analysis showed that only three variables: CO_2 , irradiance

and relative humidity explained practically all the variation in net photosynthetic rate (P_N) that was not due to treatments or populations.

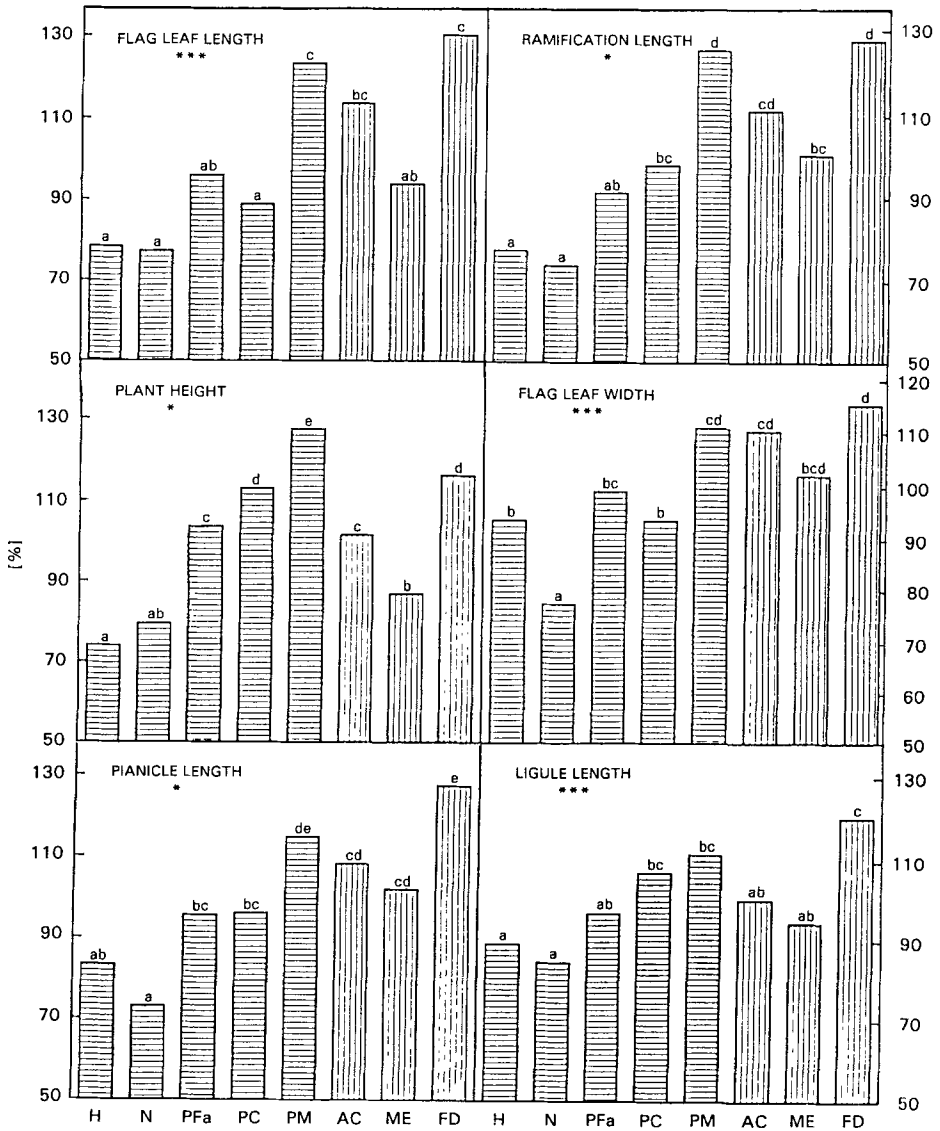


Fig. 3. Six morphological characteristics measured in the eight populations of *Dactylis glomerata* L. Data are shown as percentage with respect to the mean value. Bars with horizontal and vertical lines represent coastal and inland populations, respectively. The asterisks show significant differences between the populations: * - $P < 0.05$ and *** - $P < 0.001$ (ANOVA). The columns labeled with the same letter are not significantly different at a level of 5 % (LSD test).

In the ANOVA significant differences were seen between treatments and populations and there was a highly significant interaction between treatments and populations, so in such a way a different response to the distinct stress treatments applied was obtained in the eight populations.

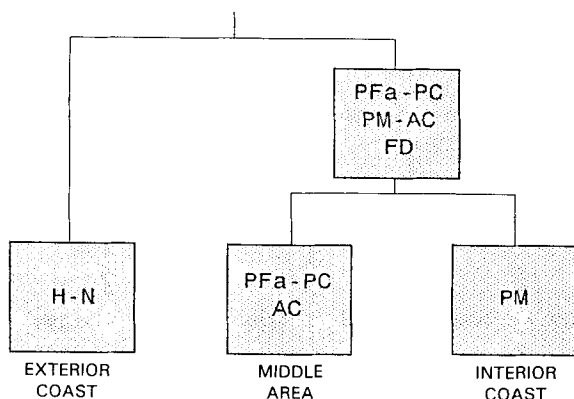


Fig. 4. Resulting dendrogram from the cluster analysis carried out for the data from the six morphological characteristics in the group of *Dactylis glomerata* L. populations. Two levels of grouping stand out which show three groups of populations according to the natural habitat: inner coastal, outer coastal, and a group between the two which contains populations of intermediate characters.

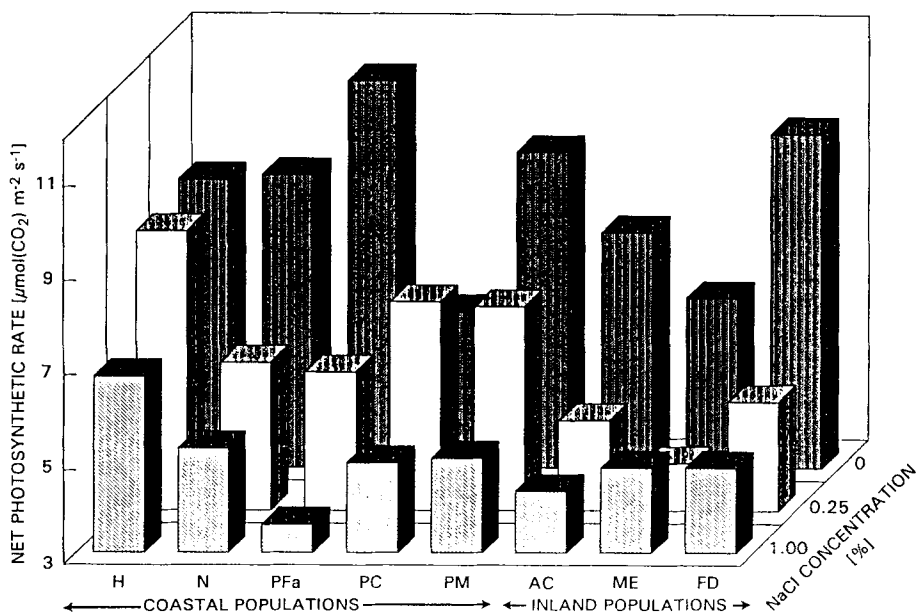


Fig. 5. Effects of salinity (0.25 % and 1 % NaCl) on the net photosynthetic rate of the eight natural Galician *Dactylis glomerata* L. populations.

Moderate salinity (0.25 % NaCl) caused a moderate decrease in P_N in the eight populations with respect to the control, but the response differs according to the original habitat as the three mountain populations are more affected, while the coastal populations appear to show in general a greater capacity to tolerate salinity, excepting PFa which was strongly affected (Fig. 5). Under 1% NaCl a marked decrease in P_N was seen in both mountain and coastal populations. Plants from the most western coastal population (H) show a great capacity to withstand both levels of salinity; only the highest salinity level depresses significantly its photosynthetic activity. There is almost no photosynthetic variation in the moderate salinity treatment compared with the control and in the strong salinity treatment the rate is the highest of the group.

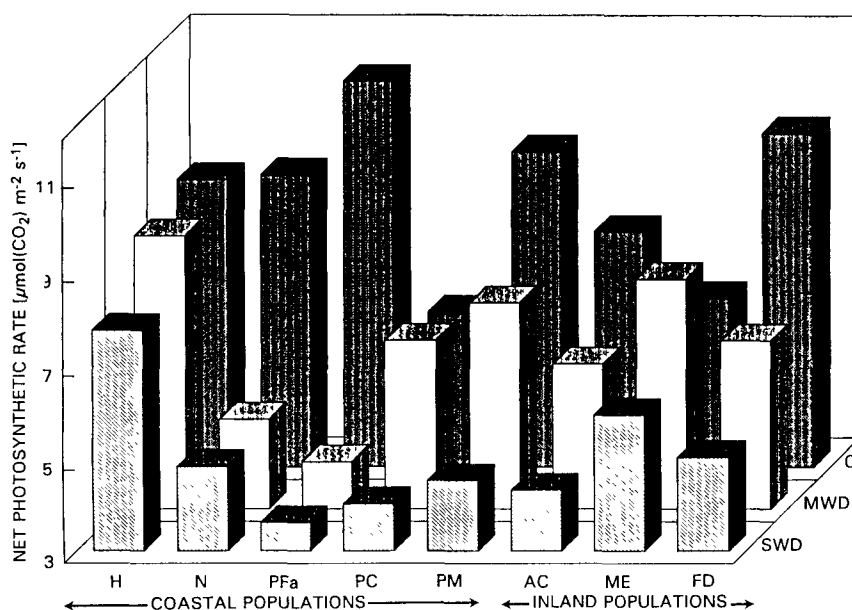


Fig. 6. Photosynthetic response of *Dactylis glomerata* L. populations to water stress: SWD - severe water deficit, MWD - mild water deficit, C - control (soil water contents 35 %, 45 % and maximum field capacity, respectively).

The photosynthetic response of the populations to water stress treatments (Fig. 6), show a similar tendency to that of the salinity stress. When the water deficit is moderate, P_N is comparable with the control, although it is unequal in each population. The populations N and PFa were low tolerant to drying and showed a dramatic decrease in photosynthetic rate both under water deficits. On the other hand the population which withstands the most severe summer drought, H, showed a great adaptive capacity to resist stress and the photosynthetic rate was hardly suppressed by the applied water stress. In conclusion, the population from the most stressing habitat was the most capable of coping with the different induced stresses.

References

- Borriol, M.: Epidermal characteristics in the diploid subspecies of *Dactylis glomerata* L. - J. Linn. Soc. Ser. Bot. **56**: 453-458, 1961.
- Carballeira, A., Devesa, C., Retuerto, R., Santillan, E., Uceda, F.: [Bioclimatology from Galicia]. - Fundación Pedro Barrié de la Maza. A Coruña 1983. [In Span.]
- Chorlton, K.H., Young, N.R.: List of grass ecotypes collected in July and August 1977 in Galicia, NW Spain. - In: Plant Genetic Resources. Rep. Welsh Plant Breeding Sta., Aberystwith 1980.
- Horjales, M., Redondo, N., Pérez, B., Brown, S.: [Presence of hexaploid *Dactylis glomerata* L. in Galicia]. - In: Boletín da Sociedade Broteriana. Vol VI-VII, in press, Universidade de Coimbra, Coimbra 1996. [In Span.]
- Laroche, J.: Nouvelle technique d'empreintes du matériel végétal. I. Etude des surfaces épidermiques et des tissus internes. - Rev. gén. Bot. **89**: 173-181, 1982.
- Levitt, J.: Responses of Plants to Environmental Stresses. 2nd Edition. Vol. II. Chapter 3. - Academic Press, New York 1980.
- Lumaret, R.: Phenotypic and genotypic variation within and between populations of the polyploid complex, *Dactylis glomerata* L. - In: Haeck, J., Woldendorp, J.W. (ed.): Phenotypic and Genotypic Variation in Plant Populations. Pp. 343-354. North-Holland, Amsterdam - Oxford - New York 1985.
- Lumaret, R., Barrientos, E., Guillermin, J.L., Jay, M., Fiasson, J.L., Ardouin, P., Delay, J., Ait Lhaj Loutfi, A., Izco, J., Amigo, J.: Signification adaptative et évolutive de la polyploïdie infraspécifique: cas des dactyles diploïdes et tétraploïdes de Galice. - In: Legay, J.M. (ed.): Biologie des Populations. Evaluation et Prospective. Vol. I. Pp. 129-133. Université Claude Bernard, Lyon 1987.
- Ortiz, S., Rodríguez-Oubiña, J.: *Dactylis glomerata* L. subsp. *izcoi*, a new subspecies from Galicia NW Iberian Peninsula. - Ann. Bot. fenn. **30**: 305-311, 1993.
- Salisbury, F.B., Ross, C.W.: Plant Physiology. 4th Edition. Wadsworth Publishing Company, Belmont 1992.
- Stebbins, G.L., Zohary, D.: Cytogenetic and evolutionary studies in the genus *Dactylis*. I. Morphology, distribution and interrelationships of diploid subspecies. - Univ. California Publ. Bot. **31**: 1-39, 1959.