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DROUGHT STRESS TESTING ON THREE GRASSES SPECIES IN CONTROLED CONDITIONS

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Abstract

Climate change effects on agriculture are estimated to be dramatically affected for the next 20 years [15]and up to 2050 and therefore it is a great need for increasing diversity in crops species for improving their ability to deal with drought, floods and harsh conditions generally. The scope of this article was to evaluate three grasses perennial species for their development under experimental drought conditions. Thus, Dactylis glomerata, Festuca pratensis and Lolium perenne were selected for this experiment as they are very important for Romanian grasslands. There have been used two types of seeds for each species: control, certified seeds and regenerated after in vitro cultivation on polyethylene glycol of these three species and obtained in a previous study [2]. Our results revealed that the plants regenerated upon in vitro cultivation on PEG are better adapted to drought conditions.

Key words**:** *Dactylis glomerata, Festuca pratensis, Lolium perenne, drought stress, dry matter, fresh weight*

INTRODUCTION

Droughts, heat waves and floods are the main challenges which will need to be answered through adaptation measures. Southern regions of the country are already exposed to desiccation phenomenon that will continue in the future. The trend of reducing the thickness of the snow, already observed in the west and northeast of the country, which could amplify in the future creates problems in management of water reserves, that are threatened by the future evolution of climate change effects. Hilly and mountainous regions are also exposed to the impact of climate change, the frequency of avalanches and landslides may change dramatically. In the context of global warming, there is a tendency in recent decades, winters in Romania become less harsh, with higher temperatures and reduced snow cover, the trend will continue in coming decades [16].
Based on the Report of the Intergovernmental Panel for Climate Change (February 2007), the average global temperature will increase until the year 2100 by about 3° C, which will cause a trigger early cycle of vegetation spring and an extension of its fall [6].
The National Soil Quality Monitoring showed that about 7.1 million hectares of farmland are affected by "frequent drought" as a major limiting factor of production [8]. Based on these authors podzolic soils, characterized by the very low natural fertility, because of the negative physical-chemical and biological traits, occupy about a quarter of the arable land, especially in regions of Carpathians such as Transylvania and Moldova, depressions foothill areas in the north, Piedmont west plateau Suceava, etc. Closely linked to the podzolic is developing the upper layers of soil acidity by removing bases from primary minerals and then clay-humic complex. Podzolic soil of high acidity, with adverse effects on crops, is accompanied by a deficiency in nutrition elements, giving a low fertility.
Due to the high content of clay, acid soils cannot store nor in wet periods than some of the water precipitation, water supplies are so scarce. In areas with podzolic soils, livestock has been and must remain the most important sector of agriculture, the share of fodder is made up of permanent pastures or meadows sown, which should provide 60-65% of the areas of perennial crops [14].
Perennial grasses and legumes are the main

components of grasslands of the vegetation of meadows. The total area of grassland in Romania is 4.9 hectares, representing about 21% of the total area of the country and 34% of agricultural land, of which 3.4 million ha are pastures and 1.5 million ha are grassland. From this point of view Romania ranks the 5th in Europe after France, England, Spain and Germany [4].

Cultivation performance, adapted to climatic and how to use them are the main means of increasing forage production, together with respect for culture technologies. Drought resistance varieties and unfavourable soil conditions is a basic element of stability production in areas affected by these phenomena.

Using a valuable germplasm and applying biotechnological technology for "in vitro" cultivation and selection and continuing with greenhouse technology for fields, is providing premises to obtain resistant varieties of perennial grasses with an increasing productivity in grasslands as a valuable alternative to harsh living conditions [5].

The main characteristics based for selection and improvement of fodder plant species are: the biomass and seed production; obtaining forage of high quality, expressed by chemical composition, energy-protein ratio, suitability to different conservation method such as: palatability, fibre content, resistance to diseases and pests, repeated harvesting resistance, resistance to limestone, adaptability to natural factors of stress. Among the environmental factors that have significant impact on the level and quality of production are water and soil pH [12].

The situation created as an effect of enhancing global warming confronts livestock farmers with new challenges. The value of forage grasses and legumes species of wild flora and their ability to exploit different production conditions were the top criteria in the selection of the most valuable biotypes and have increased the importance of a reduced number of 10 to 12 species that are part of more than 400 botanical families of two important forage base [15].

Perennial grass species that participate substantially (30-90%) to the composition of the vegetation in natural grasslands are *Festuca pratensis*, *Festuca arundinacea*, *Lolium perenne*, *Phleum pretense* and *Dactylis glomerata* [17]. Among the most valuable are perennial legumes such as *Medicago sativa*, *Trifolium pratense* (red clover) *Trifolium repens* (white clover), *Lotus corniculatus* (trefoil) and *Onobrychis viciifolia* (sulla) [10].

The scope of this article is to present our results obtained for testing in greenhouse conditions the resistance to drought of three fodders species *Dactylis glomerata, Festuca pratensis* and *Lolium perenne* that have been previously under water stress into *in vitro* conditions [2]. Fresh weight and dry matter are used as major indicator in assessing the adaptation ability of these species.

MATERIALS AND METHODS

Plant material. Certified seeds of *Dactylis glomerata* ‘Intensiv’, *Festuca pratensis* ‘Pradel’ and *Lolium perenne* ‘Mara’ as well as five clones of each species originating from previous *in vitro* experimentation after four years. These seeds are originating from regenerated plantlets upon their *in vitro* cultivation on polyethylene glycol (PEG) as a water stress factor [2]. After acclimation, these plants complete their life cycle and produces seeds that have been used in these experiments.

Experimental scheme Healthy seeds from all three species have been sown in plastic boxes for 14 weeks. Plants have been transferred after 14 weeks on pots of 15 cm in diameter. Plastic boxes and pots have been filled with field soil where these species are cultivated for more than 25 years to obtain reliable data towards control plants. The experiment was conducted in greenhouse prepared for controlling drought. Temperature was similar with that of the summer of 2014 and air drought was maintained under 10%.

Laboratory analysis were realized at the end of experiment. 15 repetitions of each were used to evaluate fresh wright (g and %) and dry matter (g and %). Dry matter was conducted in the lab oven at 60°C up to constant weight for two days.

RESULTS AND DISCUSSIONS

Most important species bear low temperatures in winter and high in the summer if vegetate on a land use enabling them to achieve a balance between the minerals that compose them. Less resistant protective layer in case of lack of snow are *Lolium perenne* and *Dactylis glomerata*. Forage grass species are generally sensitive to temperature drop during early spring time. Also, special attention should be paid to physiological phases when come in winter if sowing is performed early in autumn. The number of active grades when it is generally active ranks at around 1,000, with a higher need for species *Dactylis glomerata*. More sensitive to high temperatures is *Lolium* a perennial species in which there are varieties in very hot summer months enters vegetative rest and resume their growth in September when the temperature drops and improves the soil's water regime [11].

Important as forage, grasses species have higher water consumption with an average of 50-100% more compared to cereals due to longer vegetation period, production of biomass, evaporation during the periods after sew or grazing, large leaf surface and perspiration rate, and so on. Plants need more water with increasing elongation straw and ambient temperature. Because of this small root system plants with low capacity to absorb water, production quantitatively and qualitatively reduces. Water consumption is higher in the case of grasslands that capitalize mowing than those that grazed in which case the need is water and water more evenly throughout the growing season [1].

Analysing spreading area of forage' species it can be appreciated that they have reduced requirements to soil. However, the plants grow well in soils with medium texture, clayey or sandy loam, with a deep fertile layer with groundwater at a depth of more than 70-120 cm lower in the case of grasses and higher in vegetables. Towards the soil pH, grasses don't have outstanding claims, although they are growing the best on soils with values close to neutral, supporting wide limits the value of this indicator.

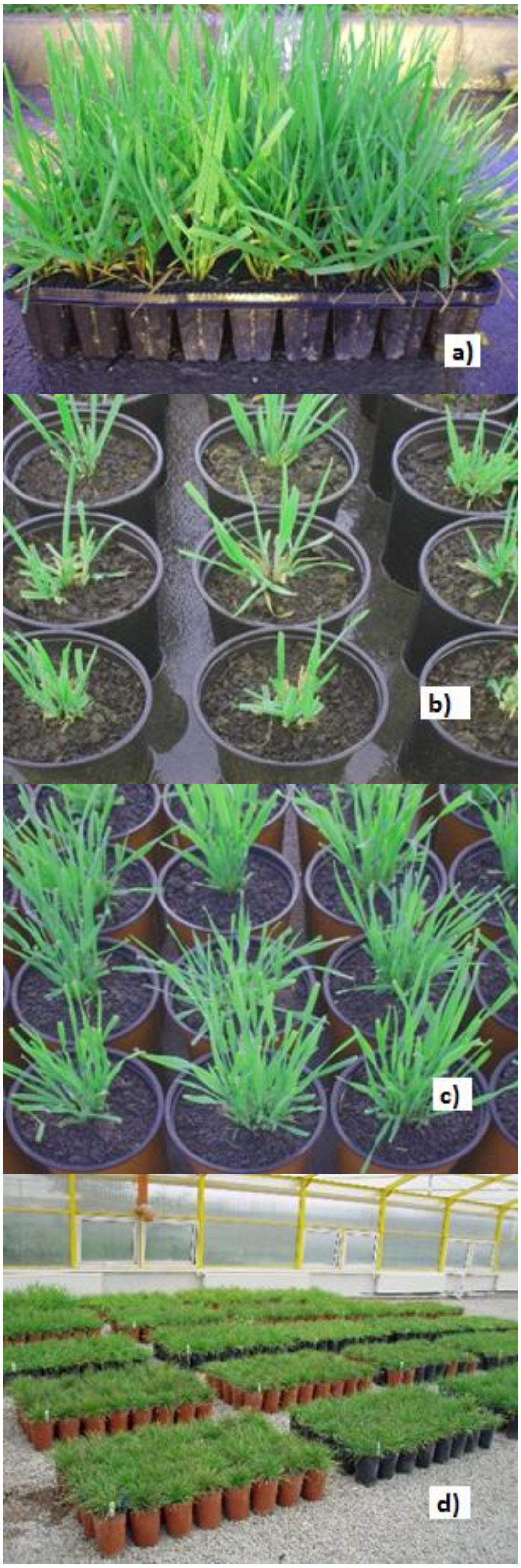

Fig. 1. Plants during the preparing of experiment: a) at 6 weeks of sowing; b) at 12 weeks; c) at 14 weeks before starting the experiment and d) after 16 weeks of starting the experiment.

Towards nutrients, grasses have moderate requirements, they managed to extract nutrients from the soil solution. Of macronutrients, grasses have high demands towards nitrogen and legumes towards phosphorus, potassium and calcium. Ensuring nitrogen supplements may increase productivity, on one hand and increasing the protein content in legumes on the other hand [9].

Table 1. The effect of drought conditions on three species *Dactylis glomerata*, *Festuca pratensis* and *Lolium perenne*

Species	Repetition clones 15 plants	Fresh weight (g)	Fresh weight (%)	Dry matter (g)	Dry matter (%)
Dactylis glomerata	1	8.60	95	4.5	123
	2	7.33	85	3.31	89
	3	7.88	90	3.45	93
	4	10.20	118	4.20	113
	5	8.65	100	3.70	100
	Average	**8.515**	**98.25**	**3.665**	**98.75**
	Control	**7.2**	**82**	**3.12**	**82**
Festuca pratensis	1	9.93	131	4.23	107
	2	7.36	97	3.25	82
	3	7.39	97	3.37	86
	4	6.94	91	2.89	73
	5	7.59	100	3.94	100
	Average	**7.842**	**103.2**	**3.532**	**89.6**
	Control	**5.32**	**81**	**2.35**	**62**
Lolium perene	1	4.94	77	2.60	75
	2	7.38	115	4.12	119
	3	7.54	117	3.85	112
	4	7.60	118	4.19	121
	5	6.44	100	3.45	100
	Average	**6.784**	**105.4**	**3.642**	**105.4**
	Control	**5.23**	**82**	**3.02**	**88**

The entire experiment started with a total of 1500 seeds: 250 of each variant and species (250 as control and 250 as treated with PEG). Each group of 250 seeds of each species have been sowed in plastic boxes filled in with soil taken from the experimental filed. The seedlings have been watered at 10 days with a nutrient solution 5% and treated with Previcur 607SL [7]. After 6 weeks of sowing, the experiment started with 200 plants presenting one or two sprouts for pot culture. 50 seeds were ensuring the success of our experiment in case of diseases or pests, or difficulties in germination. Pots have been filled with the same soil taken from our experimental field. Up to the next movement these plants have been watered at each two day and treated with nutrient solution at each 10th day up to 14 weeks (Fig 1 a, b and c). At 14 weeks of sowing all plants of each of the three species were transferred for testing the drought stress (Fig 1 d).

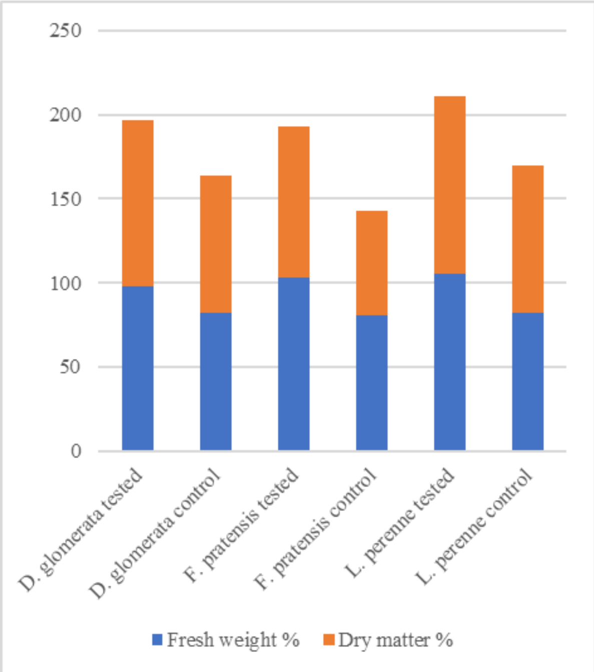

Fig. 2 Effect of induced drought on fresh weight (%) and dry matter (%) for *D. glomerata, F. pratensis* and *L. perenne* previously treated *in vitro* for water stress compared to control

All pots have been randomly disposed in five repetitions with 40 plants. Each repetition was considered a lot of plants that was placed differently inside the greenhouse. Before the drought stress, in the first stage, all plants have been abundantly watered similar with the filed conditions after heavy raining. This period was followed by an induced drought when no watering was applied and furthermore, the moister level inside the greenhouse was kept at 20%.

After 6 weeks of artificial drought induced conditions obvious effects on plants were observed such as the plant colour, vigour, turgescence and the ratio between dry leaves and shots. These preliminary observations were very important because based on them it is possible to continue new experimentations. The main indicator was dry matter for randomly selected individual plants. All results are presented in Table 1 and Fig. 2.

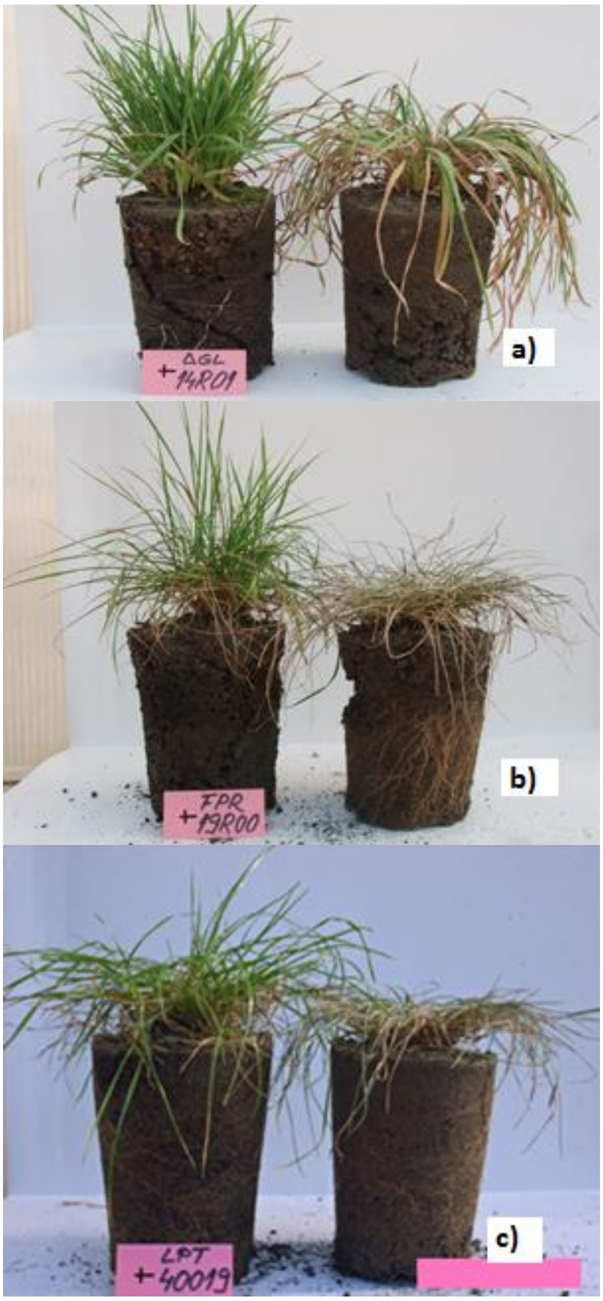

Fig. 3. General aspect of plants affected by drought: a) *Dactylis glomerata* plants after 6 weeks of drought stress (tested in left and control in right); b) *Festuca pratensis* plants after 6 weeks of drought stress (tested in left and control in right) and c) *Lolium perenne* plants after 6 weeks of drought stress (tested in left and control in right).

Based on our results all clones derived from *in vitro* experimentation presented high adaptability to experimental drought conditions compared to the control seeds using the same soil in the experiment like that of filed. Net differences between plants (i.e. vigour, colour, general appearance) of *in vitro* culture origin and control are presented in Fig. 3.

As a general result for all three species, dry matter was higher in case of seeds originating from *in vitro* testing on PEG compared to control. Thus, in case of *Dactylis glomerata* the dry matter was more than 16% higher compared to control, in case of Festuca pratensis it was recorded an increase of over 26% and in case of *Lolium perenne* over 17%. Such results are in line with previous studies [13]. Moreover, our studies support the national politics on climate change and biodiversity conservation especially for ensuring food security for long term [3].

CONCLUSIONS

Testing the effect of drought on three perennial grasses species proved that a previous testing using *in vitro* culture is beneficial in plants adaptation for ex vitro conditions after more than four years. These results further support the idea that *in vitro* techniques are valuable tools for increasing the biological diversity in species, especially related to the increase of adaptability to harsh environmental conditions.

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