

## LIMITATION IN CONDUCTING RESEARCH FOR FODDER SPECIES BREEDING

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### Abstract

Currently it is considered that food security is highly depending on the sustainable use of arable land that may be dramatically affected by drought and/or precipitations in the next 20 years. The Romanian agricultural economy is based on animal bred, crops cultivation as well as commodity trade. Fodder plants become more important when drought and acidification become limiting factors negatively impacting our own ability to bred livestock. A complex research project was implemented starting with 2007 when six fodder species (i.e. *Festuca arundinacea*, *Festuca pratensis*, *Phleum pratense*, *Dactylis glomerata*, *Lolium perenne* and *Trifolium repens*) have been tested with the scope of producing new genotypes that may develop adaptation mechanisms towards drought and acidification. Thus, the scope of this article is to emphasize the main limitations that have been encountered during project implementation. A SWOT analysis was applied for evaluating final results. Major limitations are imposed by techniques, skills and studied plant species as well as by the need of data corroboration at the international level.

**Key words:** drought stress, breeding limitations, *Festuca arundinacea*, *Festuca pratensis*, *Phleum pratense*, *Dactylis glomerata*, *Lolium perenne* and *Trifolium repens*.

### INTRODUCTION

Breeding crop species is a long-time process with a history that starts with human civilization [22]. In the end of the 19<sup>th</sup> century [31] maize starts as a subject of breeding in the so-called corn belt [6]. It can be considered that maize is among the first species as a subject for scientific breeding. However, maize is known in Romania mostly after the 18<sup>th</sup> century. Before, wheat was the major crop in Romania [28]. Thus, it was proved that wheat has a long history of over 5000 years of cultivation in the actual borders of Romania and in the inner arch of Carpathians that is relevant for the current strategies development for the country [16]. Different studies emphasized the relevance of fodder plants for animal bred as well as for nature conservation in Sibiu county including climate change negative impact of drought or precipitations [26, 27]. However, the breeding of species originating from pastures become more relevant today when considering the effects of droughts as well as the increase of soil salinity on long term [12]. Romania is passing a warming period with dramatic effects on productivity especially in agriculture due to drought conditions for long term [3]. Animal

bred will be the most affected among all agricultural sectors. Pastures and grasslands are among major food sources in Romanian agriculture and assessing the genetic diversity as well as variability of fodder species will be supportive.

The scope of this paper is to discuss some barriers encountered during the implementation of a long process of selecting and testing different fodder species that are relevant and common for all pastures in South-East Transylvania.

In 2007 it was run for a period of five years the project entitled "The identification based on biotechnological methods of new genotypes of fodder species that are resistant to drought and soil acidity". The philosophy of the process was to test based on an integrated approach from laboratory to the field, six fodder species (e.g.: *Festuca arundinacea*, *Festuca pratensis*, *Phleum pratense*, *Dactylis glomerata*, *Lolium perenne* and *Trifolium repens*), in order to access their genetic variability, to prove its existence, to isolate relevant clones and to test their resistance against drought and acid conditions.

## MATERIALS AND METHODS

**Plant material.** Certified seeds of six fodder species and varieties have been used for all experiments during 2007-2012, such as follows: *Festuca pratensis* Huds. 'Pradel', *Festuca arundinacea* Schreb. 'Kora', *Lolium perenne* L. 'Mara', *Dactylis glomerata* L. 'Intensiv', *Phleum pratense* L. 'Barpenta' and *Trifolium repens* L. 'Barblanca'. The following bred lines have been used for *Dactylis glomerata* L. (i.e. Intensiv 14R01, 83R01, 84R01 și 10R01), *Festuca pratensis* Huds. (Pradel, 19R00, 23R00, 99L2 și 77Ro7), *Phleum pratense* L. (i.e. Barpenta 34R00, 10010, 10385, 14R00 and 1Bv00), *Lolium perenne* L. (i.e. diploid: LPD and tetraploids: Mara 2002, 20020, 20062 and 2003) and *Lolium perenne* L. (i.e. tetraploids: 31A99, 40026, 40019, 30A99 and 40021).

**Methodology** Barriers will be discussed in a SWOT analysis based on published results for the six fodder plants that have been selected for *in vitro* micro propagation, testing against acidification and hydric stress and for selecting clones that are most resistant against acid or drought conditions [20, 21, 23, 24].

Some of the limits for field tests that followed the laboratory and acclimation tests will be discussed further. All field tests have been performed in pastures located in the limits of Braşov and Sibiu counties belonging to the following villages: Ucea (45°47'12"N 24°40'32"E), Dealul Luncii, Viştea (45°47'54"N 24°43'21"E), Ucea –Carmolimp, Bruuiu, Ucea- Buciumi, Ucea-Vigerox.

## RESULTS AND DISCUSSIONS

*First stage of research: Laboratory and acclimation phases.* During the laboratory phase 6 fodder plant species have been accessed all of them being in the gene bank of our institution. Based on former experience these species have been tested for their *in vitro* cultivation, setting appropriate *in vitro* micro propagation technology for each of the species [4, 13, 20, 21]. Acclimation was realized in greenhouse conditions by limiting the impact of viruses on obtained plants. The process was

realized in distinct stages: the cultivation of *in vitro* plantlets on perlite that for 10 days were acclimated from 90% humidity up to 60% as it was possible to be supplied in the greenhouse conditions. The pot second phase followed when each specimen was transferred in a single pot supplied with soil mixture rich in nutrients. In this case the humidity was set up to 30% for the greenhouse conditions to be prepared for field testing in the elite field. In this case, a major attention was oriented for handling each specimen, the presence and or persistence of pathogenic fungus or bacteria. Of major importance is the intensity of the light, especially during the first two weeks that must be between 10,000 and 6,000 lx minimum. During the pot phase the natural light will be enough but during the cloudy days, if they are longer than 10 days and ensure only 500 lx, it is relevant for two hours a day to supplement the needs for light up to 10,000 lx. The light intensity is essential for supporting the adaptation strategy of the plants towards new conditions as it was previously discussed [11, 17]. Watering will be done at the temperature of the greenhouse (25°C ±5°C) to avoid thermic shocks also in accordance with other author results [9].

The major limitations identified belong to the species, to the technique of *in vitro* culture and to the laboratory skills. Not all the species may develop fast or well into *in vitro* conditions and therefore it is a major gap to be covered for the future in this respect when distinct species are to be compared for the same culture medium cultivation. High skills for biotechnology laboratory are needed as appropriate handling of *in vitro* cultures impacts on infections. Moreover, *in vitro* plantlets' needs for passing on new culture medium request fine observations that need to support *in vitro* conditions. Testing the hormone balance is based on previous researches conducted in the laboratory.

*First stage of research: Elite field testing.*

Specimens belonging to each of the species have been planted in the elite field testing starting with 2008. In this case the common agricultural practices have been applied starting with land working, fertilizers and soil

amendments. All collected data have been used to support the control of the whole future experimentations. In parallel have been analysed the results of other research teams such as that of Peter-Schmid and collaborators [19] or from China run by Zhang and collaborators [10]. The selected cultivars proved to be valuable also for other laboratories all over the world.

Major limitations are due to climatic conditions (i.e. climatic conditions temperature, humidity, wind spread, precipitations, biotic factors). It is almost impossible to repeat the experiment under the same conditions from one year to another and therefore, in case of drought to consider only those clones or specimens that are developed under a clear measurable stress factor. Based on this experience it can be considered that marginal specimens in the tested field may not be considered if the shadow provided by rye grass from the buffering zone is impacting the rise of air humidity more compared to the rest of the tested plot areas.

*Second stage of research: Laboratory and acclimation phases.*

The second part of research is mainly based on the previous results. It is meant to test the genetic variability against drought and acidity. *In vitro* drought stress is not possible to be induced due to the height humidity in the air of each jar. However, adding a chemical agent to induce water stress such as PEG (polyethylene glycol) in different forms (i.e. PEG 6000) it is possible to stress from osmotic point of view the *in vitro* plantlets [2, 4, 15]. All six cultivars have been tested for hydric stress [2]. As a conclusion we registered that *in vitro* systems are valuable tools in our attempts to select resistant genotypes to drought conditions. The action of PEG6000 over *in vitro* plantlets is acting also as memory at the specimen level for improving the performance of selected plants towards drought conditions expressed under acclimation. These results are grounding the previously results obtained for selecting the best clones responding to hydric stress induced by the PEG 6000. The target for a 6 months period was 600 plantlets of each of the tested varieties and breed lines. Based on today

conclusion we consider that it is a feasible target. *In vitro* culture media are variants of Murashige-Skook 1962 culture medium [14] and technical parameters are comparable to those published by Gamborg and Philips [7]. The best results have been obtained for *F. pratensis* with a success of 84,60% viability of tested inocula. The rest of the genotypes and species recorded values up to 50%. In the end of the entire project it can be considered that even the chosen process is for *long term*, the results are reliable and therefore it can be recommended for future testing. In case of *D. glomerata* L. the success is obtained up to 91.50%. The second stage of *in vitro* testing was only for pH variations and decreasing the pH down to 4.5 or increasing up to 8. In case of *D. glomerata* the survival rate decreased down to 44% for a pH of 4.5 and 32% for a pH of 8. The best hormone balance was auxin / cytokinin at 1mg/l and among all six species only *D. glomerata* proved the best adaptation capacity towards hydric stress and induced acidity (fig. 1).

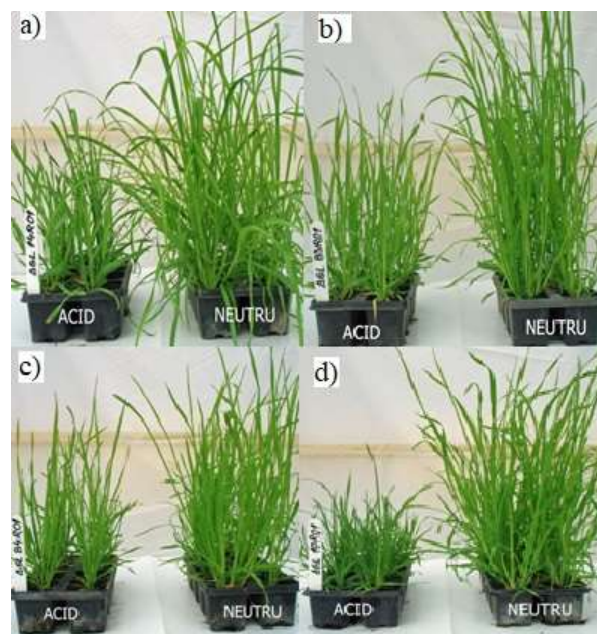


Fig. 1. *Dactylis glomerata* testing against acidity at 30 days after acclimation (a. DGL 14R01, b. DGL 83R01, c. DGL 84R01 and d. DGL 10R01)

Source: Original.

A major new limitation of the second phase may be due to the culture medium composition itself. It is possible that working more for the culture medium composition, in a unique way

for each of the species to reach different results for the future. However, it can be considered that our results need to be related to the same culture medium formula and explicitly mentioned for the results. Considering all results related to acclimation it is obvious that the best clones are those passing easily acclimation which impose to all plants drought stress conditions as well as random disposition for all variants (fig. 2). In this regard, using the PEG stressed clones it is possible to improve the selection method for producing the most appropriate genetic varieties from this point of view. However, this is not connected yet to any of quantitative genetic traits followed by agronomist in breeding the species.



Fig. 2 Random disposition of experimental tested variants for *Lolium perenne*

Source: Original.

A new major limitation may be due to the molecular mechanisms that undergoes the adaptation of each of the specimens during acclimation. However, considering the bulk of specimens belonging to one line, compared to the average value may be important only for the subjected species.

#### *Second stage of research: Elite field testing.*

Selected clones, during *in vitro* experimentation and acclimation testing have been transferred into the field testing. The target was to obtain 80 individuals per variant. Also, it was an achievable target. For *D. glomerata* have been use 15 plants of four biotypes, for *P. pratense* have been obtained 20 plants of 5 genotypes, for *F. pratensis* have been obtained 15 plants of 5 genotypes and for *L. perenne* 15 plants of 5 diploid genotypes and 5 tetraploid genotypes. Have been made observations regarding the fading index. For laboratory analysis dry content was relevant especially when considering animal

bred. Also, protein and cellulose contents were important in this regard. Major limitations are due especially to climatic conditions, the harvesting momentum and the skills of the involved team. Field testing was also complex and therefore only considering the dry mass as well as the fresh weight mass production proved to be the subject of a height variability interspecific as well as intraspecific.



Fig. 3. Isolated poly-cross of *Phleum pratense* in the field testing.

Source: Original.

In all cases acidification induced the decreased in fresh weight for all cultivars (i.e. a minimum for *P. pratense* of 3,59% and a maximum of 14,03% for *L. perenne*. In case of dry matter, it was recorded a minimum of 4,72% for *P. pratense* and a maximum of 16,38% for *L. perenne*. These results showed the height genetic variability of the species as well as limitations in others such as *Phelum sp.* Those limitations are responsible for the ratio between species in pastures and may further support the idea to access wild genotypes originating from stressing conditions related to drought and acidification [8]. Related to *D. glomerata* only two breeding lines have been considered as valuable for further experiments (i.e. DGL 83 R01 și DGL 84R01). Also, *L. perenne* diploid lines are less tolerant to acidity compared to *F. pratensis* and tetraploid lines of the same species *L. perenne* in line with current results [18, 25]. In our experiments it was the case for the following lines: *F. pratensis* FPR 77Ro7 and *L. perenne* tetraploid LPT 40019 and LPT 30A99. Among *P. pratense* lines remarkable results have been obtained in order for PHL 10385, PHL 14 R00 and PHL 1Bv00 supported also by other researchers' results [29].

The project ended in 2012 and these results appear to be reliable according to other results published in other laboratories, supporting the viability of the whole research process.

Among these fodder species *D. glomerata* line DGL 14R01 proved to maintain also highest productivity on an acid environmental conditions.

We also consider that these values can be supported by the previous *in vitro* treatments of all these lines as today is almost all the time considered the drought memory of the plants [30]. Thus, accessing the same signalling pathways creates the minimum required pools of specific compounds that may support the expression of adaptation to stress conditions [1].

Harvesting started in the elite testing field after removing path vegetation as well as the protection area meant to prevent cross-pollination represented by a field of *Secale cereale*. Each of the repetition (20 repetitions per variants) have been separately harvested 1 kg/variant for evaluating the fresh weight, the dry matter and subsequently the total protein and carbohydrates.

All free samples after first weight have been sent to the State Laboratory for Sanitary Veterinary of the County Brasov to perform the rest of analysis to remove any suspicious related to analysis conditions.

We need to underline that by the time of harvesting only *L. perenne* was entered in seed production phase as all other species were in early phase of seed production.

However, the protein content range between 12,30 % and 18,19 % for drought conditions because the diversity of the species was mainly represented by *L. perenne*, *F. pratensis* and *F. arundinacea*. The small ratio was added by *T. repens*, *D. glomerata* and *P. pretense*.

However, after the second mow the ratio of *T. repens* increased and showed dominance towards the others and explaining the increased level of total proteins.

## CONCLUSIONS

As major lessons of implementing such a long-time breeding process, with positive results, we can mention the following:

(a) we need to know, understand and acknowledge all laboratory and field limitations imposed by techniques, our own developed skills as well as by the species it selves;

(b) it is always relevant to refer our results to other results published in peer-review articles and in peer-review journals by different research teams working on the same topics to make sure the consistency of our results with the goal of breeding and;

(c) it is a great need to follow long-term breeding processes as they may contribute to the diversity of the species we need for ensuring food security for the future.

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