Use of different sucrose concentrations to model drought in alfalfa cell culture

[1,2*]Asadova Sadaqat Shirmammad, [1] Mustafayeva Gunay Hajiomar

[1]Plant Biotechnology Laboratory, Institute of Molecular Biology & Biotechnologies, Ministry of Science and Education of the Republic of Azerbaijan, 11 Izzat Nabiyev Str., Baku AZ 1073, Azerbaijan
[2] Department of Plant Physiology, Research Institute of Crop Husbandry, Ministry of Agriculture of the Republic of Azerbaijan, Pirshagi, Sovkhoz 2, Baku AZ 1098, Azerbaijan

***For correspondence:** biotexnoloqaz@mail.ru; sadagat.asadova@gmail.com*

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In order to carry out cell selection *in vitro* for drought resistance, a preliminary ranking of seeds of seven varieties of alfalfa was carried out according to the ability to sprout under different conditions of osmotic pressure. The regulation of osmotic pressure was carried out by growing seeds in sucrose solutions of various concentrations. As a result of the ranking, 4 varieties were selected: Goyazyan, Yaz chichiai, Aran, Leader. Various parts of aseptic seedlings were used as explants for introduction into in vitro culture. After obtaining the required mass, cell cultures were cultured on nutrient media containing 30 g/l, 45 g/l and 60 g/l of sucrose. Morphogenic calli were obtained for all varieties. The indicators of morphogenesis of the cell culture of the Goyazan variety, where more seeds germinated under conditions of 14 atmospheres (45 g/l of sucrose) were higher in comparison with the control variant. It is assumed that the optimization of not only the concentration of sucrose, but also the concentrations of other components of the nutrient medium, which create additional osmotic pressure, can stimulate the realization of the regenerative potential of plants in which morphogenesis is not observed under the conditions proposed by the guidelines.

***Keywords*:** *Alfalfa, in vitro culture, sucrose, modeling, drought stress*

INTRODUCTION

Alfalfa, like all leguminous crops, lives in symbiosis with nodule bacteria, thanks to which it can absorb atmospheric nitrogen and accumulate it in its biomass. It is a high-protein and vitamin-rich feed. In the green mass and hay of alfalfa there is 2-2.5 times more protein than in cereal grasses and corn. (Kotovrasov et al., 1990; Afonin, 2004). Alfalfa is the most important crop of the grass-field farming system, as a component of grass mixtures on rainfed crops, irrigated pastures in the Transcaucasus, including Azerbaijan. It is a unique predecessor for grain crops, since it enriches the soil with nitrogen biologically available to plants. In addition, alfalfa, like other leguminous crops, is used to create pastures, restore soils removed from crop rotation due to salinization and desertification (Afonin, 2004). This is a very serious problem for Azerbaijan, because currently 36 administrative regions of the country are located in zones with a high risk of desertification (az. sputniknews.ru, 2019). To restore such lands, it is necessary to use drought-resistant plants, since according to the IPCC, climate change that has engulfed the planet will lead to prolonged droughts (IPCC., 2015; Summary for Policymakers, 2019), and during drought, significant changes in the regulation of its growth and development occur in the plant body, which ultimately ends with the death of the plant (Zair et al., 2003; Takahashi et al., 2020).

The purpose of this work was to evaluate various alfalfa species for drought tolerance and in vitro validity; pick up and include these plants in cell selection.

MATERIALS AND METHODS

Seeds of 7 promising varieties of alfalfa of various origins were used as the material of the study: Aran, Absheron, Agstafa 1, Agstafa 2, Goyazan, Yaz chichiai, Leader. According to the guidelines (Udovenko, 1988), after sterilization the seeds of each of these varieties were planted in sterile Petri dishes with aqueous solutions of sucrose, the content of which varied from 7% to 20%. After the ranking, 4 varieties were selected for introduction into *in vitro* culture: Aran, Goyazan, Leader and Yaz chichiai.

After the sterilization process the seeds of these varieties were grown on the artificial nutrient medium of Hamburg (B_5) in order to obtain aseptic

seedlings. Callus cultures were obtained from aseptic seedlings, which were divided into 3 parts and planted on B_5 nutrient medium with the addition of 30 g/l of sucrose (control, variant 1), 45 g/l of sucrose (variant 2) and 60 g/l of sucrose (variant 3).

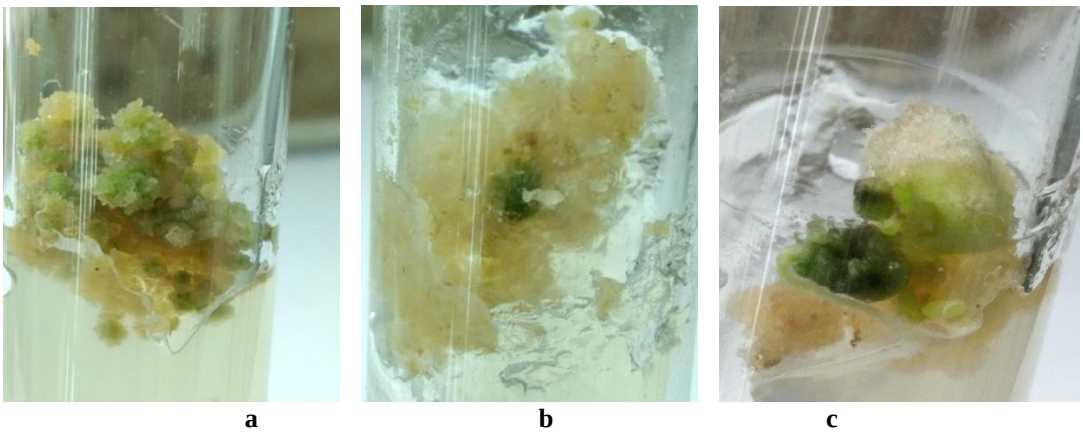
After 10 days of cultivation, the calli were transferred to a medium for induction of morphogenesis containing 0.2 mg/l of 6-benzylaminopurine (BAP). The resulting embryoids for germination were transferred to B_5 medium without phytohormones.

RESULTS AND DISCUSSION

It is known that the drought resistance of a plant depends on its genotype, the ecological conditions of cultivation, the type of drought, its duration, etc. But there are methods for assessing mass drought resistance based on the germination of plant seeds in osmotic solutions, which, while holding water, imitate its deficiency, creating a high osmotic pressure (Udovenko, 1988). Sucrose is a commonly used and available osmolyte, which began to be used as early as the beginning of the 20th century, and certain concentrations of sucrose solutions were determined for a number of crops, reflecting their level of drought tolerance (Drozdov, Udovenko, 1988). So, for leguminous crops: peas, beans, vetch, lupins, soybeans (Workshop on plant growth and resistance, 2001; Petrenko, 2017; Dzyubenko, 2017), - the concentration of sucrose, reflecting their drought resistance, created an atmospheric pressure of 7-9 atmospheres (8.7-10.8%). Since there are no such recommendations for alfalfa, we decided to test the ability of seeds of these varieties to germinate in solutions of 7%-20% sucrose, which corresponds to an osmotic pressure of 6-14 atmospheres. It should be noted that at low concentrations of sucrose, which create an osmotic pressure of 6–9 atmospheres in the germination medium, there were no significant differences in the number of germinated seeds between varieties. Significant differences between varieties in the number of germinating seeds were noted at an osmotic pressure reaching 14 atmospheres, and at a pressure of 20 atm, normally germinated seeds were not found in any variety. Therefore, the ranking was carried out using a sucrose solution with a concentration of 15%, at which an osmotic pressure of 14 atmospheres was created in the germination medium.

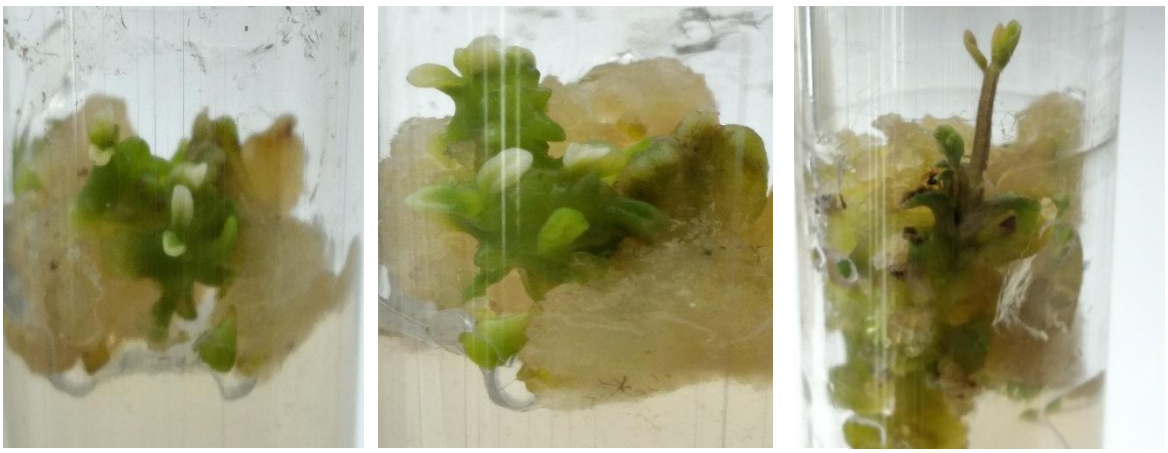
In this case, the varieties were arranged in the following order: Goyazyan - 16.7%; Yaz chichiai - 8.3%; Absheron 6.7%; Agstafa-2 - 6.7%; Agstafa-1 - 6%, Aran - 2% and Leader - 0%. The germination of seeds in such solutions demonstrates that the ability to germinate in less water is a genetically fixed trait, and also indicates a high value of the sucking power of the cells, due to which the required amount of water is absorbed faster. Good sucking power is the guarantor of the formation of a stable root system of the seedling. And the properties of the seedling significantly affect the formation of drought resistance in an adult plant (Dolgikh et al., 1994; Zair, 2003; Edmeades, 2008; Yegorova, 2013; Takahashi, 2020). Therefore, after ranking, 4 varieties were selected for involvement in cell selection for drought resistance: Goyazyan, Yaz chichiai, Aran and Leader. The cultivars differed in their ability to regenerate under *in vitro* conditions. The regeneration required for cell selection was observed in the Leader and Aran cultivars. The morphogenetic abilities of the varieties Goyazyan and Yaz chichiai were lower, but the seeds of these varieties sprouted under conditions of an osmotic pressure of 14 atmospheres.

Aseptic seedlings were obtained from the seeds of the selected varieties, and calli were obtained from the seedlings. After increasing the required mass, they were divided into 3 parts and transplanted into 3 variants of the B5 medium with a sucrose content of 30 g/l (1 variant, control), 45 g/l (2 variant) and 60 g/l (variant 3).



a **b** **c**

Fig. 1. Formation (a) and germination of embryoids (b, c) in morphogenic calli.



a **b** **c**

Fig. 2. Formation of regenerative plants : a -Yaz chichiai; b- Leader; c- Goyazan,

After 10 days of cultivation, the calli were transferred to a medium for induction of morphogenesis containing 0.2 mg/l of BAP. After 5-6 days, the appearance of meristematic foci was observed (Fig. 1). Morphogenesis was noted for all varieties. When embryoids began to form in calluses, the phenomenon of embryogenesis and morphogenesis was observed (Fig. 2).

Especially it is necessary to note varieties the Goyazan (variants 2 and 3) and Leader (variants 1 and 2). Considering that the seeds of the Goyazan variety turned out to be the most resistant to germination under conditions of increased osmotic pressure, it can be assumed that the weak regeneration potential observed in this variety under conditions corresponding to the guidelines can be stimulated by increasing the osmotic pressure in the regeneration medium. There is information that by creating the required osmotic pressure, the authors obtained the desired result (Polevoy, 2001). As for the Leader variety, it was characterized by a fairly good realization of the regeneration potential in the control and when assessing varieties for competence in *in vitro* conditions. The presence of morphogenesis in the second and even third (in one test tube) experimental variants may indicate the possible appearance of somaclonal forms. Therefore, after obtaining regenerated plants, it is necessary to assess their ability to form normal fertile seeds. It can be assumed that the optimization of not only the concentration of sucrose, which plays the role of an energy source and affects the change in osmotic pressure (Asadova, 2001; Yarmanov, 2017) but also the concentrations of other components of the nutrient medium that create additional osmotic pressure, can stimulate the realization of the regenerative potential of plants in which morphogenesis is not observed under the conditions proposed guidelines.

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Yonca hüceyrə kulturasında quraqlığın modelləşdirilməsi üçün saxarozanın müxtəlif qatılıqlarının istifadəsi

[1,2] Əsədova Sədaqət Şirməmməd qızı, [1] Mustafayeva Günay Hacıömər qızı

[1] Azərbaycan Respublikası Elm və Təhsil Nazirliyi, Molekulyar Biologiya və Biotexnologiyalar İnstitutunun Bitki biotexnologiyası laboratoriyası, Bakı, Azərbaycan
[2]Azərbaycan Respublikası Kənd Təsərrüfatı Nazirliyi Əkinçilik Elmi-Tədqiqat İnstitutunun Bitki fiziologiyası şöbəsi, Bakı, Azərbaycan

In vitro hüceyrə kuturasında quraqlığa davamlılıq seleksiyasının aparılması məqsədilə 7 yonca sortunun toxumlarının müxtəlif osmotik təzyiq şəraitində cücərmə qabiliyyəti qiymətləndirilmişdir və bunun əsasında sortların sıralaması aparılmışdır. Osmotik təzyiq toxumların cücərməsi üçün istifadə edilən süni qida mühitlərinə əlavə olunmuş saxarozanın müxtəlif qatılıqları vasitəsilə tənzimlənmişrdi. Siralanma nəticəsində hüceyrə seleksiyasina 4 sort cəlb olunmuşdur: Göyəzən, Yaz çiçəyi, Aran, Lider. Eksplant kimi in vitro kulturaya sortların aseptik cücərtilərinin müxtəlif hissələri daxil edilmişdir. Tələb olunan kütlə əldə edildikdən sonra hüceyrə kulturaları tərkibində 30 q/l, 45 q/l və 60 q/l saxaroza olan qidalı mühitlərdə becərilmişdir. Bütün sortlar üçün morfogen kalluslar alınmışdır. Göyəzən sortunun kalluslarında 14 atmosfer (45 q/l saxaroza) şəraitində morfogenez nəzarət variantına nisbətən daha intensiv gedirdi. Güman edilir ki, təkcə saxarozanın konsentrasiyası deyil, həm də qida mühitində əlavə osmotik təzyiq yaradan digər komponentlərin konsentrasiyasının optimallaşdırılması adı şəraitdə (metodikaya uyğun) in vitro morfogenez müşahidə olunmayan bitkilədə regenerasiya potensialının reallaşdırılmasına təkan verə bilər.

Açar sözlər: *Yonca, in vitro, saxaroza, modelləşmə, quraqlıq stresi*