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Application of different techniques for garlic tissue culture initiation

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Abstract

Garlic (*Allium sativum* L.) is a vegetatively propagated crop of high nutritional, medicinal, and economic importance, nonetheless its conventional conservation in the field is limited by pathogen accumulation and genetic instability. This study aimed to establish the first *in vitro* garlic collection in Bosnia and Herzegovina and to evaluate two methods of culture initiation: the stem disc method and the clove method. Seven accessions collected from different regions of the Republic of Srpska were introduced into *in vitro* culture under controlled conditions. Both methods enabled culture initiation, but significant differences were observed in contamination rates and regeneration success. The stem disc method showed superior results, with a lower contamination rate (18%) and higher regeneration efficiency compared to the clove method, where over 50% of explants were contaminated. All non-contaminated explants from both methods demonstrated the capacity to regenerate shoots and roots, indicating their potential for long-term conservation. The findings suggest that the stem disc method, in combination with refined sterilization protocols and careful selection of plant material, represents a more reliable approach for establishing *in vitro* garlic collections. This study provides the first insights into *in vitro* conservation of garlic in Bosnia and Herzegovina and contributes to global efforts in safeguarding plant genetic resources and supporting sustainable agriculture.

***Key words**: Allium sativum* L., Conservation, Gene bank, Germplasm.

Introduction

Garlic (*Allium sativum* L.) is a highly valuable plant with a wide range of applications in gastronomy, traditional and modern medicine, and sustainable agriculture. Since garlic is a vegetatively propagated crop with very limited seed production, traditional methods of conservation, such as maintaining collections in the field, are often insufficient to ensure genetic stability and diversity. Prolonged vegetative propagation under field conditions frequently results in the accumulation of viral, fungal, and bacterial pathogens, as well as increased risk of genetic drift. In contrast, the application of *in vitro* techniques enables the maintenance of healthy, disease-free plant material under controlled laboratory conditions, ensuring both the preservation of unique genotypes and their availability for future breeding, biotechnology applications, and research programs (Tkalec Kojić et al., 2023; Tirado et al., 2023; Rajech et al., 2024).

In vitro conservation of garlic represents an effective and sustainable approach for the long-term preservation of its valuable genetic resources. Moreover, *in vitro* conservation provides significant advantages over conventional field maintenance, such as reduced risk of pathogen infection, lower space requirements, and the possibility of long-term storage under minimal growth conditions (Benke et al., 2025). Various methods have been used for the initiation of garlic tissue culture, each with its own advantages and limitations (Tirado et al., 2023; Benke et al., 2025). Different explant types have been successfully tested, including meristem tissues (Greedharry et al., 2024), stem-disc culture (Ayabe and Sumi, 1998; Keshari et al., 2018), clove explants (Nandariyah et al., 2021; Karn et al., 2022), and somatic embryogenesis (Kereša et al., 2021). These techniques differ not only in their regeneration efficiency but also in their ability to eliminate pathogens and provide stable, vigorous plantlets.

The aim of this research is to determine which method, stem-disc culture or clove explants, provides better results under *in vitro* conditions. In addition, the study seeks to compare their efficiency in terms of plant regeneration, growth performance, and the production of healthy, pathogen free material. By evaluating the outcomes of different approaches, this research will contribute to identifying the most reliable and effective technique for establishing *in vitro* collections of garlic. The findings will also provide valuable insights for optimizing protocols that can be applied in gene banks, breeding programs, and commercial production systems. Ultimately, the results will strengthen efforts to conserve garlic genetic resources while ensuring their sustainable utilization in agriculture and food systems.

Material and Methods

The experiment was carried out during spring 2025, focusing on the development of *in vitro* collection of garlic accessions at the Institute of Genetic Resources, University of Banja Luka. Seven garlic accessions were collected from different regions of the Republic of Srpska (Nevesinje, Gacko, Laktaši, Bileća, Banja Luka, Mrkonjić Grad) and registered in the Gene Bank database. The first step involved the preparation of plant material for the *in vitro* culture initiation. Cloves from each accession were surface-sterilized by rinsing under running water for 20 minutes with the addition of liquid soap and Tween-20, followed by immersion in 70% ethanol for 1 minute. Subsequently, the cloves were treated with a 10% sodium hypochlorite (NaClO) solution for 20 minutes and then rinsed three times in sterile water for 10 minutes. The surface-sterilized material was transferred with sterile forceps onto a sterile surface for further processing. Two methods of *in vitro* initiation were applied: the stem disc method and the garlic clove method. For the stem disc method, five cloves from each accession were used, and each clove was dissected into five discs, which were subsequently inoculated into test tubes containing MS medium (Murashige & Skoog, 1962) supplemented with 3% sucrose, without hormones. In the garlic clove method, five cloves of each accession were inoculated into test tubes containing the same MS medium supplemented with 3% sucrose without hormones. The experiment was conducted under controlled conditions at 22 °C, with a light intensity of 13,000 lux and a photoperiod of 16 hours light / 8 hours darkness. The entire procedure was carried out under strictly sterile conditions and lasted eight weeks.

Results and Discussion

Seven garlic accessions collected in the Republic of Srpska were used to establish an *in vitro* garlic collection at the Institute of Genetic Resources, University of Banja Luka. Two methods were applied for this purpose: the stem disc method and the whole clove method. Culture initiation was successfully achieved in all explants, regardless of the method employed. However, several explants exhibited signs of contamination a few days after inoculation, which directly influenced the overall success rate of culture establishment. In the case of the stem disc method, the contamination rate was relatively low, with only 18% of explants affected, while 82% remained uncontaminated and initiated cultures without visible signs of pathogens or pests (Fig. 1).

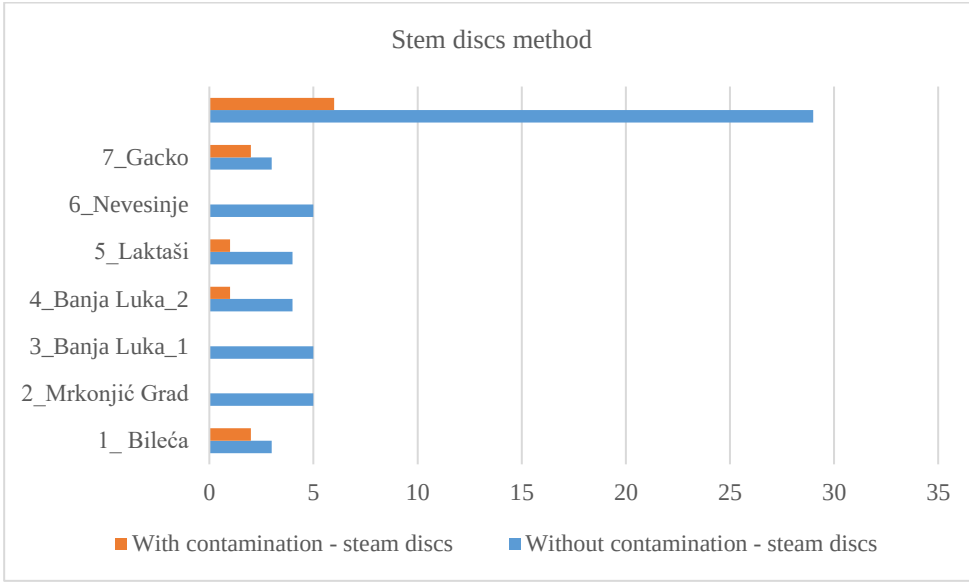

Fig. 1 - Presence of contamination on garlic explants from seven accessions by using the stem discs method

These results indicate that the stem disc method provides relatively clean starting material for *in vitro* culture, thereby ensuring a higher probability of survival and subsequent regeneration. It is significant that, by using this method, each clove was divided into five discs, and in every accession where contamination was observed, all five discs became contaminated.

When compared with the stem disc method, the clove method demonstrated a considerably higher contamination rate. Specifically, 51.4% of all inoculated cloves were contaminated, while only 48.6% remained without contamination (Fig. 2). These results highlight the challenges of using whole cloves as explants, since the outer tissue layers are more prone to harbouring microorganisms.

Despite this limitation, the stem disc method overall proved to be more efficient for culture initiation and maintenance under aseptic conditions. The highest regeneration rates without contamination were recorded in accessions collected from Mrkonjić Grad, Banja Luka_1, and Nevesinje. On the other hand, the highest contamination levels were found in accessions originating from Bileća and Gacko (Herzegovina region), suggesting possible differences in field conditions or microbial load associated with the planting material. In contrast, when the clove method was applied, contamination was observed across all accessions. The most severe case was in the accession from Bileća, where all explants were contaminated and no healthy cultures were obtained. The

accession from Laktaši showed a contamination rate of 66.6%, while accessions from Mrkonjić Grad, Banja Luka_1, Banja Luka_2, Nevesinje, and Gacko had contamination levels of approximately 40%. Interestingly, despite the relatively high contamination rates, all non-contaminated explants from both methods were able to successfully initiate the development of both shoots and root systems, confirming their regeneration potential under sterile conditions.

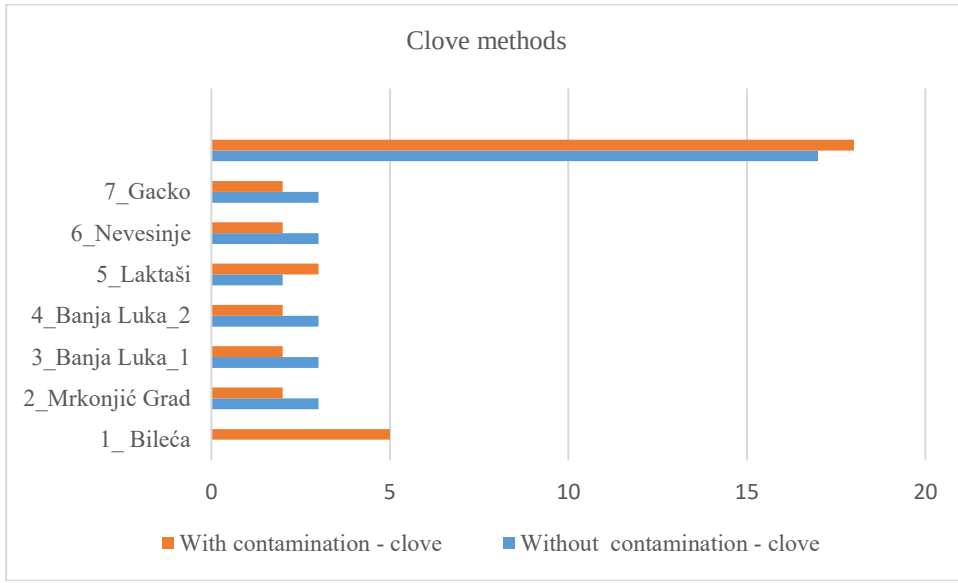

Fig. 2 - Presence of contamination on garlic explants from seven accessions by using the clove method

This demonstrates that, once contamination is controlled or eliminated, both methods are capable of producing viable *in vitro* plant material suitable for long-term conservation and further use in garlic breeding or propagation programs, which has previously been confirmed in different studies on *in vitro* initiation culture (Haque et al., 2002; Haque et al., 2023; Rajesh et al., 2024). The results obtained suggest that the stem disc method is more effective for establishing a clean *in vitro* garlic collection, although the presence of the basal plate is essential for successful regeneration. The clove method, while simpler in practice, poses higher risks of contamination and therefore requires additional sterilization steps or modifications to be considered a reliable alternative. The introduced plant material will be used in future research with the aim of defining the best protocols for obtaining healthy material, which will be directed toward both short-term and long-term conservation.

Conclusion

The results obtained represent the first findings in Bosnia and Herzegovina on the establishment of an *in vitro* garlic collection. Experiments on the introduction of garlic accessions into *in vitro* culture demonstrated that both methods, the stem disc and clove methods, can be successfully applied for establishing such a collection. However, the stem disc method yielded better results and is therefore recommended for further use in expanding the garlic collection at the Institute for Genetic Resources. A crucial factor in this process is surface sterilization, which plays a decisive role in the success of culture establishment. The results highlight the need to refine sterilization protocols and carefully select plant material, as accessions from the Bileća locality have not produced satisfactory outcomes. This study underlines the importance of choosing appropriate explants and applying a meticulous approach to regeneration in order to secure a stable, long-term garlic collection. Such efforts are vital for the conservation of plant genetic resources and their sustainable use in agriculture. Ultimately, *in vitro* conservation contributes to global initiatives focused on safeguarding plant genetic resources and ensuring food security.

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Примјена различитих техника за иницирање културе ткива бијелог лука

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Сажетак

Бијели лук (*Allium sativum* L.), као култура која се вегетативно размножава, је од великог нутритивног, љековитог и економског значаја, али његово конвенционално очување на пољу ограничено је акумулацијом патогена и генетичком нестабилношћу. Циљ овог истраживања био је успостављање прве *in vitro* колекције бијелог лука у Босни и Херцеговини и процјена двије методе иницирања културе: методе диска стабљике и методе чена. Седам принова сакупљених из различитих региона Републике Српске уведено је у *in vitro* културу под контролисаним условима. Обје методе омогућиле су иницирање културе, али су уочене значајне разлике у стопама контаминације и успјешности регенерације. Метода диска стабљике показала је супериорне резултате, са нижом стопом контаминације (18%) и већом ефикасношћу регенерације у поређењу са методом чена, код које је више од 50% експлантата било контаминирано. Сви неконтаминирани експлантати из обје методе показали су способност регенерације изданака и корјена, што указује на њихов потенцијал за дугорочно очување. Резултати сугеришу да метода диска стабљике, у комбинацији с унапријеђеним протоколима стерилизације и пажљивим одабиром биљног материјала, представља поузданији приступ за успостављање *in vitro* колекција бијелог лука. Ово истраживање пружа први увид у *in vitro* конзервацију бијелог лука у Босни и Херцеговини и доприноси глобалним напорима у очувању биљних генетичких ресурса и подршци одрживој пољопривреди.

***Кључне ријечи**: Allium sativum* L., конзервација, банка гена, гермплазма.

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