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Conservation, genetic characterization, phytochemical and biological investigation of black calla lily: A wild endangered medicinal plant

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#### ABSTRACT

Scientists continue to search for and conserve plants whose medicinal properties have become crucial in the fight against diseases. Moreover, lessons from folk medicine, indigenous knowledge and Chinese medicine on crude extracts points to possible findings of novel promising and strong pharmaceutically bioactive constituents. *Arum palaestinum*, commonly known as black calla lily, is one of the most important medicinal plants belonging to the family Araceae, which has not been well studied. Little is known about its pharmaceutically bioactive constituents and the effective conservation through the use of biotechnology. Thus, *Arum Palaestinum* is selected and reviewed for its phytochemical analysis and biological activities. Besides, the tissue culture and genetic characterization developed for effective conservation of the plant were also summarized.

1. Introduction

As a result of the unfavorable climatic conditions, salinity, drought, desertification and urbanization, medicinal plants are subjected to endangerment. For the majority of the world's population, medicinal plants are the main source of drugs that cure many diseases and save many lives. In this context, biotechnological techniques are very vital in selection, conservation and multiplication of the important genotypes of medicinal plants<sup>[1]</sup>. *In vitro*, regeneration plays an important role in the production of high-quality plant-based medicine.

Araceae, also called Arum family, belongs to monocotyledonous flowering plants, in which flowers are borne on a type of inflorescence called spadix. The spadix is usually accompanied by leaf-like bract. The members of Arum family are often known

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as aroids. This family consists of approximately 3 700 species of 107 genera distributed in the north temperate regions and the old and new world tropics<sup>[2]</sup>. Araceae species are often tuberous or rhizomatous and are often found to contain calcium oxalate crystals. In this family, many plants are so thermogenic that their flowers can reach up to 45 °C. This unusually high temperature attracts insects to pollinate the plant and prevents tissue damage in cold regions<sup>[3]</sup>. The *Arum* genus has 31 species of plants that have been already identified in nature.

Black calla lily, the English name of *Arum palaestinum* Boiss. (*A. palaestinum*) is one of the most important plants in the family Araceae, which is native to Northern Africa, Europe and Western Asia with wide distribution in the Mediterranean region[4]. *A. palaestinum* is a typical cryptic species producing a smell of rotten fruit, since its appendix emits mainly ethyl acetate. In many countries, its aerial parts are edible after drying or soaking in salty water. *A. palaestinum* acts as a kind of ornamental plants and animal fodders and is used traditionally to cure several chronic diseases such as stomach acidity, atherosclerosis, diabetes and cancer[5]. It grows naturally in mountains and hills near water in rocky places, in the upper Jordan Valley and in many spots such as Irbid, Wadi Shua'ib, Jarash, Ajlun, Amman and Al Balqa[5.6]. The plant is endangered due to many factors like forestry practices, excessive

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collection, pollution, inappropriate agriculture, urbanization, habitat destruction, conflicts, climatic changes, desertification, degradation and seed germination difficulty<sup>[6]</sup>. Therefore, there is a need to establish a reliable strategy for conservation and multiplication of this plant germplasm<sup>[7]</sup>.

#### 2. Tissue culture on Arum plants

The term plant tissue culture broadly refers to the aseptic cultivation of any plant segments, single cell and tissue organs<sup>[8]</sup>. During the last few decades, plant cell and tissue culture have arisen as new techniques in plant biology for many purposes, among which genetic germplasm conservation and *in vitro* production of bioactive substances are used in pharmaceutical industries<sup>[9]</sup>. Herbal medicines are generally perceived as safe products<sup>[10]</sup> and some of these safe therapies are used to treat liver diseases, diabetes and jaundice<sup>[11]</sup>. In the search for alternatives to traditional agriculture in the large scale production of bioactive plant metabolites and biotechnological techniques specially, plant tissue culture is found to be very useful in the production of desirable medicinal compounds from plants<sup>[1]</sup>.

Many studies have reported on tissue culture of the Araceae family such as the in vitro rapid propagation of Acorus calamus, Aglaonema commutatum, Anthurium andreanum, Caladium bicolor, Homalomena aromatica, Homalomena pineodora[12-18]. There are few records concerning tissue culture of A. palaestinum[19,20]. Shibli et al.[19] obtained somatic embryogenesis from black lily callus which induced from sterilized corm bud sprouts cultured on basal medium containing 5.4 µmol/L 1-naphthaleneacetic acid and 4.4 µmol/L 6-benzyladenine. The germinating embryos were developed into rooted plantlets, then grown in the greenhouse and adapted effectively with 95% survival rate. Furthermore, the same survival rate of acclimatized plantlets under ex vitro conditions was obtained from tissue culture of A. palaestinum seeds[20]. Murshage and Skoog medium supplemented with 5.0 mg/L benzyl adenine and 0.1 mg/L naphthalene acetic acid was the best medium for shoots proliferation. The Murshage and Skoog medium containing 1.0 mg/L naphthalene acetic acid and 2.0 g/L charcoal was the best medium in rooting of the regenerated shoots.

### 3. Biochemical analysis of black calla lily

Polyacrylamide gel electrophoresis (PAGE) is a technique widely used to separate biological macromolecules like nucleic acids or proteins according to their electrophoretic mobility which is a function of charge of the molecule, the length and conformation<sup>[21]</sup>. Sodium dodecyl sulfate (SDS) is an anionic detergent applied to protein sample to impart a negative charge to linearized proteins. This procedure is called SDS-PAGE. Isozymes are enzymes that catalyze the same chemical reaction, but differ in the amino acid sequence. There is only one report concerning protein analysis of *A. palaestinum*<sup>[20]</sup> and suggesting that a real genetic change might be occurred due to the difference in protein profiles in the tissue culture explants and mother plant. There are few studies reported on Araceae family, such as the analysis of the phylogenetic relationships and geographical differentiation of Indian taro, *Colocasia esculenta*  (L.) by isozyme of seven enzyme systems<sup>[22]</sup> and also the study of taxonomic relationships, genetics and breeding in *Zantedeschia aethiopica* and *Zantedeschia elliottiana* leaf protein by the analysis of karyotype, SDS-PAGE and DNA content<sup>[23]</sup>.

# 4. Molecular profiling using DNA markers

Advances in DNA technology in the last three decades have created a wealth of new data that revolutionized biological studies and the molecular markers generated by the new possibilities of genome genotyping. In the last two decades, several PCR-based molecular markers have been made available for biological research<sup>[24]</sup>. The most common methods are random amplification of polymorphic DNA, inter simple sequence repeats (ISSR) and amplified fragment length polymorphism (AFLP).

There are many reports concerning the molecular analysis to determine the genetic stability, differences and phylogenetic relationship using random amplification of polymorphic DNA, ISSR and AFLP molecular markers of many plants in Araceae family, such as *Acorus calamus, Amorphophallus albus, Amorphophallus rivieri, Anthurium andreanum, Colocasia esculenta, Syngonium podophyllum*[22,25-30].

The use of molecular markers to assess levels of genetic variation of Arum species was studied by Makhadmeh et al.[5]. Twenty one locations were surveyed to assess the genetic variation. Ten plants from each location were collected and measured directly in the field. In total, 210 plants were studied and three Arum species (Arum palaestinum, Arum dioscoroides and Arum hygrophilum) were found in these locations. AFLP technique was used to study the genetic variation between Arum species. Six combinations of selective primers generated a total of 2882 reproducible and clearly scorable bands of different sizes. About 98.6% of them were polymorphic. The overall genetic distance between Arum species was 25%. The results showed that Arum populations of the same species or having a common genome were grouped in the same cluster regardless of the collection site. The wide range of genetic distance was represented by the high level of DNA polymorphism occurring among Arum species. The results attained in this study will be of great importance for further work aiming at establishing a conservation strategy for preserving and maintaining the germplasm of this plant using in and ex situ gene banks, while Farid et al.[20] studied the molecular variation of tissue culture explants and control plant of A. palaestinum and the obtained results of ISSR showed variations between the control plant and tissue culture regenerated plantlets.

## 5. Phytochemical analysis of A. palaestinum

The genera of the family Araceae contain various classes of secondary metabolites including flavones, flavonols, C-glycosyl flavone, polyphenols, alkaloids and proanthocyanidins<sup>[31,32]</sup>. Moreover, the phytochemical screening of *A. palaestinum* extracts revealed the presence of terpenoids, flavonoids and phenolic acids<sup>[33]</sup>. Its phenolic constituents were investigated using high performance liquid chromatography analysis leading to the

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