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Wild Golden Iris (*Iris aurantica*) in Syria

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Abstract

Iris aurantica is a rhizomatous perennial, from the south mountain of Syria (JabalAl Drouze), it has a compact rhizome and falcate leaves. It has a slender stem with flowers golden yellow to coppery-brown. The golden iris in Syria is becoming rare due to destruction of their natural habit. In this paper, plant classification, botanical description, morphological, geographical, chemical composition, *in vitro* propagation and conservation, and cryopreservation of *Iris aurantica* are investigated.

Keywords: Morphological; Botanical description; Micro propagation; Conservation; Chemical composition

Abbreviations: MS: Murashige and Skoog; HF-MS: Hormon Free Murashige and Skoog; PVS2: Plant Vitrification Solution 2; LN: Liquid Nitrogen

Introduction

Syrian flora has 3247 species [1]. There are many of endemic species, some of which belong to genus Lilium, Crocus, Tulips and Iris. Iris is the largest and most complicated genus of iridaceae, which includes over 300 species [2]. This includes some of the world most popular and varied garden flowers that are originated in both Japan and the Mediterranean [3].

In Syria, *Iris* is considered as a wild perennial herbaceous plant that subjected to strict protection, though Iris grows naturally in many regions of Syria. It presents some 30 species grown in Syria [1]. There are five subgenus found in the world. Apogon, Pogonias, Xiphion, Guno and oncocyclus which includes most of the Syrian species, that are considered as rare endemic plants, characterized by special, beautiful forms that have a great importance in applied studies for genetic biodiversity, such as *Iris aurantiaca* Dinsm.

Iris aurantica, of Syria was first discovered by Dinsmor on the Tell Quleib in Syria. Mouterde found it in several other places in Djebel Druze, Tell Qouleib, Kafer, Tell Jaffna, Mayamas, Sahwet-El-Khodr [1] and Distribution in the Djebel Druze at about 1600m [4]. Dr. Werkmeister, Professor of Botany at the Botanical Institute, Geisenheim am Rhein, Germany which had the opportunity to collecting the golden Iris in 1961,and cultured it in his garden. The golden Iris flowered in Europe in June one month later than in their natural habit in Syria [5].

Iris plants in Syria are becoming rare due to both ongoing destruction of their natural habit, as well as over harvesting of wild species and the influencing of modernization, i.e., urbanization, migration, determental climatic and environmental changes, adding the huge destruction of plant biodiversity by the hard war since 5 years [6].

In this paper, Plant classification, botanical description, morphological, geographical, chemical composition, genetic variability, *in vitro* propagation and conservation, and cryopreservation of *Iris aurantica* were investigated.

Scientific Classification

Iris aurantica L is one of the important species that belongs to the family *Iridaceae*, endemic to Jabal Al `Arab, rhizomatous (with thick, creeping underground stems) [7].

A. Kingdom: Plantae.

B. Unranked: Angiosperm, Monocots. Order *Asparagales*.

C. Family: Iridaceae. Subfamily: Iridoideae, Tribe: *irideae*.

D. Genus: Iris, Subgenus: Oncocyclus. Species: *Iris aurantica.*

Description of *Iris aurantica*

Iris aurantica is a perennial plant, growing from compact rhizome that reaches up to 10-14 cm long. Rhizome develops from axillary buds, it allows new shoots to grow upwards. The golden iris rhizomes planted underground about 5-10cm in rocky soils (Figure 1). It uses rhizomes to store nutrients like proteins, starches and lipids, these nutrients become useful for the plant when new shoots will be formed in early spring.

Figure 1: Rhizome of *iris aurantica*.

Figure 2: Leaves and roots of *iris aurantica*.

Iris aurantica has green falcate leaves, they can grow up to 15-25cm long and between 1-1.5cm wide (Figure 2). Leaves can sheath up to haft of the stem after the iris has bloomed. At the summer sun with dry conditions, leaves fade and die. Golden Iris has a slender stem, which can grow up to 30-50cm tall. The stems hold terminal flowers (top of stem), blooming during May and Jun, they can flower for up of month long [4].

Figure 3: *Iris aurantica* flower parts.

Golden Iris has a slender stem, which can grow up to 30-50cm tall. The stems hold terminal flowers (top of stem), blooming during May and Jun, they can flower for up of month long [4]. The flowers like other irises, it has two pairs of petals, three large sepals, known as the falls and three inner smaller petals or sepals, known as the standards. The standards are golden yellow to coppery-brown, 8-9cm long, 5-5.5 wide, with fine purple veins. The falls are oblong-shaped, 7cm long, and 4cm wide, abovate, with minute purplish red spots and very fine reddish veins. It has golden yellow with fine purple. Style branches (stigma) keeled, and have lobes tips that are a similar color like falls (Figure 3). After the iris has flowered, it produces a seed capsule, 8cm long, rather narrow.

The golden iris flowered in Europe in June, a whole month later than in their habitat in Syria. The unexpectedly cold European weather in the spring of 1962 had nearly everywhere in Europe killed the flowers on the iris species. The only exception and a unique consolation, was the plants of Iris aurantica from Syria, which gave us much joy with their flowers [5].

Golden Iris Propagation

The propagation of *Iris* species is usually accomplished vegetatively through bulbs or splitting of rhizomes (*rhizomatous Iris*). In rhizomatous *Iris*, splitting the rhizomes gives a maximum of 10 plants per year per rhizome [8]. Furthermore, the propagation of *Iris* species through seedlings is known to be difficult due to a poor fruit set and a very low germination rate.

Plant tissue culture is a powerful alternative technique for propagation and conservation of plants, especially for those that are rare and difficult to propagate by conventional methods. Therefore, a new trend has evolved to propagate these species through tissue culture techniquein order to preserve it from deterioration and to study the possibility of using them as a medical or ornamental plant.

Micro propagation is the aseptic culture of cells, pieces of tissue, or organs. It is possible to regenerate new plants from small pieces of plant tissue identical to the plant from which it was derived.

The process of micro propagation can be divided into four stages:

A. Initiation stage: The objective of this stage is to achieve an aseptic culture. An aseptic culture is one without contaminating bacteria or fungi. Base of leaves and shoot tips of rhizomes in *Iris aurantica* (after surface disinfection by chlorox 3%) were cultured on solidified MS medium containing 30g/l sucrose, and supplemented with 2mg/lBAP and 0.2mg/lIBA (Abouzedan and Al-Batal 2015). Results showed, after one month of culture, that using shoot tips of rhizomes resulted in the highest growth percentage (35.76%) in initial stage [9].

B. Multiplication stage: A growing ex plant can be induced to produce vegetative shoots by including a cytokinin in the medium and different media. In *Iris aurantica*, the highest average number of shoots per ex plant was found (3.43) under BAP at a concentration of 3.0mg/l (Figure 4), and MS media resulted in the highest multiplication rate and shoot length with significant difference compared with Heller media, Subculture of the plantlets on the same medium resulted in increasing multiplication rate and shoot length in *Iris aurantica* [9] the

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treatment of high concentrations of cytokinins (5 and 10mg/lBAP) consist of appearance of vitrification.

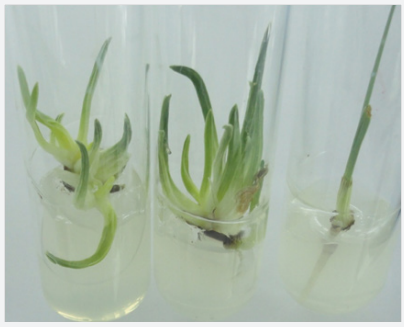

Figure 4: Multiplication stage in *Iris aurantica.*

C. Rooting stage: Growing shoots can be induced to produce adventitious roots by including an auxin in the medium. *In iris aurantica*, the highest root percentage (88.5%) was obtained on medium containing 3mg/lIBA (Figure 5). The highest root number (4.25) was recorded when using the concentration 0.5mg/lIBA [9] (Table 1).

Figure 5: Rooting stage, 1mgdm^{-3} IBA.

Table 1: The best media of tissue culture in iris aurantica.

Compositions of Growth Medium	Initiation Stage	Multiplication Stage	Rooting Stage
Medium	MS	MS	½MS
Sucrose	30g dm^{-3}	30g dm^{-3}	30g dm^{-3}
Agar	7g dm^{-3}	7g dm^{-3}	7g dm^{-3}
Thiamine(vitamin B1)	1mg dm^{-3}	1mg dm^{-3}	1mg dm^{-3}
Myo- inositol (B8)	100mg dm^{-3}	100mg dm^{-3}	100mg dm^{-3l}
Hormones			
BAP	2mg dm^{-3}	3.0mg dm^{-3}	0
IBA	0.2mg dm^{-3}	0.1mg dm^{-3}	3.0mg dm^{-3}
Other Compounds			
Activated Carbon	3g dm^{-3}	-	-

D. Acclimatization: A growing, rooted shoot can be removed from tissue culture and placed in soil. When this is done, the humidity must be gradually reduced over time because tissue-cultured plants are extremely susceptible to wilting. The acclimatization in vivo was achieved easily with high percentage of success (86.95%) in *iris aurantica* (Figure 6). Two months, later, plantlets were cultured in greenhouse and the average length of shoots were 23. 25 cm [10].

Figure 6: Acclimatization stage.
A: plantlet after one week, B: plantlet after 2 months, C: plantlets in greenhouse.

In Vitro Conservation

Conservation is a very simple in vitro technique that permits conservation plants material for periods ranging from 6 months to 5-7 years, depending on species [11]. This technique is based on reducing the growth rates of the tissue cultured plant and yet increasing the intervals between subcultures [12]. Research was conducted, to develop an *in vitro* technique for short-term conservation and relieve of growth and increase the period of time between transfers of *Iris aurantica*, The best osmotic agents for *in vitro* conservation was sucrose compared with (mannitol and sorbitol) and the best medium concentration was 1/10 MS [10] in *iris aurantica* the cultured stored at (3 ˚C) gave the highest survival (93.33%) and lengthening the time period between transfers up to 6 months [10]. ABA was found to regulate expression of many genes that are responsible for the syntheses of proteins needed for osmotic adjustment in the cell, such as, membrane stabilization proteins, and the LEA proteins that would modify water state in the cell to cope with osmotic stress [13]. Some researchers reported that ABA is responsible for low temperature tolerance capacity of plant tissues [14]. In our research Iris *aurantica* micro shoots were conserved for more than nine months at normal growth room conditions in the media supplemented with 0.5 to 3.0mg/lABA, also ABA significantly decreases growth of shoots in medium when compared with control.

Results showed that the best survival after three, six and nine month were obtained in media supplemented with 2 and 3mg/l ABA with significant difference compared to the control at 3 ˚C and normal growth room conditions. The best treatments on germ plasm conservation were in 3mg/l ABA at 3 ˚C, in these treatments the survival rate was 60%.

Long - Term Conservation (Cryopreservation)

The principle of cryopreservation is the storage of plant material at ultra low temperature (-196 ˚C) that takes a place in a cryogenic condition which is liquid nitrogen [15]. At this

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temperature, all forms of cellular divisions and metabolic activities of plant cell are ceased and consequently plant material can be stored unaltered for an indefinite time scale [16,17]. Two techniques of cryopreservation were used in the case of Golden iris:

Vitrification

Vitrification is based on three major phases, the loading phase, dehydration with the highly concentrated vitrification solutions, and unloading phase [18]. In loading phase samples are exposed to cryo protectants or diluted vitrification solutions [19] the samples are dehydrated by a highly concentrated vitrification solution before being plugged in LN, [20]. In unloading phase vitrification solution is drained out of the cryovials after rapid thawing, and then replaced routinely with 1.2 M sucrose for 10-20 min [21]. Plant vitrification solution 2 (PVS2) is commonly used in most vitrification protocols [22].

In our research Shoot-tips in *Iris aurantica* were excised aseptically from in vitro grown plants and incubated for 3 days on solid (HF-MS) media supplemented with 0.3 M sucrose under complete darkness at 24 ± 1˚C.in vitrification, shoot-tips were loaded in 0.4 M sucrose and 2 M glycerol for 20 min followed by desiccation with different combinations and concentrations of PVS2, before immersion in LN. Results showed that Subjecting *I. aurantica* shoot-tips to gradual increase in PVS2 concentrations (20, 40, 60, and 100%) before plunging them in LN gave the highest survival for the non-cryo preserved shoot-tips (50%), and cryo preserved shoot-tips (30%), respectively, Using of 2 M glycerol plus 0.4 M sucrose resulted in the highest survival (80%, 25%) for the non cryo preserved and cryo preserved *Iris aurantia* shoot-tips, respectively, The highest survival for the non cryo preserved *I. aurantica* shoot-tips was recorded after 30 min of loading.

Encapsulation-Dehydration

This method, developed [23] in which shoot tips, somatic embryos or callus cells are encapsulated within alginate beads and subsequent culture in a medium containing elevated concentrations (0.7-1.5M) of sucrose for 1 to 3 days [24,25]. The beads are then allowed to dehydrate using silica gel or by air under the laminar air flow until the moisture content drops to 20-30% before being immersed in LN [23].

In our study shoot-tips in *Iris aurantica* were encapsulated in 3% calcium alginate and dehydrated under laminar air flow cabinet for0, 2, 4, or 6h. the highest survival were obtained after pre-treating encapsulated non-cryo preserved shoot-tips for 3 days in 0.5M sucrose supplemented media with 4h dehydration.

Chemical Analysis

The medicinal parts of Iris (orris) species are the rhizomes with the roots. They contain volatile oil (α, β, γ, irons) giving the odor of violets triterpenes, isoflavonoids, flavonoids, xanthones and starch [26]. Some of Iris species were reported for their medical use. The isolated compounds from some species were demonstrated to have pesticide, anti neo plastic and anti tuberculosis properties [27]. The extract of I. germanica was found to have central anti serotonin activity [28].

Gas chromatography–mass spectrometry (GC-MS) analyses of the essential oil have indicated the presence of 23 compounds in *Iris germanica*, and 19 in *Iris aurantica* [29]. The major compound in these essential oils was Myristic acid (61.42%, 70.67%) in *Iris germanica*, and *Iris aurantica* respectively with no significant differences [29]. The findings here agreed with those obtained [30] which noted that the myristic acid was the major compound of the oil of the fresh and naturally aged rhizomes in *Iris pallida* which has antifungal properties [31]. The other sub major compounds obtained were Lauric acid, Decanoic acid (Capric acid), Palmitic acid methyl ester, Octadecanoic acid methyl ester, Elaidic acid methyl ester (9- Octadecenoic acid methyl ester (E) and Palmitic acid [29].

The highest percentage of Lauric acid was obtained (6.97%) in *I. aurantica*, with no significant differences comparing with *Iris germanica* (5.69%), these findings give us the possibility to investigate the use of *iris aurantica* for medical purposes [29].

Iris Cultivation

Wild *Iris aurantica* is very difficult to cultivate. It can withstand the cold and the heat as long as it is dry. Golden iris needs well drained soil and at least 6-8 hours sunlight. If the soils are heavy, sand or humus may be added to improve drainage. The ideal pH is less than 7, slightly acidic. Iris should be planted in September or October, when the weather starts to cool, it is preferably to be divided and planted at least six weeks before the first frost in any area.

The rhizomes produce more rhizomes, which in turn lead to more leaves and flowers. One rhizome of golden iris can give more than ten flowers. When the bloom production slows and it is necessity to divide the plants, removing and replanting the baby rhizomes in spacing 15-25 cm. Close planting results in immediate effect, faster clump formation, and makes dividing clumps a necessity in two to three years. New plantations of irises need moisture and fertilizer to help their root systems becomes established. Watering depends on the soil quality and the climatic conditions (Figure 7). It is preferable to give deep watering at long interval is better than shallow watering.

Figure 7: Soil of Mayamas Al Suwayda Syria.

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