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Research Article

In vitro culture initiation of *Cleome droserifolia* glandular trichomes for potential biotechnological applications

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Abstract

Cleome droserifolia (Forssk.) Del., belongs to family Cleomaceae. It is an aromatic shrub covered with glandular hairs that give off a distinct volatile smell of its medicinal important chemicals. The initiation of glandular hairs cultures of *Cleome droserifolia* was successfully achieved on Murashige and Skoog medium containing a combination of BA (1mg l^{-1}) with 2, 4D (10 mg l^{-1}) and IAA (6mg l^{-1}) with Kinetin (4mg l^{-1}) in *in vitro* cultures**.** The obtained result will open the door for new biotechnological applications for the production of fine chemicals. Stem and leaf petiole glandular hairs expanded and formed small side daughter hairs perpendicular to the mother trichome. Multiplication of single trichome is obtained from leaf lamina and added new divisions from glands. The best response for multiplications and branching of daughter glandular trichomes was observed on MS medium containing 1mg l^{-1}BA and 10mg l^{-1} 2, 4 D.

Keywords: *Cleome droserifolia*, Glandular trichomes (GT), Tissue culture, Multiplication and branching.

Introduction

Cleome droserifolia (Forssk.) Del., wild perennial herb about 60cm high belongs to Family Cleomaceae. It is an aromatic shrub characterized by its orbicular leaves. The leaves have a characteristic slightly disagreeable odour and bitter taste. The plant is covered with glandular hairs that give off a distinct smell. The upper-side of the leaves is green and the lower-side is a lighter yellowish-green. Flowers are yellow with pinkish stamen filaments and are about 1 – 1.5 cm. It grows in Egypt, Libya, Palestine, and Syria in stony and sandy soil (Täckholm, 1974; Zohary, 1966; Boulos, 1999; Batanouny, 1999). It is known in Egypt as Samwah, Afein or Reeh-El-Bard, is used by herbalists in Egypt as a hypoglycemic agent, and its decoction is widely used by the Bedouins of the southern Sinai for the treatment of diabetes. This plant has a great fame as an antihyperglycemic agent (Yaniv et al., 1987; Yang et al., 1990; Nicola et al., 1996; Abdel-Hady, 1998; Abdel-Kawy et al., 2000; Motaal et al., 2011). Although considerable research efforts confirmed its utility as a hypoglycemic herb, the chemical basis underlying this therapeutic

property is unknown so far. Recently has been found to contain antioxidant and hepatoprotective constituents (Nassar & Gamal El-deen, 2003; Abdel-Kader et al., 2009). Treatment of hepatocytes culture with the plant extract resulted in a reduction in cell viability (Saad et al., 2006). Used medicinally also by local Bedouin people to treat bee stings, to cleanse internal and external infections on the skin or eyes of people and animals. *C. droserifolia* leaves extract has anti-schistosomiasis activity (El-Shenawy et al., 2006). Previous phytochemical study of *C. droserifolia* indicated the presence of sesquiterpene, steroids and flavonoids (Hussein et al., 1994; Fushiya et al., 1999; El-Askary, 2005).

Trichomes are epidermal extensions deriving from the Greek word "trichos" which means hair and are single-cell or multicellular structures (Wagner et al., 2004).They may have the ability to give secretions of which trichomes are divided into two general categories glandular or non-glandular. Glandular trichomes are the most important for study and possess two main types peltate or capitate which differ depending on their head size and stalk length. Peltate trichomes are defined as short stalked (unicellular or bicellular); whereas capitate trichomes have a stalk whose length is more than half the head height. It is clear that trichomes play a role in plant defence, by possessing glands which exude phytoanticipins which are olfactory or gustatory repellents especially with regard to phytophagous insects. In numerous species there is a negative correlation between trichome density and insect feeding and oviposition responses, and the nutrition of larvae. Protection some groups of plants against large mammals is achieved by the presence of stinging trichomes (Donald, 1973; Fahn, 2000). Essential oils are produced from glandular trichomes of Lamiaceae plants, such as mint (*Mentha x piperita*), thyme (*Thymus vulgaris*), basil (*Ocimum basilicum*) and oregano (*Origanum vulgare*) (Schilmiller et al., 2008). Glandular trichomes of the annual wormwood *(Artemisia annua)* produced, artemisinin, a sesquiterpene lactone that is used for the treatment of malaria (Weathers et al., 2011). The ability of some plants to tolerate high levels of metals is correlated with their ability to sequester these compounds in their trichomes, as shown for the rough hawkbit (*Leontodon hispidus*)which can sequester calcium (De Silva et al., 1996) and tobacco (*Nicotiana tabacum*) which is able to secrete cadmium and zinc via its trichomes (Sarret et al., 2006). Intraspecific variation for trichome type and density is known in many species, and often is clinal in accordance with geographic parameters. Scanning electron microscopy of *C.droserifolia* and *C.trinervia* petioles and leaf surfaces showed that the two species are coated with capitate-type glandular hairs consistent with productivity of such species with essential oils (Muhaidat et al., 2015). Plant genetic resources are a vital part of the world's biodiversity and an essential source for the human well-being. Plant tissue culture is widely used to produce clones of a plant in a method known as micropropagation. Tissue culture is useful for multiplying and conserving the species, which are difficult to regenerate by conventional methods and save them from extinction and also play a vital role in search for alternative to production of desirable medicinal compounds from plants (Ramachandra & Ravishankar, 2002). In the last decade, *C.droserifolia* plant has been subject to severe overexploitation to be used in folk medicine for diabetes. It has been eradicated from vast areas, especially in the Sinai and the Eastern Desert. However, in the far south of the Eastern Desert, the plant is still flourishing and is growing in many wadies in hot desert areas. The main objective of this study is to initiation of glandular hairs cultures of *C. droserifolia,* so we focused on attempts to use isolated single glandular trichome of plant tissues (leaf lamina, petiole, and stem) as explant. This would be achieved through using Murashige and Skoog medium containing combination of BA with 2,4D and IAA with Kinetin in *in vitro* cultures. It is desirable to develop this technology to assist future

studies on the bio-production of fine chemicals, physiology, and biochemistry of plant with potential application for medicinal purposes.

Materials and Methods

Collection of plant material

Plant material was collected from Plant material Red sea wadies in Egypt. The plant was also grown in the university greenhouse to be used in experiments.

Sterilized plant material

Aerial parts (stem and leaves) of *C. droserifolia* were washed under continuous flashing of running tap water for 1h to remove all the adhering dust particles from the surfaces and then sterilized by immersing in 70% ethanol for 1 min followed by sterilizing with 5% sodium hypochlorite for 5 min, then washed five times by sterilized distilled water to remove minor amounts of disinfection liquid.

Culturing of trichomes

The trichomes were taken from stem and leaf as explant. The explants were placed on petri dishes (9cm) containing MS medium (Murashige & Skoog, 1962) supplemented with vitamins, sucrose, and plant growth regulators that were combination between BA (Benzyl Adenine,1mg/L) with 2,4D(2,4D-Dichlorophenoxyacetic acid, 10mg/L) and also between Kintein(4mg/L) with IAA (Indole acetic acid, 6mg/L). The PH was adjusted at 5.8 before adding 8g/L Agar by using NaOH or/and HCl before autoclaving at 120 °C for 20 min. Cultures were placed in darkness for 3 days, then incubated in growth chamber at 28°C with 16/8h photoperiod provided with light intensity of 2,000 lux provided by white fluorescent lamps. After 4 weeks the resulting of these cultures were examined by stereomicroscope (Olympus SZ61) and photographs were taken using Olympus soft imaging solutions GMBH camera model LC20 (Munster, Germany).

Result and Discussion

C. droserifolia possess two morphologically distinct trichomes, long and thin & short and thick. These trichomes occur on the adaxial and abaxial surfaces of leaves with short trichomes dominant on the adaxial surfaces (Fig.1, C,D) and long trichomes on the petiole and stem (Fig .1, E-H). Trichomes in *C. droserifolia* leaf lamina appeared denser and shorter than in petiole and stem that might be related to differences in oil composition and percentage profiles of secondary metabolites (Muhaidat et al., 2015). An efficient procedure for high frequency of trichomes multiplication was developed and the ability of combination between auxins and cytokinins with specific concentration to induce multiple trichomes was documented. Initiation of daughter glandular hairs from mother single glandular hair cultures was successfully achieved (Fig.2). These initials developed normally into glandular trichomes and the densities of trichomes were significant in which multiplication of single trichome that obtained from leaf lamina and added new divisions from glands (Fig.2, A-E). But while in trichomes that obtained from stem and petiole which might be related to length of trichomes initiated by different signs by the elongation mother GT base and forming small daughter hairs perpendicular to the mother of trichomes , the formation of branches, and the well established daughter glandular hair (Fig.2, F-L).

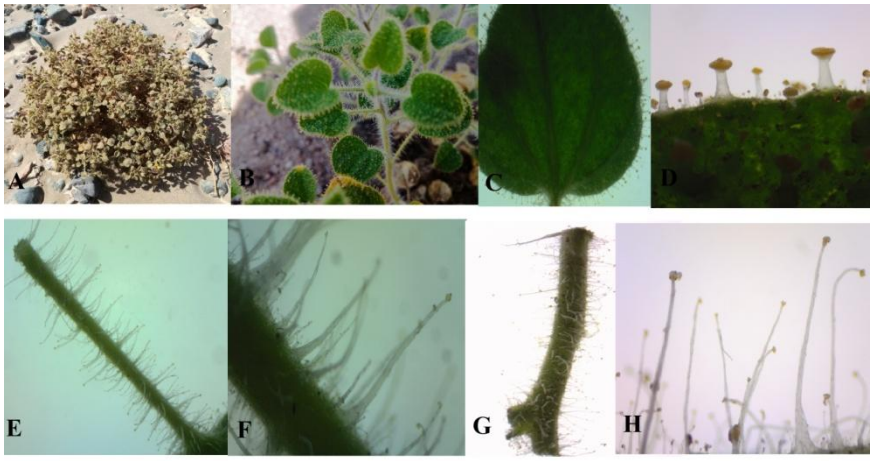

Figure 1. Morphology, distributions and density of glandular trichomes **(A-H). (A)** Wild accessions of *Cleome droserifolia* **(B)** Cultivation of *C.droserifolia* in green house with high densities of glandular trichomes that confer resistance to several pests, **(C, D)** Short capitate glandular trichomes on the adaxial and abaxial surfaces of leaf lamina, **(E)** leaf petiole of *C. droserifolia***, (F)** close view of petiole with trichomes, (G,H) Stem of C. *droserifolia* and glandular trichomes on it . Bars :500µm .

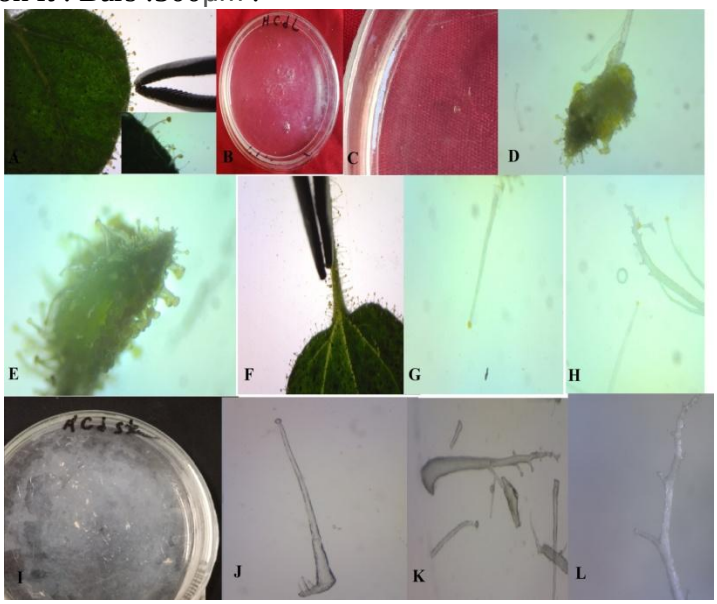

Figure 2. *In vitro* cultures showing the gradual developmental changes of glandular trichomes (GT) of leaf lamina, leaf petiole and stem. **(A-C)** *in vitro* culturing GT on **MS** medium containing combination of BA (1mg l^{-1}) plus 2,4D (10 mg l^{-1}). **(D, E)** multiplication of single trichome that obtained from leaf lamina and added new divisions from glands**. (F-H)** *in vitro* culturing GT from petiole on MS medium. **(H)** Formation of branches of new small daughter glandular trichomes on original mother GT. **(I-L)** *in vitro* culturing glandular trichomes from stem. **(J)** GT expand and formation of new small daughter GT perpendicular to the mother trichome. **(K)** Trichome branching from GT on MS medium containing combination of Kintein (4mg l^{-1}) and IAA (6mg l^{-1}). **(L)** Formation of new GT branching on MS medium containing combination of BA (1mg l^{-1}) plus 2,4D (10 mg l^{-1}) showing well establishment of new GT daughter.Bars:200 µm.

The initiation of trichomes proceeds basipetally, with the first trichomes forming near the distal end that directly attached with the organ. Kim et al (2007) showed the concentration of growth regulators also plays a significant role in the development or inhibition of trichomes. In the present study, however; using of combination of 2,4D plus BA and IAA plus KN in different concentrations, resulted in similar grades of glandular trichome branching and development. The best response for multiplications and branching of single glandular trichomes was observed on Murashiage ans Skoog medium containing 1mg l^{-1} BA and 10 mg l^{-1} 2,4D. In which, 2, 4-D high concentration might be led to an increase in endoreduplication in trichomes calli. Multiplication of lamina trichome cultured only was obtained result on MS medium added with 1mg BA L^{-1} and10 mg 2, 4 D L^{-1}. The effect of BA concentrations alone on shoot multiplication from shoot tip or nodal explant was investigated by (Hassan, 2014) on *C. droserfolia*; the author found that the highest axial shoot length after 6 weeks was resulted from shoot tip explants with BA at 1.0 mgl^{-1}. IAA plus kinetin resulted in rapid cell division and change the cells to a meristematic course (Raven et al., 1981). It has been also reported that some common hormones, from it 2,4D, affect the density of glandular trichomes with regard to the yield and composition of essential oils in aromatic plants or alkaloid response in plants (Zenk et al., 1977; Stahl & Wollensah, 1986; Baldwin,1989; Chien & Sussex, 1996). Bhaumik & Datta (1989) found in Japanese mint (*Mentha arvensis var.piperescens*) that each of GA, NAA, and 2,4D had an optimum dose for increasing the glandular hairs. The ability to synthesize and secret a variety of secondary metabolites is attributed to the glandular trichomes function (Huchelmann et al., 2017). Producing volatile oils from glandular trichomes is important for pesticide, pharmaceutical, flavoring, perfumery and in the fragrance and cosmetic industries (Zeybek & Zeybek, 2002). Glandular trichomes are producing valuable chemicals and improving productivity can be achieved by increasing the glandular trichomes density. There are few reports on the micro propagation of *C. droserifolia* and genus *Cleome* as a general but no reference to *C.droserifolia* trichomes cultures. Because of its traditional usage and medicinal importance, trichomes rapid glandular hair culture initiation of *C. droserifolia* in this study promoted efficient survivability and provided a data baseline for future potential to produce value-added secondary metabolites in tissue culture but the extent of endoreduplication in glandular trichomes is still mostly uncharacterized. So far, published data suggest that multicellular trichome formation probably occurs through different transcriptional regulatory networks from those regulating trichome formations in *Arabidopsis*, so mere orthologous relationships may not concluded (Payne et al., 1999; Serna & Martin, 2006; Yang et al., 2015; Liu et al., 2016). In trichomes initials, cell-specific transcriptional control of gene expression and cell cycle regulation results in the beginning of a controlled cell division and trichome morphogenesis program, most of which is still not so well understood. The field of glandular trichome development lacks a unique and robust model system due to the difficulty of finding such appropriate system. Over the long term, the ability to modulate the density and productivity of such secreting structures in plants would be of great biotechnological interest. This requires the identification and characterization of the genes initiating, regulating, and driving the development of such glandular structures and their metabolic pathways.

Conclusion

The main result of this study is a rapid initiation of glandular trichomes. The findings concluded that the present protocol could be efficiently used for enhancing one glandular hair to initiate new small daughter glandular hairs by multiplication or branching. The previous studies demonstrated the initiation and development of glandular trichomes from epidermal cells of field grown leaves and also how 2.4D affect significantly on glandular trichomes density. Initiation of complete

trichomes from glandular trichome itself as explant will generate new leads to improve production of important specialized metabolites further.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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