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REVIEW ARTICLE

Citrullus colocynthis (L.) Schrad. (colocynth): Biotechnological perspectives

Jaime A. Teixeira da Silva[1]*, Abdullah I. Hussain[2]

[1]P. O. Box 7, Miki-cho Post Office, Ikenobe 3011-2, Kagawa-ken, 761-0799, Japan, [2]Department of Applied Chemistry and Biochemistry, Natural Product and Synthetic Chemistry Laboratory, Government College University, Faisalabad 38000, Pakistan

ABSTRACT

Citrullus colocynthis (L.) Schrad. is commonly known as colocynth. The fruit pulp of colocynth has medicinal properties while the seeds have nutritive qualities. *C. colosynthis* is resistant to high temperatures and grows in the desert regions of North Africa, the Middle East and Western Asia. *C. colocynthis* likely carries genes of interest that could be explored for inducing abiotic stress resistance in transgenic plants. Although the tissue culture and molecular biology of this species have been explored, the latter has been primarily used to resolve taxonomic relationships with other members of the *Citrullus* genus and curcubits. Genetic mining of the plant is scarce while genetic transformation protocols are also rare. The aim of the present review is to present a brief overview of the biotechnological perspectives of *C. colocynthis*.

Keywords: Abiotic stress-resistance; Colocynth; *Cucurbitaceae*; Medicinal properties; Plant growth regulators; Tissue culture

INTRODUCTION

Citrullus colocynthis (L.) Schrad. (*Cucurbitaceae*) has medicinal and ornamental purposes, the former derived primarily from the fruit pulp (de Smet, 1997). Common names for this plant include colocynth, bitter gourd, bitter apple, and bitter cucumber in English while it is known as Koloquinthe in German and *coloquinte* in French (de Smet, 1997). *C. colocynthis* has only one accepted name but six synonyms (The Plant List, 2017). In India and Pakistan, it is known as *tumba* (Mahajan and Kumawat, 2013; Hussain et al., 2014).

Early literature indicates that *C. colocynthis* was the closest relative or progenitor of watermelon (*Citrullus lanatus* (South African watermelon) and *Citrullus vulgaris* L. (Linnaeus' watermelon)) (Assis et al., 2000), but molecular phylogenetic analyses combined with herbarium sample analyses conducted by Chomicki and Renner (2015) revealed that in fact this was not true. Furthermore, what was referred to as "Egusi" melon by Ntui et al. (2009, 2010a, 2010b) as *Colocynthis citrullus* L., may represent an incorrect inversion of the Latin name and possibly a completely different plant, since Chomicki and Renner (2015) indicated that "Egusi" melon is *Citrullus mucosospermus* (formerly *C. lanatus* subsp. *mucosospermus*; Levi and Thomas, 2005), a position supported by morphological and phenetic analyses (Achigan-Dako et al., 2015) and genetic studies (Paris, 2016) (Table 1). Jarret and Newman (2000) also showed that *C. colocynthis* and *C. mucosospermus* clustered separately using internal transcribed spacer (ITS) sequences. Until formally resolved, for all effective purposes, the authors consider, within this review, the plant reported by Ntui et al. (2009, 2010a, 2010b) to be *C. colocynthis*.

Dane et al. (2007) employed three cpDNA regions and the nuclear *G3pdh* transit peptide section with intron 2 in a using molecular phylogeography study to show how *C. colocynthis* accessions migrated from xerophytic habitats in Algeria, Chad and Egypt to Israel, Cyprus and the Middle East, then further east to Iran, Afghanistan, Pakistan, and India, whereas Moroccan accessions migrated to Australia while Israeli accessions migrated to Ethiopia.

IMPORTANCE AND USES

According to Hussain et al. (2014), *C. colocynthis* has the following traditional medicinal uses: "diabetes, leprosy, common cold, cough, asthma, bronchitis,

***Corresponding author:**
Jaime A. Teixeira da Silva, P. O. Box 7, Miki-cho Post Office, Ikenobe 3011-2, Kagawa-ken, 761-0799, Japan. E-mail: jaimetex@yahoo.com

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Table 1: Taxonomy of *Citrullus colocynthis* (L.) Schrad*

Tanonomy and classification of *Citrullus colosynthis* (Renner et al. 2014; Chomicki and Renner, 2015)	
Citrullus colocynthis var. *capensis* Alef.	
Citrullus colocynthis var. *insipidus* Pangalo	
Citrullus colocynthis subsp. *insipidus* (Pangalo) Fursa	
Citrullus colocynthis var. *lanatus* (Thunb.) Matsum. & Nakai	
Citrullus colocynthis var. *stenotomus* Pangalo	
Citrullus colocynthis subsp. *stenotomus* (Pangalo) Fursa	
English name	**Species**
Dessert watermelon	*C. lanatus* (Thunb.) Matsum. & Nakai
Citron watermelon	*C. amarus* Schrad.
Egusi watermelon	*C. mucosospermus* (Fursa) Fursa
Colocynth	*C. colocynthis* (L.) Schrad.

*Synonyms from The Plant List (2017), and within the *Citrullus* genus, based on Paris (2016)

jaundice, joint pain, cancer, toothache, wound, mastitis, and in gastrointestinal disorders such as indigestion, constipation, dysentery, gastroenteritis, colic pain and different microbial infections." Also, according to the same authors, who wrote a comprehensive review on several properties of *C. colocynthis*, indicated that there are multiple medicinal and biological activities, including antidiabetic, anticancer, cytotoxic, antioxidant, antilipidemic, insecticidal, antimicrobial and anti-inflammatory. De Smet (1997) also reviewed earlier literature on the medicinal properties of *C. colocynthis*.

The seeds of *C. colocynthis* contain edible oil, 56% of which contained linoleic acids and 25% of which contained oleic acids (Sawaya et al., 1983). Fruits hold bioactive chemical constituents such as glycosides, flavonoids, alkaloids and terpenoids while "curcurbitacins A, B, C, D, E, I, J, K, and L and Colocynthosides A, and B" have also been isolated (Hussain et al., 2014). Several accessions have shown resistance to several viruses and diseases (Dabauza et al., 1997). The identification of such genes would assist in breeding virus and disease resistance in other cucurbits such as watermelon. To this end, Dabauza et al. (1997) developed an *Agrobacterium tumefaciens*-mediated genetic transformation protocol in which 7-day-old seedling cotyledons were infected with strain LBA4404 carrying the binary vector pBI121, harboring the β-glucuronidase (*gus*; reporter) and the neomycin phosphotransferase (*nptII*; marker) genes. Based on GUS expression, 14% of explants were shown to be transformed. PCR confirmed the integration of the *gus* and *nptII* genes while Southern blot analysis conferred transmission of the *gus* gene to several transgenic plants obtained by selfing.

C. colocynthis is able to withstand extreme desert temperatures through a high rate of transpiration to lower leaf temperatures below lethal temperatures (Althawadi and Grace, 1986). In the Thar Desert in Pakistan, the perennial plant develops an extensive root system, and despite only receiving only 35-40 mm of rainfall per hectare, can produce as much as 1-1.5 t of seed, but as much as 40-fold more if rainfall is high (Mahajan and Kumawat, 2013). Exploring this ability of *C. colocynthis* to grow in arid climates, Schafferman et al. (2001) assessed 28 accessions growing wild in Negev, Arava and Sinai Deserts in Israel, and found that the mean seed oil yield ranged from 17.1% to 19.5% (v/w) in five high-yielding lines (dry seed weight basis). In addition, across the 28 accessions, linoleic acid (C18:2) was dominant ($\bar{x}$ = 70.1%), followed by oleic acid (C18:1; $\bar{x}$ = 13.1%), palmitic acid (C16:0; $\bar{x}$ = 10.1%), and stearic acid (C18:0; $\bar{x}$ = 6.7%). With a seed yield of 1.5-2.1 kg/10 m^2, *C. colocynthis* may be a potential seed oil-yielding plant for desert and arid regions (Schafferman et al., 2001).

C. colocynthis seeds exhibit strong dormancy, and even strong chemical or physical treatments are unable to release the seeds from this state of dormancy (Koller et al., 1963; El-Hajzein and Neville, 1993), although scarification of the testa using sandpaper resulted in 61% germination (Saberi and Shahriari, 2011). Mahajan and Kumawat (2013) observed 51.4% germination of seeds taken from fresh fruits and placed at 30°C and 95% relative humidity whereas 48 h fermentation increased germination to 58.8%, and decreased to 16.4% after storage for 12 months at room temperature. Sen and Bhandari (1974) achieved 98% germination after 4.5 h of treatment with concentrated sulphuric acid and germination in the dark since exposure to light inhibited germination.

TISSUE CULTURE AND GENETIC TRANSFORMATION

The success of a plant genetic transformation protocol involves a reliable *in vitro* regeneration protocol (except for *in planta* transformation), an effective vector to transmit the desired gene into target tissue, stable integration and transgene expression without transgene silencing and only as a single gene copy (Teixeira da Silva et al., 2016).

As described next, the first aspect, i.e., effective *in vitro* regeneration protocols, have been developed for *C. colocynthis* and tissue derived from *in vitro* plantlets is available all-year round and is suitable for both *Agrobacterium*-mediated and bombardment-induced introduction of transgenes.

There have not been many studies published on the tissue culture of *C. colocynthis* (Table 2). Dabauza et al. (1997) induced callus and shoots from seedlings' cotyledons, with 81.1% of explants being organogenic, and 68.3% forming shoots, 80% of which could root on IBA-containing medium. El-Baz et al. (2010) also used seedling tissue to induce callus, mostly from stems, less so from leaves and least from roots, but in all cases with more than 90% of explants inducing callus from one tissue or another. Verma et al. (2013) used disinfected shoot buds and nodes from wild plants to induce shoots (as many as 18-20/explant) and roots.

Ntui et al. (2009) induced shoots from cotyledon explants within 12 days (4.4 shoots/explant in 'NHC1-130') but hypocotyl explants failed to form shoots and only induced callus. In 'Ejagham', 86.3% of explants induced shoots, similar to improved cultivar NHC1-130. Averaged across all four cultivars, 65% of shoots rooted on PGR-free MS medium, and although acclimatized plants had a normal appearance, *in vitro*, mixoploid and tetraploid shoots formed. This regeneration protocol served as the basis for genetic transformation experiments by Ntui et al. (2010b) in which cotyledonary explants of 'Ejagham' and 'NHC1-130' were infected with *Agrobacterium tumefaciens* strain EHA101 harboring one of two plasmids, pIG121-Hm, carrying the *gus*, hygromycin phosphotransferase (*hpt*) and *nptII* genes, or pBBR*acdS*, harboring the same three genes as well as the 1-aminocyclopropane-1-carboxylate (ACC) deaminase gene. Based on PCR of kanamycin-resistant shoots, transformation efficiency ranged from 2.4% to 9.9%, depending on the cultivar and bacterial strain. Expanding upon this protocol, Ntui et al. (2010a) introduced the chimeric defensin gene from *Wasabia japonica* into cotyledons of 'Ejagham' and 'NHC1-130' using *A. tumefaciens* strain EHA101 with plasmid pEKH1-WD, with a 25-27% transformation efficiency. Transformed plats were seen to be growing in medium in which heavy fungal contamination was observed.

Meena et al. (2010, 2014) induced as many as 23 shoots per shoot tip. Savitha et al. (2010a, 2010b), Shasthree et al. (2012, 2014) and Ramakrishna and Shasthree (2016) noted that seedling-derived leaves formed callus more than stems (65% vs 45% of explants) while stems formed shoots more than leaves (75% vs 51% of explants). Satyavani et al. (2011a) induced shoots indirectly from callus, which was induced from stem explants and a maximum of 20 shoots/explant could be induced. Taha and Mutasher (2014) also found that seedling-derived leaves and stems were effective (induced in 83 (leaves) - 85% (stems) of explants) explants for callus induction. Gharehmatrossian (2015) induced shoots indirectly from callus that had been induced from seedling explants, but the outcome was not quantified. More details of these studies may be found in Table 2. Shasthree et al. (2009) found that *in vitro*-derived regenerants showed considerable somaclonal variation, including of leaves, flowers, fruits and seeds.

MOLECULAR BIOLOGY AND ABIOTIC STRESS TOLERANCE

Molecular biology in *C. colocynthis* research has primarily been used in taxonomic and phylogenetic classification through the use of molecular markers while considerable work has been done on the characterization of genes and transcription factors involved in abiotic stress tolerance.

Alatar et al. (2012) optimized a protocol for the isolation of high-quality DNA from *C. colocynthis* from Saudi Arabia. The extracted DNA was totally digestible with 1 U *Cfo*I per μg DNA and showed no detectable RNA contamination, obtaining 10-20 μg DNA per tube in the 2-10 Kb range. This DNA is useful for plant species fragment amplification and microsatellite analysis.

The colocynth plant material used in experiments must be precise identified to avoid confusion, especially form interspecific hybrids with *C. lanatus*, in the field (Fulks et al., 1979), or as controlled crosses (Shimotsuma, 1960; Sain et al., 2002). However, randomly amplified polymorphic DNA (RAPD) and UPGMA cluster analyses are able to differentiate *C. colocynthis* from *C. lanatus* (Levi et al., 2001). RAPD was also used, alongside ISSR, to confirm the genetic stability of *in vitro* regenerants (Verma et al., 2013). Shaik et al. (2015) used two gene regions, the nuclear *G3pdh* gene and the chloroplast *ycf6–psbM* intergenic spacer region, to differentiate three *Citrullus* species (*C. colocynthis*, *C. lanatus* (camel melon), and *C. myriocarpus* (prickly paddy melon)) invasive to Australia, discovering that a western and an eastern introduction of *C. colocynthis* may have taken place. Mary et al. (2016) used DNA barcoding with the *trnH-psbA* intergenic spacer to characterize *C. colocynthis* relative to other curcubit genera, placing it as a distinct phylum. Molecular markers thus serve as useful tools in taxonomic differentiation and to assess evolutionary events. Nimmakayala et al. (2011) offer a comprehensive overview of the use of molecular markers in phylogeny and taxonomy of *Citrullus*.

Si et al. (2009, 2010a; Table 3) found that 18 drought-responsive genes related to abiotic and biotic stresses

Table 2: Tissue culture conditions for *Citrullus colocynthis* (L.) Schrad

Cultivars and sources	Disinfection process	Culture conditions*	Optimal medium**,***	Reference
R309	Testa removed from seeds and soaked in 50% commercial bleach with 50 g/l active chlorine for 30 min. Rinse 3 times in SDW. Cotyledons from 7-d-old seedlings cut in half length-wise to yield two explants	16-h PP. Grolux bulbs. 90 μmol m^{-2} s^{-1}. 26±2°C. pH NR. 1% (SG) or 3% (SIM) sucrose. 0.8% (SG) or 1% (SIM) agar. Plant acclimatization NR	MS in darkness for 28 d (SG). MS+100 mg/L *myo*-inositol+25 μM BA (CIM, SIM). MS+2.5 or 5 μM IBA (RIM)	Dabauza et al. 1997
Three local (Nigerian) cultivars (Ejagham, Sewere and Barablackedge); one improved cultivar (NHC1-130)	Seeds without testa dipped in 70% EtOH for 2 min, 1% NaOCl for 20 min and rinsed with SDW. Cotyledons of 4- or 8-d-old seedlings cut into 1/2, 1/4 and 1/6 strips. Hypocotyls cut into 1 cm long segments	16-h PP. 30-40 μmol m^{-2} s^{-1}. 25±1°C. pH 5.8. 1% (SG) or 3% (SIM) sucrose. 0.8% agar. Plants acclimatized in autoclaved vermiculite	PGR-free MS (SG, RIM). MS+5 mg/L BA (SIM). MS+1 mg/L BA (SEM)	Ntui et al. 2009, 2010a, 2010b
Wild plants from Wadi Soule, Sinai, Egypt	NR. Stems, leaves and roots of 2-w-old *in vitro* seedlings used as explants	16-h PP. 80 μmol m^{-2} s^{-1}. 24±2°C. All other *in vitro* conditions NR	MS+2 or 6 mg/L 2,4-D+2 or 4 mg/L Kin; MS+0.1 mg/L BA+5 mg/L NAA (CIM)	El-Baz et al. 2010
Mature plants growing in Jaipur, India	Terminus of stems with shoot tips in RTW+0.1% Labolene for 5 min then 0.05% $HgCl_2$ for 2-3 min. Washed with SDW 4-5×	16-h PP. LI NR. 25±2°C. pH 5.8. 3% sucrose. 0.8% agar. Plants acclimatized in sterilized soil : vermiculite (3:1)	MS+0.5 mg/L BA+0.5 mg/L NAA (SIM). MS+4 mg/L IBA+0.2% AC (RIM)	Meena et al. 2010, 2014
Wild plants from river valleys in Andhra Pradesh, India	Leaves and stems in RTW for 5-10 min, 0.1% $HgCl_2$ for 1-2 min, DDW	16-h PP. 2000 lux. 25±1°C. pH 5.7. Carbon source and gelling agent NR. Plants acclimatized in garden soil : FYM (1:1)	MS+1.5 or 2 mg/L 2,4-D+1 mg/L BA (or 0.5 mg/L TDZ) (2010) or 2.5 mg/L 2,4-D+1 mg/L Zea (2014) (CIM, SIM). MS+2 mg/L IBA+1.5 mg/L NAA (RIM)	Savitha et al. 2010a, 2010b, Shasthree et al. 2014
Wild plants from Tamil Nadu coast, India	Stems immersed in 70% EtOH for 1-5 min, 0.1% $HgCl_2$ for 3 min, then washed with SDW 4×and cut into 1.0 cm long explants	8-h PP. 20-30 μmol m^{-2} s^{-1}. 25±2°C. 60±10% RH. pH 5.8. 3% sucrose. 0.8% agar. Plants acclimatized in sterilized soil : vermiculite (3:1)	MS+1 mg/L BA+0.5 mg/L IAA+0.5 mg/L 2,4-D (CIM). MS+1.5 mg/L BA+0.5 mg/L NAA (SIM). MS+3 mg/L BA+0.2% AC (RIM)	Satyavani et al. 2011a
Wild plants from Eturnagaram Tribal, India	Seeds germinated *in vitro* (protocol NR). Leaves, stems and cotyledons washed with 1% Labolene, RTW for 30 min, 0.1% $HgCl_2$ (time NR), then washed with SDDW. Explant size NR	pH 5.6. All other *in vitro* conditions NR	MS+1 mg/L 2,4-D+0.5 mg/L IAA or 2 mg/L BA+0.5 mg/L NAA (CIM, SIM)	Shasthree et al. 2012
Wild plants growing in Jaipur, India	Shoot buds and nodal segments in RTW for 15 min, Extran (detergent) for 5 min then 0.1% $HgCl_2$ for 3 min. Washed with SDW 4-5×	16-h PP. Cool fluorescent tubes. 25 μmol m^{-2} s^{-1}. 25±1°C. pH 5.8. 3% sucrose. 0.9% agar. Plants acclimatized in garden soil : organic manure (1:1)	MS+2.2 μM BA (SIM). MS+4.9 μM IBA (RIM)	Verma et al. 2013
Wild plants from Iraqi desert	Seeds in 90% EtOH for 30 s, SDW several times, 0.5% NaOCl for 5-10 min, SDW. Leaves and stems of 3-w old seedlings served as explants	16-h PP. 1000 lux. 25±2°C. pH, carbon source, gelling agent NR	PGR-free ½MS (SG). MS+2 mg/L BA+0.5 mg/L NAA (CIM)	Taha and Mutasher 2014
Source NR.	Seeds treated with 10 mg/L GA_3 for 24 h. 10-d-old seedlings (all parts) used as explants	Constant darkness (CIM) or 16-h PP (SIM). 30°C. pH 5.7. 3% sucrose. 0.8% agar. Plants acclimatized in soil	PGR-free MS (SG). MS+1 mg/L Kin+1 mg/L IAA or MS+2× MS vitamins+2 mg/L BA+0.1 mg/L NAA (CIM, SIM). MS+1 mg/L IAA (RIM)	Gharehmatrossian 2015

(Contd...)

Table 2: *(Continued)*

Cultivars and sources	Disinfection process	Culture conditions*	Optimal medium**,***	Reference
Wild plants from Koonoor river in Warangal, Telangana State, India	Similar protocol to Savitha et al. 2010a, 2010b; Shasthree et al. 2012, 2014	Similar protocol to Savitha et al. 2010a, 2010b; Shasthree et al. 2012, 2014	MS+1 mg/L 2,4-D+1.5 mg/L IBA (CIM). Callus on MS+2 mg/L NAA+1 mg/L IBA (RIM)	Ramakrishna and Shasthree 2015
Seeds from wild plants in Basar and Koonoor river valleys, Nizamabad and Warangal, Telangana State, India	Similar protocol to Savitha et al. 2010a, 2010b; Shasthree et al. 2012, 2014	Similar protocol to Savitha et al. 2010a, 2010b; Shasthree et al. 2012, 2014	MS+1 mg/L 2,4-D+1.5 mg/L BA (SEIM)	Ramakrishna and Shasthree 2016¶

2,4-D - 2,4-dichlorophenoxyacetic acid; AC - activated charcoal; BA - N^6-benzyladenine (BA is used throughout even though BAP (6-benzylamino purine) may have been used in the original, according to Teixeira da Silva (2012a); CIM - callus induction medium; DDW - double distilled water; EtOH - ethyl alcohol (ethanol); FYM - farmyard manure; $HgCl_2$ - mercuric chloride; IAA - indole-3-acetic acid; IBA - indole-3-butyric acid; Kin - kinetin (6-furfuryl aminopurine); LI - light intensity; MS - Murashige and Skoog (1962) medium; NAA - α-naphthaleneacetic acid; NaOCl - sodium hypochlorite; NR - not reported in the study; PGR - plant growth regulator; PP - photoperiod; RH - relative humidity; RIM - root induction medium; RTW - running tap water; SDW - sterilized (by autoclaving) distilled water; SDDW - sterilized (by autoclaving) double distilled water; SEM - shoot elongation medium; SEIM - somatic embryo induction medium; SG - seed germination; SIM - shoot induction medium; TDZ, thidiazuron (*N*-phenyl-*N*'- 1,2,3-thiadiazol-5-ylurea); w - week (s); Zea, zeatin. *The original light intensity reported in each study has been represented since the conversion of lux to µmol m^{-2} s^{-1} is different for different illumination (main ones represented): for fluorescent lamps, 1 µmol m^{-2} s^{-1}=80 lux; the sun, 1 µmol m^{-2} s^{-1}=55.6 lux; high voltage sodium lamp, 1 µmol m^{-2} s^{-1}=71.4 lux (Thimijan and Heins 1983). **Percentage values of solids as w/v (weight/volume) and of liquids as volume/volume (v/v). ***Even though calli was used in the original, the term callus has been used here based on the recommendation of Teixeira da Silva (2012b). ¶Claims of somatic embryogenesis without sufficient proof (cytological, histological, genetic), i.e., only photos of macromorphology

were upregulated in the shoots of seedlings of an Israeli accession in response to PEG-induced stress. Two NAC (no apical meristem, Arabidopsis transcription activation factor 1 and 2, cup-shaped cotyledon 2) transcription factors were shown to be involved in this abiotic stress response, *CcNAC1* and *CcNAC2* (Wang et al., 2014a), being influenced by the spectral light quality (Wang et al., 2014b). The ability to isolate and clone *CcNAC1* and *CcNAC2* into other plants without any drought resistance might be an effective way of exploring arid and water-stressed regions for expanded horticulture with drought-resistant crops. In fact, the ability to transmit drought-resistant signals from scion to rootstock using *C. colocynthis* and watermelon (*C. lanatus*) (Si et al., 2010a, 2010b) holds great promise for research on the drought-resistance mechanism of *C. colocynthis*. Transcriptomic analyses of the leaves of *C. colocynthis* during drought stress revealed 2545 genes that changed significantly during drought stress (Wang et al., 2014c), giving promise to the mining of this plant for drought stress-related genes.

FUTURE PERSPECTIVES

Colocynth is a rich source of functionally important bioactive compounds and therapeutics such as polyphenols, glycosides, triterpenes and cucurbitacins and its fruit has been widely used for the treatment of many diseases including diabetes, rheumatism, paronychia, ulcer and cancer (Hussain et al., 2014). However, the biotechnology of *C. colocynthis* is still underexplored. Although some tissue culture studies and genetic transformation experiments exist, biotechnological research into this plant would benefit from the use of the following techniques: *In vitro* flowering (Teixeira da Silva et al., 2014) for controlled crosses *in vitro*, use of magnetic fields (Teixeira da Silva and Dobránszki, 2015), ultrasound or sonication (Teixeira da Silva and Dobránszki, 2014), or thin cell layers (Teixeira da Silva and Dobránszki, 2013) to explore alternative pathways for growth and development *in vitro*. Given the heat-tolerant nature of *C. colocynthis* (Althawadi and Grace, 1986), and ability to grow in water stress, mining the genes of this plant would perhaps reveal genes that could be cloned into other plants to induce heat stress resistance. The ability to micropropagate and mass produce uniform plant material *in vitro*, independent of season, or through the use of bioreactors, would allow callus to be constantly harvested for silver nanoparticle production (Satyavani et al., 2011b) with multiple uses in agriculture, medicine and industry. The cryopreservation of *C. colocynthis* seeds has already provided one such possibility for the long-term preservation of germplasm (Alsadon et al., 2014). Using callus that they had induced from the leaves of *C. colocynthis* (Satyavani et al., 2011a), Satyavani et al. (2011b) produced

Table 3: 18 drought-responsive genes coding for functional and regulatory proteins that were differentially expressed in the roots of *C. colocynthis* after treatment with polyethylene glycol (modified from Si et al., 2009)

Name of gene sequence	Homologous function	Homologous plant	GenBank accession number
CC4	Heat shock protein 70	*Cucumis sativus*	FK707354
CC85	Heat shock 22 kDa protein, mitochondrial	*Glycine max*	GH626170
CC47	grpE like protein	*Arabidopsis thaliana*	GH626164
CC61	Putative pathogenesis-related protein	*Cucumis sativus*	GH626166
CC26	APC11 (anaphase promoting complex subunit 11)	*Arabidopsis thaliana*	GH626159
CC36	Putative alpha7 proteasome subunit	*Nicotiana tabacum*	GH626162
CC37	RBOHD (respiratory burst oxidase)	*Arabidopsis thaliana*	EU580727*
CC38	VIP2 (VIRE2-INTERACTING PROTEIN2)	*Arabidopsis thaliana*	GH626163
CC16	ABC transporter-like protein	*Arabidopsis thaliana*	FK707355
CC48	Synaptobrevin-related protein	*Pyrus pyrifolia*	GH626165
CC24	Toc34-1 (translocon outer envelope of chloroplast)	*Zea mays*	GH626158
CC64	Beta-amylase	*Prunus armeniaca*	GH626167
CC32	Pyruvate kinase	*Arabidopsis thaliana*	GH626161
CC75	TIP1 (TIP GROWTH DEFECTIVE 1)	*Arabidopsis thaliana*	GH626168
CC19	Leucine-rich repeat transmembrane protein kinase	*Arabidopsis thaliana*	GH626156
CC23	Protein kinase	*Fagus sylvatica*	GH626157
CC27	Hairy meristem	*Petunia* x *hybrida*	GH626160
CC76	NAC 2	*Glycine max*	GH626169

*The *Ccrboh* gene in Si et al. (2010b), encoding a respiratory burst oxidase

Fig 1. Colocynth (*Citrullus colocynthis* (L.) Schrad.) growing in a field in Faisalabad in winter (January, 2017). Despite the harsh conditions, i.e., dry, cold and arid, the plant is able to set fruit. This makes colocynth a suitable winter cash crop. All photos unpublished.

silver nanoparticles, which were able to reduce the toxicity of Human epidermoid larynx carcinoma (HEp-2) cell lines by as much as 50%. Khan et al. (2016) purified a low molecular weight serine protease with high catalytic activity that has many possible industrial applications from *C. colocynthis* seeds.

CONCLUSIONS

Citrullus colocynthis (L.) Schrad., an important fruit species with medicinal and nutritional value, would serve as a valuable crop species in arid regions (Fig. 1) such as North Africa and the Middle East. To increase production, micropropagation protocols need to be refined and to fortify salt- and drought-tolerance, genetic engineering may offer a valuable solution, especially since several genes related to drought-tolerance have already been identified. Basic studies on the biology and biotechnology of this plant are needed to fortify the application of molecular marker technology, which is fairly well developed for this plant.

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Conflicts of interest

The authors declare no conflicts of interest.

Author contributions

Both authors contributed equally to all parts of the development and revisions of this review.

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