# Chapter 5
# Water Spinach (*Ipomoea aquatica* Forsk.) Breeding

**Moumita Gangopadhyay, Anup Kumar Das, Subhendu Bandyopadhyay, and Samanwita Das**

**Abstract** *Ipomoea aquatica* Forsk. (Convolvulaceae) is a commonly grown vegetable in the Americas, Africa and especially Southeast Asia, including India. Due to the presence of numerous secondary metabolites, this plant has considerable therapeutic as well nutraceutical value and is categorized among highly prioritized but neglected leafy vegetables. Proper identification of the higher quality genotypes of *I. aquatica* will help scientists explore major genes to develop future high-quality varieties. Therefore, an integrated approach combining traditional and molecular plant breeding should be carried out to strengthen future breeding programs. Identification of traits controlling genes by extensive database searching with bioinformatics, followed by genomics and transgenic approaches, opens a new possibility to use these beneficial vegetables as potent nutraceuticals, especially in developing countries where malnutrition is a matter of concern. Application of plant cell culture technique can be an attractive field of research for this plant species. In this context micropropagation is the best choice for producing year around pilot-scale production within a short time span. In vitro plantlets can also be conserved as artificial seed to maintain elite plant lines with augmented secondary metabolites. Screening by the use of hairy root culture under photoautotrophic condition to detect contaminants and pollution can assure cultivars are safe to consume. This chapter presents an overview of the origin, distribution, botanical classification, breeding through classical and molecular approaches, tissue-culture practices like rapid micropropagation for high frequency regeneration, use of elite clones and conservation by alginate entrapment, prospects of using hairy root culture, recent developments and future scope of biotechnology and molecular biology using bio-

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informatics and transgenic approaches and their application for improvement of *I. aquatica*.

**Keywords** Biotechnology · Breeding · Cultivars · *Ipomoea* · Molecular diversity · Secondary metabolites · Vegetables

## 5.1 Introduction

*Ipomoea aquatica* Forsk. is an aquatic plant possessing long and hollow stems containing large numbers of air passages and often with rooting present at the nodes. Leaves of the plant are elliptical in shape; white or pale purple flowers are funnel- or cone-shaped; the fruit is in the form of a capsule (Anonymous 1959; Edie and Ho 1969; Gamble 1921; Payne 1956; Synder et al. 1981).

*Ipomoea aquatica* is believed to have originated in China (Umar et al. 2007; Edie and Ho 1969). It is found throughout Tropical Asia, India, Sri Lanka, Africa and Australia, as shown in Fig. 5.1 (Kirtkar and Basu 1952). The plant grows as a weed in India and the USA (Anonymous 1959; Reed 1977) while the plant is grown commercially in Southeast Asia (Candlish et al. 1987; Chen et al. 1991).

The plant contains vitamins such as A, $B_1$, $B_2$, $B_6$, $B_{12}$, C, E and K (Igwenyi et al. 2011) in addition to S-methylmethionine, a reputed treatment for diseases of the gastrointestinal tract (GIT) (Roi 1955). It also contains secondary metabolites such as flavonoids, amino acids, alkaloids, lipids, steroids, saponins, phenols, reducing sugars, tannins, β-carotene, glycosides and minerals (Bergman et al. 2001; Pandjaitan et al. 2005).

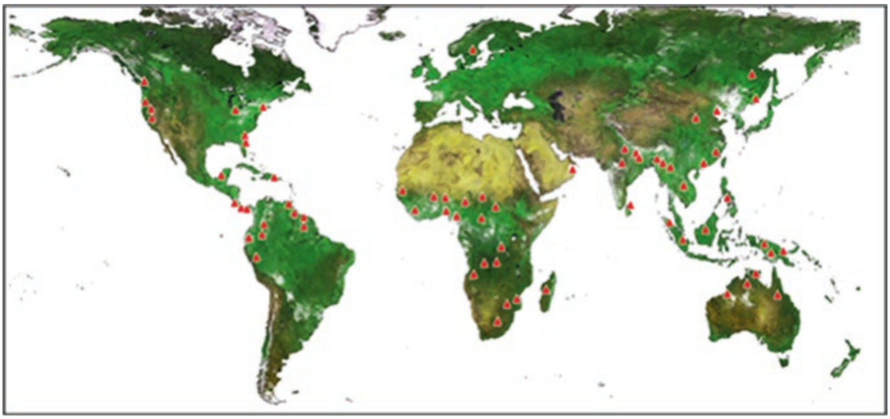


**Fig. 5.1** World distribution of *Ipomoea aquatic*. Red triangle indicates the area of occurrence. (Source: Austin 2007)

In traditional medicine, it is used to treat constipation, migraine and sleep-related disorders (Burkill 1966). It is also recommended for the treatment of liver-related disorders (Badruzzaman and Husain 1992), diabetes, mental illness and intestinal problems (Samuelsson et al. 1992). It is a treatment for nosebleeds and high blood pressure (Duke and Ayensu 1985; Perry and Metzger 1980), as an anthelmintic (Nadkarni 1954), antiepileptic and hypolipidemic agent (Dhanasekaran and Muralidaran 2010), and antimicrobial and anti-inflammatory agent (Dhanasekaran and Muralidaran 2010).

The plant is also said to inhibit prostaglandin generation (Tseng et al. 1992). The plant extract is purported to be effective against arsenic poisoning. Poultices of plant extract are also reputed to be effective against itching (Khare 2007).

The above facts illustrate that it is a nutritious comestible plant with a high potential medicinal significance, but is usually neglected due to the lack of scientific knowledge. Therefore, this book chapter provides a complete overview of water spinach taxonomic description, chemistry, cultivation and restoration preservation methodologies and socioeconomic benefits of this vegetable so that people become more aware of its attributes, include it in their diet and begin to promote its cultivation on a larger scale.

### *5.1.1 Classification*

Kingdom: Plantae
Family: Convolvulaceae
Genus: *Ipomoea* Lour.
Species: *Ipomoea aquatica* Forssk.
Synonyms:

- *Ipomoea clappertonii* R. Br.
- *Ipomoea incurve* G. Don
- *Ipomoea natans* Dinter & Süsseng.
- *Ipomoea repens* Roth
- *Ipomoea reptans* Poir.
- *Ipomoea sagittifolia* Hook. & Arn.

Local names: Swamp morning glory, water spinach, water convolvulus and swamp spinach.

### *5.1.2 Botanical Aspects*

*Ipomoea aquatica* is a sedentary plant that can grow beyond 1 year. Water spinach plants have a rooting system, which can spread in all directions and can penetrate the soil to a depth of 60–100 cm, and expand horizontally to a radius of 150 cm or more. Roots are small to medium sized having a woody or soft core. Branching of roots is minimal.

Water spinach is an annual or perennial, aquatic herb whose stem is hollow, spongy and 2–3 m long. It trails or floats on water and is glabrous, or hairy at the nodes. The stem also contains a milky sap. The surfaces of the leaves are even and arrowhead-shaped. The blades extend up to 5–15 cm and are 2–6 cm broad, whereas the petiole is about 3–14 cm in length. The leaves generally float on water. The fruits are oval-shaped and enclose 1–4 seeds. Seeds may differ in color ranging from gray, brown to black. The plants accumulate heavy metals when cultivated near polluted water, thus posing a risk of biomagnifications. The flowers are bisexual, funnel-shaped and are white to light purple in color. They are found either singly or in clusters between the petiole and stem; the different plant parts are shown in Fig. 5.2.

### 5.1.3 Habitat Description

*Ipomoea aquatica* generally grows on moist soils along stagnant streams, fresh water bodies or near wet rice fields. It occurs in both wild and cultivated forms and can be easily propagated through stem cuttings. Within few weeks of planting, it grows rapidly and produces a dense mass of foliage. Water spinach is considered to

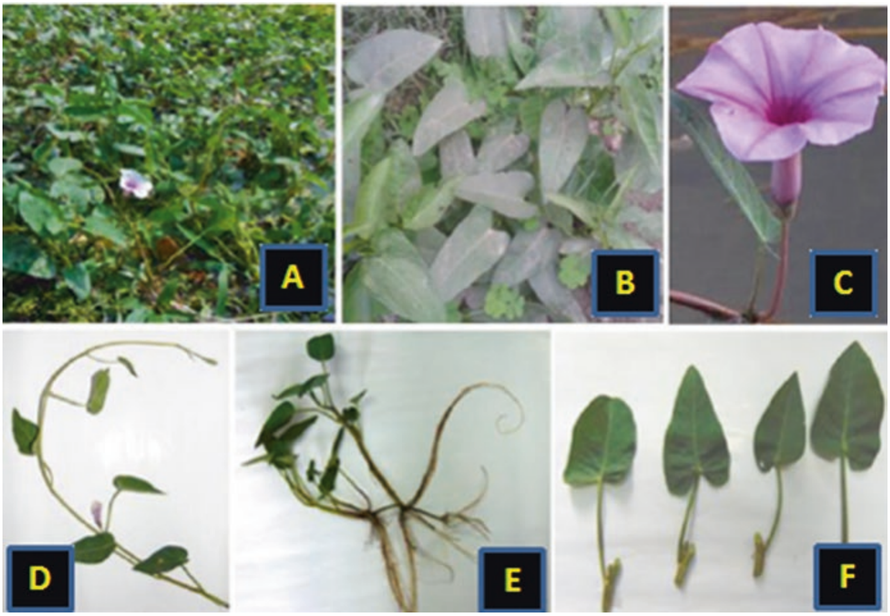



**Fig. 5.2** Different plant parts of water spinach, *Ipomoea aquatica.* The morphology of I. aquatica Forssk. (**a**) The plant floating on water, (**b**) The plant creeping on moist soil; (**c**) The funnel form flower of I. aquatica, (**d**) flowering twig with simple and alternate leaves, (**e**) rooting at nodes, (**f**) leaves arise from nodes. (Source: Dua et al. 2015; https://translational-medicine.biomedcentral.com/articles/10.1186/s12967-015-0430-3)

be among the most accepted leafy vegetable (Kala and Prakash 2004; Anonymous 1959; Candlish et al. 1987; Chen et al. 1991; Edie and Ho 1969; Payne 1956; Synder et al. 1981; Wills et al. 1984).

### *5.1.4 Origin and Distribution*

The scientific literature reveals that *Ipomoea aquatica* may have originated in China (Edie and Ho 1969; Umar et al. 2007). Its distribution is mapped and presented in Fig. 5.1. Water spinach is found throughout Tropical Asia, Africa and Australia (Kirtkar and Basu 1952). In India and the USA it grows wild as a weed (Anonymous 1959; Reed 1977) while it is cultivated commercially in Southeast Asian countries (Candlish et al. 1987; Chen et al. 1991).

### *5.1.5 Nutritional Components*

The leaves of water spinach are known to contain proteins, carotenoids, amino acids, vitamins and polyphenols (Chen and Chen 1992; Chitsa et al. 2014; Chu et al. 2000; Daniel 1989; Miean and Mohamed 2001; Ngamsaeng et al. 2004; Rao and Vijay 2002; Umar et al. 2007; Wills et al. 1984). Water spinach has higher antioxidant activity than land spinach with high phenol/flavonoid content (Mariani et al. 2019). Vitamin C, starch, dietary fiber and minerals such as sodium, potassium, calcium, iron, magnesium and zinc contents for *Ipomoea aquatica* were studied by Kala and Prakash (2004) and Wills et al. (1984). Candlish et al. (1987) studied the dietary fiber and starch content in *I. aquatica*. The tocopherol content of *I. aquatica* was also compared and analyzed by Candlish (1983). Imb and Pham (1995) detected lipids, fatty acid and triglycerides content of water spinach. Vitamin C and iron content were investigated by Duc et al. (1999). Munger (1999) compared the nutritional value of *I. aquatica* with rice, sugarcane and maize. It can be argued that investment in water spinach may be an appropriate and an effective way to supplement human nutritional deficiency in developing countries. Irrespective of human nutritional aspect, this leafy vegetable also act as a protein source for indigenous pigs in Vietnam.

## 5.2 Cultivation

*Ipomoea aquatica* is a tropical plant native to China, but widely cultivated in East Asia and the Indian subcontinent (Naskar 1990), as well as in California, Texas and the U.S. Virgin Islands (Daniel 2007). As a tropical plant, water spinach produces its highest yield under conditions of high temperature and full sunshine. Below

500 m elevation and 25 °C, plant growth is very slow. This plant is adapted in variable soil condition but organic soils with pH 5.3–6.0 have shown best results (Westphal 1993). Three types of cultivation practices are used for water spinach:

(a) Plants are grown in small lakes or ponds, floating on the water surface.
(b) Wetland cultivation is practiced on hydric soil or saturated ground where seeds or stem cuttings are planted. This cultivation is popular in Southeast Asia and some parts of Africa, but is declining because upland cultivation is more productive. Planting is done by cutting or transplanting from nursery beds. A water level of 15–20 cm is suitable for cultivation and plants are cut 5–10 cm above ground level. The major problem of this form of cultivation is serious losses due to diseases, pests or weeds.
(c) Plants are cultivated on dry soil like normal land plants. However, here special attention should be taken by providing additional fertilizers (mostly N fertilizer) and excess irrigation which is very expensive.
(d) First harvesting is generally done 1 month after planting and thereafter harvesting of leaves can be done at weekly intervals; additional top dressing is recommended after each cutting. Apical dominance is broken by removal of the apical part of the shoot; due to profuse lateral branching of the plant, it forms a bushy architecture (Kaur et al. 2016). Once the plants are established in a particular area, cuttings of can be used as secondary source for cultivation (Edie and Ho 1969).

White rust (*Albugo ipomoeae-panduratae)* is the most common disease of water spinach. Damping off (*Pythium* sp.), root-knot nematodes (*Meloidogyne* spp.) are also reported. Caterpillars like *Spodoptera litura* and *Diacrisia strigatula,* and aphids cause serious threats to this plant.

## 5.3 Traditional Breeding

The presence of high variability in chromosome numbers and structural ecogeographic variations are observed factors that can be exploited in the implementation of a water spinach breeding program. Although this leafy vegetable grows well under tropical condition, due to its high nutritive value, strategies are needed to develop it for cultivation as well in temperate climatic zones. Up to now, cultivation in temperate regions is only possible under controlled condition using aeroponics or hydroponics (Hoang and Böhme 2001; Pinker et al. 2004) which are expensive and require skilled technicians. Westphal (1993) reported the selection of seeds from Southeast Asia landraces with low quality, low germination rate and high variability, but not for cultivation in temperate regions. Therefore, a holistic approach for developing new varieties by searching and incorporating genes from locally-adapted high elevation varieties into commercial varieties is of utmost importance for water spinach breeders. Researching genetic variability of locally-adapted biotypes began worldwide in the 1980s and 1990s (Synder et al. 1981). Rich genetic variability was observed among biotypes in Southeast Asia, the Americas and also in Africa, by several earlier researchers. Westphal (1993) reported two main wild biotypes in Southeast Asia on the basis of color variation. One is called Red with green/purple stems, dark green leaves with sometimes purple petioles or veins; another called White characterized by green/white stems, green leaves and green/white petioles. In the USA (Florida), two floating biotypes grown in fresh water or ponds were identified (Van and Madeira 1998) by color differentiation. One is a Red type with red flowers and another White type with white flowers. An Upland cv. was also identified and cultivated commercially in Florida. All of these morphological variations followed by cytogenetic differentiation can be use in breeding programs, especially using genes from nutritionally-rich floating cultivars to upland cultivars to develop nutritionally-enhanced cultivars to grow on a large scale under organized field cultivation.

As water spinach is a highly nutritious and low-cost leafy vegetable, breeding of new spinach varieties with high nutritional value as well as low accumulation of health hazardous compounds at their edible part is the main challenge for genetic improvement programs of this plant species. The work of Chauhan et al. (2017) has shown that various quantitative characters specially related to leaf characteristics like foliage color, yield, leaf length, leaf width, dry matter, chlorophyll content exhibited a strong genotype dependent variation in terms of phenotypic (PCV) and genotypic coefficient of variance (GCV) for selection by separating out environmental influence from total variability. This may be used by plant breeders for the improvement of leaf characteristics (since leaf is the economic part) through selection. This study also revealed that the degree of association in terms of heritability coupled with high genetic advances for genotype-specific leaf traits is due to additive gene effects that may successfully be used in breeding programs towards the improvement of green foliage yield in water spinach. Using $D^2$ analysis of 25 water spinach genotypes for foliage characteristics, Chauhan and Singh (2018) clustered genotypes into 5 groups suggesting that crossing between genotypes of distant clusters resulted in better recombinants with wider spectrum genetic variability is the promising choice for breeding program for better heterosis in successive generations.

In Africa, there have also been observed different ecotypes with morphological and cytological differences (Ogunwenmo and Oyelana 2009). Two perennial savanna-restricted ecotypes exhibit slightly larger cotyledons and germinated within 2 weeks, while a sporadic annual forest type with smaller cotyledons usually germinates in soil after 6–8 weeks. In normal cases the chromosome number of this species is $2n = 30$ (Fedorov 1969), but perennial and annual biotypes showed chromosome number (2n) 30 and 28, respectively; the chromosome size ranged from 1–2.5 μm and 1.5–3 μm, respectively.

In *Ipomoea aquatic*, two cytotypes (https://shodhganga.inflibnet.ac.in/bitstream/10603/15812/7/07_chapter%202) with different morphological traits exist. Broad-leaved aquatic Variant I bears large flowers with a bitter tasting leaves, while a narrow-leaved terrestrial Variant II has small flowers and a sweet taste. Both variants possess significantly different karyotypes and idiograms in their somatic chromosome. Variant I showed 18 metacentric, 12 submetacentric and 2 pairs of satellite chromosome ($m_{18} + sm_{12} + st_o + t_o = 2n = 30$) whereas Variant II possessed 16 metacentric, 14 submetacentric with 4 pairs of satellite chromosome ($m_{16} + sm_{14} + st_o + t_o = 2n = 30$).

Singh et al. (2016) analyzed 10 genotypes of water spinach to evaluate nutritional and anti-oxidant properties in terms for protein, sugar, chlorophyll, carotenoid, phenol, proline, flavonoids and ascorbic acid contents. This work clearly demonstrated that biochemical traits related to nutritional and anti-oxidant potentialities are largely monitored by additive genes and less influenced by the environment. The high heritability along with high genetic advances indicate genotypic-dependent expression which can be utilized for genetic improvement program through convention plant breeding.

From an integrated metabolomics approach a correlation with phenotypic and metabolic, as well antioxidants level, were observed among different biotypes in terms of nutritional quality (Lawal et al. 2017). Proton nuclear magnetic resonance ($^1$H NMR) spectroscopy combined with multivariate data analysis revealed that Special Pointed Leaf (K-11) cv. had high phenolic content and was most active due to the presence of epicatechin, 4,5-dicaffeoylquinic, protocatechuic acid and rutin as compared to two other cultivars namely Broad Leaf (K-25) and Bamboo Leaf (K-88). To the contrary, K-88 had higher sugars and some amino acids while K-25 possessed a higher content of organic acids. Water spinach breeders need to develop nutritionally enhanced cultivars combining all desirable traits by gene pyramiding, aimed at enhancing trait performance (nutritional quality) using hybridization-based breeding programs.

Today's consumers have specific preferences with regard to the quality of the product, e.g. plant structure, nutritional quality, plantation schedule. East-West Seed Company in Thailand has developed a few popular cultivars for commercial uses. A few examples are for single harvest such as cv. Yangtze with broad leaves and grown year-round, in contrast to cv. Chinwin, suitable for multiple harvesting, cv. Salween with small bamboo-like leaves and suitable for the hot rainy season and cv. Liao with bamboo-like leaves for the dry season (http://www.eastwestseed.com).

Concerning the gene pool resources with morpho-cyto and chemical polymorphism conservation of indigenous resources, or different ecotypes, these are important criteria for strategic implementation in future breeding programs. In this context in situ or field conservation is an essential approach for assembly and preservation of various indigenous land races with genetic diversity. AVRDC (World Vegetable Centre in Taiwan) has already begun this venture, not only for germplasm conservation but also to support research and cultivation (http://203.64.245.173/avgris/).

### *5.3.1 Breeding for Pollution Safe Cultivars*

Water spinach is a valuable, low-cost, leafy vegetable useful as a powerful model plant for phytoremediation by accumulating heavy metals from contaminated water (Li et al. 2016; Zhang et al. 2014) or soil (Ng et al. 2016a, b). Although from the nutraceutical point of view, higher accumulation of heavy toxic metals (Rai and Sinha 2001) becomes hazardous to human health via bioaccumulation and biomagnifications along the food chain. Therefore, developing pollution safe cultivars (PSCs) to ensure food safety with higher nutritional qualities and adaptabilities is the biggest challenge of plant breeders. PSCs are safe for consumption due to the lower accumulation of specific pollutants in their edible parts which is a practical method of minimizing the concentrations of heavy metals in crops (Grant et al. 2008). The PSC strategy is based on the fact that genotypic variation of edible parts accumulating pollutants is large enough at the cultivar level.

Cadmium (Cd) is one of the most toxic and non-degradable health hazardous heavy metals that accumulate in soil and water used in agriculture and transmit through food chain (Lane et al. 2015). Cultivars of water spinach vary widely in Cd concentration in the cellular vacuoles of the edible part, mainly the shoot portion (Xin et al. 2010). Xiao et al. (2015) and Huang et al. (2016) reported that Cd detoxification, i.e. inhibition in translocation from root to shoot in water spinach, is highly genotype dependent. Henceforth breeding strategies should focus on screening and development of cultivars with lower accumulation of Cd in edible part to assure safety for human consumption. After investigating 38 water spinach genotypes for low Cd accumulation in edible part, Tang et al. (2018) selected 4 genotypes, i.e. *JXDY*, *GZQL*, *XGDB*, and *B888* as pollution safe cultivars without health risk. A Low Shoot Cd cv. (QLQ 56) and a High Shoot Cd cv. (Taiwan 308) of water spinach were documented by Wang et al. (2009) and Xin et al. (2010), validating the molecular mechanism of the differentially-expressed genotypes for cadmium accumulation. Cd-induced gene expression differences of the two water spinach cultivars (QLQ 56, Taiwan 308) have been investigated using suppression subtractive hybridization (SSH) (Huang et al. 2009a, b). Almost 13.3% of the cultivars were found to be vulnerable to Cd contamination in soils (non-Cd-PSC), including cvs. Taiwan 308, Xianggangdaye, Sannongbaigeng, and Jieyangbaigeng, while 6 cvs., Daxingbaigu, Huifengqing, Qiangkunbaigu, Qiangkunqinggu, Shenniuliuye and Xingtianqinggu, were treated as typical Cd-PSCs (Grant et al. 2008). The work of Lian et al. (2010) revealed that the discrepancy in Cd accumulation among cultivars is genotype-dependent rather than from edaphic factors. It is hypothesized that genotyping dependent Cd accumulation of water spinach is mainly controlled by root processing mechanisms (Xin et al. 2013a). Moreover, Xin et al. (2013b) clearly indicated that this is due to the presence of cultivar specific thicker phloem and outer cortex cell walls in the roots which restricts root to shoot translocation. Although there are some studies (Milner et al. 2014; Papoyan and Kochian 2004) focusing on the molecular mechanisms of root processing regarding Cd hyperaccumulation in several species, but the molecular mechanisms of cultivar dependent difference in Cd accumulation of crops are still not adequately studied (Yamaguchi et al. 2010). Huang et al. (2018) reported the role of *metallothioneins* for differential cultivar-dependent Cd accumulation and the mechanism of detoxification. The work of Li et al. (2015) has revealed that unlike Cd, other heavy metals such as Pb and Zn also share similar transport uptake mechanism.

Just like in Cd, differential abilities of lead (Pb) accumulation by different cultivars were observed (Lian et al. 2010). Genotype dependence Pb accumulation at the cultivar level (Xin et al. 2010, 2011) as well root-specific uptake has been reported (Huang et al. 2009a, b). It is assumed that high variability among the cultivars led to the explanation of intraspecific species (Folorunso et al. 2013) being involved in the mechanism of evolution and variability. However, relevant information regarding hereditary patterns of heavy metal accumulation is quite limited.

## 5.4 Molecular Diversity

The genus *Ipomoea* belongs to the family Convolvulaceae, consisting of 650 species distributed throughout different parts of the world and comprising shrubs, herbs and small trees, with the majority of species being twining, climbing plants (Mabberley 1997; Miller et al. 2004). Austin (2007) found a close alignment of *Ipomoea aquatica* with other species like *I. cairica, I. ochracea* and *I. obscura* in section *Leiocalyx* (Van Oostroom 1940) due to the polyphyletic nature of section *Erpipomoea*. Ogunwenmo (2003) reported a study based on morphometric and qualitative characters of mature cotyledons in 18 taxa of *Ipomoea*; they identified these species at seedling stages with different morphological traits. In another study, Das and Mukherjee (1997) investigated 12 species of *Ipomoea* and traced their homology and linkage through isozyme markers such as esterase and peroxidase. They performed a comparison between isozyme data and morphological data and successfully determined their interrelationships.

*Ipomoea aquatica* is an important member of this genus, often consumed as a vegetable. It has also several medicinal properties due to the presence of vitamins, minerals, flavonoids, alkaloids and many secondary metabolites (Malakar and NathChoudhury 2015). Therefore, genetic diversity analysis in different populations of *I. aquatica* it is very important to assess their nutritional as well as medicinal potential for proper utilization.

Genetic diversity analysis with morphological and molecular markers has not been done in great detail for *Ipomoea aquatic*; there are only few reports available for the species. Van and Madeira (1998) conducted a study on three biotypes of *I. aquatic* from Florida using RAPD markers and differentiated all three biotypes, although no clear cut distinction was made for wild types and cultivated biotypes (Fig. 5.3). ITS region and waxy gene sequence based on phylogenetic analysis in *Ipomoea* genus revealed that *I. aquatica* and *I. diamantinensis* are a sister group to *I. quamoclit*; *I. Aquatic* has also been found to be placed consistently in section *Erpipomoea* and is native to the Old-World tropics (Miller et al. 1999). Pollen and flower morphology-based analysis of some *Ipomoea* species in Nigeria classified all the species successfully and two important traits emerged for classification (Jayeola and Oladunjoye 2012).

In another interspecies study conducted by Das (2011), based on morphological and RAPD markers within 12 species of *Ipomoea* using 12 different sets of RAPD primers among which only 4 primers gave amplification for all the examined accessions. The data obtained with the primer OPA 1(5'CCGGCCCTTC3') is shown here in Fig. 5.4 with 11 different lanes corresponding to different *Ipomoea* species

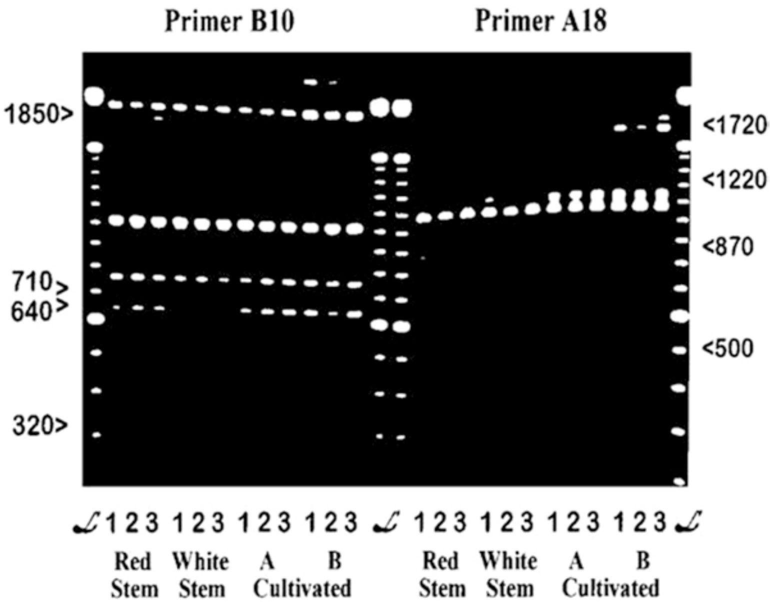
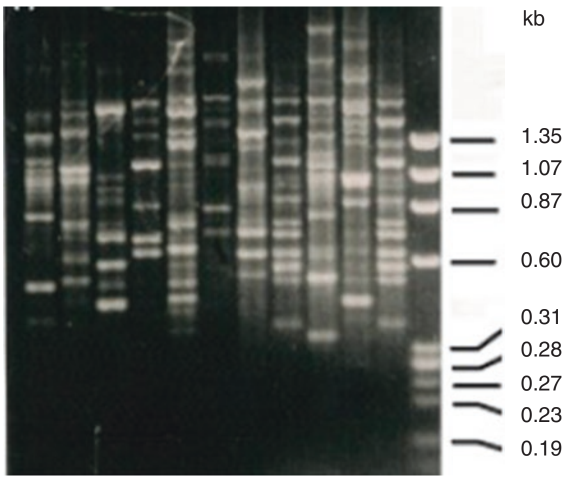



**Fig. 5.3** A study on three biotypes of *Ipomoea aquatic* of Florida using RAPD. Lane 6 – *Ipomoea aquatica* RAPD profile. All the other lanes correspond to different *Ipomoea* species. (Source: Van and Madeira (1998))

**Fig. 5.4** RAPD fragment profile of different *Ipomoea* species with OPA 01 primer. Lane 1-11, 1. *I. quamoclit*, 2. *I. hederifolia*, 3.*I. pes-caprae*, 4. *I. fistulosa*, 5. I. *aquatica*, 6. *I. sepiria*, 7.*I.nil*, 8. *I. obscura*, 9. *I. chryseides*, 10.*I. pes-tigridis*, 11.*I. triloba*, M-DNA marker**.** (Source: Das (2011))



including *I. aquatica*. Moreover, the species *I. hispida* produced least number of bands. It has also been found that the maximum number of amplified fragments were obtained for *I. aquatica* and a sharp congruence was obtained between morphological and molecular data. Nahar and Alam (2016) in their study Anthelmintic based on karyotyping and RAPD markers estimated the effect of industrial effluents on the chromosomes of different samples of *I. aquatica*. They opined that industrial wastes contributed to significant DNA damage in the species. Chauhan et al. (2017) collected 25 genotypes of *I. aquatica* plants from different sites in the district of Chattisgarh, India and studied 15 parameters related to heritability and genetic advance to select a better genotype. Among the 15 parameters, researchers found that traits like leaf length, leaf width and green foliage yield could be successfully for future improvement of the green foliage yield in water spinach.

Here a short account of molecular diversity reports has been provided for related *Ipomoea* species, *I. batata* L. (Sweet potato) which is a species from the same genus and is considered as least risky vegetables to grow due to its rapid growth rate. It is also highly nutritive due to high starch level and elevated content of vitamins and minerals (Moulin et al. 2012). In one study, estimation of genetic variation of different germplasms collected from the local market of Rio de Janeiro, Brazil has been conducted using RAPD and ISSR markers. The results of this study showed that traditional farmers maintain sweet potato genotypes with good genetic diversity (Moulin et al. 2012). Another study reported the genetic diversity analysis between 18 different cultivars each from both South Africa and Papua New Guinea. Using RAPD markers, the study demonstrated the low genetic variation in Papua New Guinea cultivars (Zhang et al. 1998). Molecular diversity report for another species named *Ipomoea triloba*, an annual plant and a close relative to *I. aquatica* (used as a vegetable in West Africa and several other countries) have been investigated by Rane and Patel (2015) using RAPD. The study clearly showed similarity between different species of *Ipomoea* by constructing a dendrogram with UPGMA method based on RAPD data. Molecular evaluation of another species *Ipomoea lacunose* L. generally a weedy species and a close wild relative of *I. batata*, has been done with 14 ISSR primers and their evolutionary diversification was assessed in mid-south of USA (Burgos et al. 2011). Besides, a study has been done using RAPD markers for generating DNA fingerprints of 12 *Ipomoea* species to the morphologically classified sections *Pharbitis, Quamoclit* and *Batatas*. The greatest genetic distance found to be between section *Batatas* and the two ornamental sections (*Pharbitis* and *Quamoclit*). The section *Quamoclit* showed a rather high heterogeneity of the comprised species (Ardelean et al. 2004).

In view of the food value of the species, more molecular marker-based inter-population studies are required to properly distinguish genotypes with higher food value. Exact identification of the higher quality genotypes will also help scientists to explore major nutrition responsive genes to develop high-quality varieties in future. Therefore, association analysis for morphological and nutraceutical traits in *Ipomoea aquatica* should be done to strengthen future breeding programs.

## 5.5 Water Spinach Tissue Culture

### *5.5.1 Problems in Conventional Agriculture*

Water spinach, being a highly nutritive and low-cost plant with the presence of most of the important essential amino acids, has attracted the attention of researchers for use as an ideal dietary protein and food supplement in place of soybean or eggs (Faruq et al. 2002; Gupta et al. 2005; Hongfei et al. 2011; Kala and Prakash 2004; Ogle et al. 2001) especially for the underprivileged. This neglected vegetable reproduces by sexual and asexual means. Asexual reproduction through vegetative fragmentation is slow due to the dependency on some carrier for their separation of a propagule from the main body and its migration to a different location for establishment (Patnaik 1976). The major problems in sexual reproduction in water are poor seed germination rates and slow initial seedling development (Edie and Ho 1969; Palada and Crossman 1999). Conventional cultivation seems unpromising due to problems of seasonal availability and the endemic nature of cultivars (South Asia, India, South China). In this context the in vitro micropropagation technique is well suited to meet ever growing market demand to produce year around pilot-scale production within a short time span for commercial exploitation.

### *5.5.2 Application of Plant Tissue Culture*

Carotenoids and other antioxidants are present in *Ipomoea aquatic*. Prasad et al. (2005) isolated and characterized a compound 7-o-β-Dglucopyranosyl-dihydroquercitin-3-o-α-D-gluco-pyrannoside having potential antioxidant properties. Prasad et al. (2006) also reported callus-mediated tissue culture with high antioxidant potential. Kiradmanee et al. (2006) and Cha-um et al. (2007) utilized in vitro culture systems as a means to study how the various environmental conditions of photoautotrophic cultivation affect the growth of water spinach under temperature and salt stress, so as to select stress-tolerant clones. Tissue-culture raised transgenic plants developed through nodal co-cultivation with *Agrobacterium tumefaciens* bearing a gene of interest, was reported by Masanori et al. (1997). Normally salinity adversely affects germination process as well normal growth development by hampering physiological processes in plants. However, researchers using in vitro system as testing model with *Ipomea aquatic* Forssk. stated that salt tolerance of water spinach increased as the response towards increasing salinity (Ibrahim et al. 2019) which impart possibility of growing this plant in saline prone ecosystem. Plant regeneration through in vitro tissue culture remains necessary for rapid multiplication, in vitro conservation as well genetic manipulation and source for the isolation of bioactive compounds. Therefore, development of specific protocols especially for the identification of optimized culture media recipe for tissue culture establishment is very important. Stephen and Bopaiah (2014) demonstrated formulation of an ideal culture medium for the in vitro propagation of *Ipomoea palmata* Forssk. (Synonym: *Ipomoea cairica.* L. Sweet).

### 5.5.3 Photoautotrophic Hairy Root Culture

Due to the potential of active and rapid propagation, and a high metabolic rate induced by *Agrobacterium rhizogenes*, hairy root culture can be used as an effective tool to develop high yielding cell lines. Several researchers established hairy root culture for the production of antioxidative enzymes like peroxidase (Taya et al. 1989) and superoxide dismutase (Kino-oka et al. 1991). Several researchers reported that higher illumination elicits the accumulation of more chlorophyll and develops greener (Fig. 5.5) transformed root clones (Masahiro et al. 1996; Nagatome et al. 2000; Taya et al. 1994). Hirofumi et al. (2000) reported the development of a phototropic cell line with augmented chlorophyll and activities of 5-ribulose-biphosphate carboxylase content. Kiuo-oka et al. (1996) presented a kinetic model regarding hairy root growth and chlorophyll formation in a photoautotrophic condition under continuous illumination. Ninomiya et al. (2001) investigated differential elongating behavior in photoautotrophic hairy roots and reported changes in chlorophyll content under a photoautotrophic condition (Ninomiya et al. 2002). From his work on green hairy root of pak bung (water spinach), a correlation between growth pattern and oxygen uptake (Ninomiya et al. 2003) was established.

Currently, phytoremediation has become an important sustainable technique for the removal of environmental pollutants (Agostini et al. 2013). Since hairy roots have become a tool for screening the accumulation potentialities of pollutants in different cultivars to tolerate or accumulate, pak bung (*Ipomea aquatica*) hairy roots were used as a model system for assessing pollutant absorption capacity**.** Since herbicides are potent environmental pollutants, green pak bung hairy roots showed

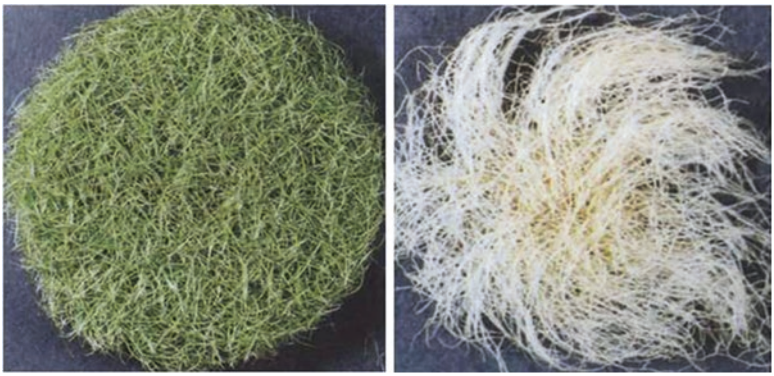


**Fig. 5.5** Photoautotrophic and heterotrophic hairy roots of *Ipomea aquatica*. (**a**) Green hairy root under illumination, (**b**) Hairy roots under dark condition. (Source: Taya et al. 1994)

differential herbicidal response in comparison to heterotrophic hairy roots when grown under herbicidal stimuli using image analysis (Ninomiya et al. 2002, 2003).

### 5.5.4 In Vitro Conservation Through Synthetic Seed

A major downside to commercialization of conventional tissue culture is the need for continuous subculturing, which often produces undesirable results and the plant material is not easily exchangeable (West et al. 2006). Artificial seed or synthetic seed technology offers an efficient technique for exchange of plant germplasm, short- or long-term storage, and direct transfer of in vitro material to ex vitro conditions (Ara et al. 2000; Germana et al. 2011; Rai et al. 2009; Standardi and Piccioni 1998). By virtue of this method, artificially encapsulated micropropagules can be used as seeds and directly planted in the nursery and grown into a plant under in vitro or in vivo conditions. Nodes with axillary buds from *Ipomoea aquatica* have been successfully encapsulated and were well established when planted in the field (Tang et al. 1994). As this plant possesses high nutritive values, the alginate entrapment process can also be used as a potential tool for enhancing plant secondary metabolites with health benefits like other plants where immobilized cells showed a higher accumulation of secondary metabolites (Hall et al. 1989; Hussain et al. 2012). Table 5.1 provides a list of plants showing high levels of secondary metabolite accumulations using alginate entrapment.

## 5.6 Transgenic Approaches for Phytoremediation Studies

Phytoremediation is the removal of toxic compounds from polluted soil or water bodies by plants. Environmental pollution is a present-day threat to human lives in ever-greater proportion. In this context identification of plants with phytoremedial potential is of high concern and scientists are continuing work on it. *Ipomoea aquatica* is such a plant with immense potential as a metal chelator, which has already been proved in several scientific reports.

In view of the phytoremedial qualities of water spinach, and its use as a vegetable, gene transfer studies are very important as they will help in the development of better quality genotypes with higher food qualities or better pollutant removal qualities. In this context it is important to note that very few genetic engineering studies in *Ipomoea aquatica* have been done to date. A genetic transformation protocol was standardized by Khamwan et al. (2003), using cut cotyledons to infect with *Agrobacterium* harboring the *GUS* gene. The resulting *I. aquatica* plants showed stable *GUS* expression, indicating a successful transformation system. Sakulkoo et al. (2005) developed transgenic *I. aquatica* plants capable of hyperassimilation of sulfate and showing tolerance to toxic levels of sulfide and cadmium. This study demonstrated introduction of the *Arabidopsis* adenosine *phosphate sulfate*

**Table 5.1** Some plants producing various secondary metabolites using alginate entrapment

| Common and scientific names of plants | Secondary metabolites | References |
|---|---|---|
| Madagascar periwinkle (*Catharanthus roseus*) | Ajmalicine | Brodelius et al. (1979) |
| Pepper (*Capsicum annuum*) | Capsaicin | Johnson et al. (1991) |
| Opium poppy (*Papaver somniferum)* | Codeine | Furuya et al. (1984) |
| Foxglove (*Digitalis lanata*) | Digitoxin | Alfermann and Reinhard (1980) |
| Velvet bean (*Muczma pruriens*) | L-DOPA | Wichers et al. (1983) |
| Egyptian henbane (*Hyoscyamus muticus*) | Solavetivone | Ramakrishna et al. (1993) |
| Kalmegh (*Andrographis paniculata*) | Andrographolide | Chauhan et al. (2019) |
| Vinca (*Catharantus roseus*) | Ajmalicine | Akimoto et al. (1999) and Lee-Parsons and Shuler (2005) |
| Vinca (*Catharantus roseus*) | Indole alkaloid | Zhao et al. (2001) |
| Datura (*Datura innoxia*) | Tropane alkaloids | Gontier et al. (1994) |
| California poppy (*Eschscholzia californica*) | Benzophenanthridine alkaloid | Villegas et al. (2000) |
| Buckthorn (*Frangula alnus*) | Anthraquinones | Sajc et al. (1995) |
| Egyptian henbane (*Hyoscyamus muticus*) | Sesquiterpenes | Curtis et al. (1995) |
| Flax (*Linum usitatissimum*) | Neolignan | Attoumbré et al. (2006) |
| Tobacco (*Nicotiana tabacum*) | Scopolin | Gilleta et al. (2000) |
| Tobacco (*Nicotiana tabacum*) | Phenolics | Shibasaki-Kitakawa et al. (2001) |
| Parthenocissus tricuspidata | methyl(2R,3S)-2- benzamido-methyl-3- hydroxybutanoate | Shimoda et al. (2009) |

*transferase* gene of a sulfur assimilation pathway that strengthened the sulfur assimilation capabilities of the plant. Meerak et al. (2006) reported the development of transgenic *I. aquatica* plants coexpressing the *Arabidopsisserine acyltransferase* gene and a rice cysteine synthase gene which conferred them higher sulfur assimilation qualities. Furthermore, Moontongchoon et al. (2008), reported that two transgenic lines of *I. aquatica* plants coexpressing *Arabidopsis* serine acyltransferase gene and rice cysteine synthase gene effectively mitigated detrimental effects of cadmium toxicity by efficiently developing and storing sulfur compounds.

*Ipomoea aquatica* is known as an accumulator of different environmental pollutants like heavy metals, sulfates and phosphates (Ng et al. 2016a, b; Rai and Sinha 2001). Hence, for better exploration of the species in relation to phytoremediation, two *I. aquatica* cultivars have been compared at the transcriptome level to understand the molecular mechanisms underlying the property of cadmium accumulation (Huang et al. 2016). The Low Shoot Cd (QLQ) cv. showed higher expression for cell wall biosynthesis genes, such as *GAUT* and *laccase*, and three Cd efflux genes (*Nramp5*, *MATE9*, *YSL7*). The High Shoot Cd cv. (T308) highly expressed sulfur and glutathione metabolism pathway genes like sulfate transporter and cysteine synthase. Exploration of metallothioneins (MTs) in a Low Shoot Cd (QLQ) cv. and a High Shoot Cd (T308) cv. has been done by Huang et al. (2016). Results showed that three *IaMT* genes, *IaMT1, IaMT2* and *IaMT3*, have been variably expressed in response to Cd stress in different cultivars. All three genes have been cloned and characterized in bacterial system where their roles have been properly elucidated. *IaMT1* was found to be unresponsive towards Cd stress whereas *IaMT2* and *IaMT3* increased Cd accumulation in *Escherichia coli*. Chuang Shen et al. (2017) demonstrated the role of miRNAs in cadmium accumulation and translocation in a Low Shoot Cd (QLQ) cv. and a High Shoot Cd (T308) cv. They reported that five different miRNAs have exclusively been regulated in the QLQ cultivar among them miRNA395 was shown to be upregulated, hypothesized to enhance the Cd retention and detoxification. Apart from miRNA395, several others, miRNA5139, miRNA1511 and miRNA8155, showed altered expression during Cd stress and were found to be involved in attenuation of Cd translocation into the shoot of QLQ. In the T308 cultivar, complex responses of miRNAs were revealed; miRNA397 regulation was found to be related to Cd influx, whereas miRNA3627 was involved in the efflux of Cd. These studies provided a new standard for molecular-assisted breeding of Low Cd cultivars for leafy vegetables.

In an updated and very recent review by Prasad (2019), phytoremedial prospects of high biomass producing aquatic plants have been discussed including transgenic approaches. It has been suggested that industrial-waste water which contains several toxic metal ions can be made free from these toxic elements with the help of aquatic plants like *I. aquatica*. Molecular biological studies can be performed for overexpression of genes for accumulation and detoxification of toxic metals and metalloids in these plants for phytoremediation purposes. In this respect, several important genes (like metallothionins, phytochelatins and several other uptake and transport related genes) can be used for making transgenic *I. aquatica* plants so that these modified plants can be used for phytoremediation purposes in future.

## 5.7 Bioinformatics

Bioinformatics is the study of biological samples using computer science and information technology. In the current genomic era, sequence information obtained from different scientific projects related to genome sequencing has generated huge quantities of data that need proper handling and interpretation. In this context bioinformatics databases and tools provide wide-ranging opportunities to analyze and discover new biological insights. In crop development, bioinformatics has played a very significant role. The genomes of staple crops like rice, wheat and maize have all been sequenced, as well as important vegetables and legumes like tomato (Sato et al. 2012), potato (Xu et al. 2011), soybean (Schmutz et al. 2010) and chickpea (Varshney et al. 2013). Sequencing of these genomes has provided vast information that will provide important information regarding future improvement of these crop plants, which in turn will benefit humankind.

The full genome of *Ipomoea aquatic* has not yet been sequenced; a literature search revealed one in silico study of antibacterial and antioxidant potential of the plant and another such analysis of compounds blocking bacterial life cycle receptors. Gas chromatography based-MS analysis demonstrated the presence of five major compounds subjected to in silico analysis with bacterial receptors such as LuxS (1JVI), FtsZ (1S1J), FtsZ (3VOB) and LsrB (1TM2) from *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhii*, respectively, by using autodock 4.2 and Cygwin. Results from the in silico study supported the fact that compounds from *I. aquatica* exhibited antibacterial properties (Chandra and Shamli 2015).

The nucleotide database of National Centre for Biotechnology Information (NCBI) contains 219 entries for *Ipomoea aquatica* (Table 5.2). Among them, several full-length genes are present (MG471389, *Ipomoea aquatic* metallothionein MT3 (MT3) mRNA;MG471388, *Ipomoea aquatic* metallothionein MT2 (MT2) mRNA; MG471387, *Ipomoea aquatic* metallothionein MT1 (MT1), mRNA), several partial gene sequences are also available (MH792116.1, *Ipomoea aquatica* voucher DMB 8 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence). Given the importance of the species the information available in the database was found to be inadequate; hence, there is ample scope for more work in this field.

## 5.8 Conclusions and Prospects

*Ipomoea aquatica* is a low cost vegetable increasingly gaining attention around the world because of its high nutritional potential. Scientific findings on this plant reveal a treasure trove of several bioactive compounds with nutraceutical as well pharmaceutical importance. Phytochemical screening of the plant as well as investigation on its hyperaccumulation qualities has been done in great detail, but molecular data for the plant are available in a much smaller amount. There is a broad scope of work to be done regarding molecular-marker based identification of different genotypes, biotypes and cultivars through which we can pinpoint high value genotypes. An organized and targeted specific breeding program or gene pyramiding effort is needed, as well as marker-assisted selection using locally-adapted cultivars

**Table 5.2** Different genes expressing various proteins in different accessions *of Ipomoea aquatic*

| Accession No. | Gene/Protein name | Features | Source |
|---|---|---|---|
| GU135247.1 | Ribulose1,5-bisphophate carboxylase/oxygenase large subunit (rbcL); partial sequence | 567 bp linear DNA | Abbott JR et al. Submitted (27-OCT-2009) Florida Museum of Natural History, University of Florida, P.O. Box 117800, Gainesville, FL 32611-7800, USA |
| GU135418.2 | Psba gene; partial sequence | 468 bp linear DNA | |
| GU135084.1 | matK gene; partial sequence | 827 bp linear DNA | |
| MK309395.1 | Ribulose1,5-bisphophate carboxylase/oxygenase large subunit (rbcL); partial sequence | 567 bp linear DNA | Sasikumar, K. and Anuradha, C. Submitted (18-DEC-2018) Department of Botany, Periyar Evr College, Khajamalai, Thiruchirapalli, Tamil Nadu 620023, India |
| MK347242.1 | matK gene; partial sequence | 655 bp linear DNA | |
| MH189790.1 | Ribulose1,5-bisphophate carboxylase/oxygenase large subunit (rbcL); partial sequence | 545 bp linear DNA | Zhang W and Zhao H Submitted (10-APR-2018) Marine College, Shandong University (Weihai), 180 Wenhua Xilu, Weihai, Shandong 264209, China |
| MN153541.1 | SKK-004 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence | 534 bp linear DNA | Kadam S Submitted (09-JUL-2019) Department of Biochemistry, Shivaji University, Shivaji university, Kolhapur, Maharashtra 416004, India |
| MH796546.1 | *Ipomoea aquatica* voucher DMB 8 ribulose 1,5-bisphosphate carboxylase/ oxygenase large subunit (rbcL) gene, chloroplast partial sequence | 1249 bp linear DNA | Ranathunga APDT et al. Submitted (24-AUG-2018) Department of Molecular Biology, University of Peradeniya, Peradeniya, Kandy, Central 20000, Sri Lanka |
| MH796544.1 | *Ipomoea aquatica* voucher DMB 6 ribulose 1,5-bisphosphate carboxylase/ oxygenase large subunit (rbcL) gene, chloroplast partial sequence | 1249 bp linear DNA | |
| MG471389.1 | *Ipomoea aquatica* metallothionein MT3 (MT3) mRNA, complete sequence | 518 bp linear mRNA | Huang Y-Y Submitted (11-NOV-2017) School of Life Sciences, Sun Yat-Sen University, Xingang Xi Road 135, Guangzhou, Guangdong 510275, China |
| MG471388.1 | *Ipomoea aquatica* metallothionein MT2 (MT2) mRNA, complete sequence | 596 bp linear mRNA | |
| MG471387.1 | *Ipomoea aquatica* metallothionein MT1 (MT1) mRNA, complete sequence | 656 bp linear mRNA | |

(continued)

**Table 5.2** (continued)

| Accession No. | Gene/Protein name | Features | Source |
|---|---|---|---|
| MH792116.1 | *Ipomoea aquatica* voucher DMB 8 small subunit ribosomal RNA gene,partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence | 633 bp linear DNA | Ranathunga AP et al. Submitted (24-AUG-2018) Molecular Biology and Biotechnology, University of Peradeniya, Peradeniya, Kandy, Central 20000, Sri Lanka |
| MH792115.1 | *Ipomoea aquatica* voucher DMB 7 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence | 633 bp linear DNA | |
| MH792114.1 | *Ipomoea aquatica* voucher DMB 6 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence. | 633 bp linear DNA | |
| AY100958.1 | *Ipomoea aquatica* ribulose-1,5-bisphosphate carboxylase/ oxygenase, large subunit (rbcL) gene, partial cds; chloroplast gene for chloroplast product | 1372 bp linear DNA | Stefanovic S, Krueger L and Olmstead,R.G. Submitted (30-APR-2002) Botany, University of Washington, Box 355325, Seattle, WA 98195-5325, USA |
| AY100856.1 | *Ipomoea aquatica* PsbE (psbE) gene, partial cds; PsbF (psbF) and PsbL (psbL) genes, complete cds; and PsbJ (psbJ) gene, partial cds; chloroplast genes for chloroplast products | 758 bp linear DNA | |
| AY100749.1 | *Ipomoea aquati*ca ATP synthase beta subunit (atpB) gene, complete cds; chloroplast gene for chloroplast product | 1473 bp linear DNA | |
| AF111127.1 | *Ipomoea aquatica* granule-bound starch synthase (waxy) gene, partial sequence | 589 bp linear DNA | Miller RE, Rausher MD and Manos PS Submitted (07-DEC-1998) Botany, Duke University, Durham, NC 27708, USA |

(continued)

**Table 5.2** (continued)

| Accession No. | Gene/Protein name | Features | Source |
|---|---|---|---|
| HQ142108.1 | *Ipomoea aquatica* UDP glucose flavonoid 3-glucosyltransferase (UF3GT) mRNA, partial sequence | 806 bp linear mRNA | Toleno DM, Durbin ML, Lundy KE and Clegg MT Plant Species Biol 25 (1): 30–42 (2010) |
| HQ142087.1 | *Ipomoea aquatica* MADS (MADS) mRNA, partial sequence | 402 bp linear mRNA | |
| HQ142068 | *Ipomoea aquatica* alpha isopropylmalate synthase (IPMS) mRNA, partial sequence | 822 bp linear mRNA | |
| HQ142048.1 | *Ipomoea aquatica* isopropylmalate dehydrogenase (IMDH) mRNA, partial sequence | 672 bp linear mRNA | |
| HQ142029.1 | *Ipomoea aquatica* isolate 1 DFRB (DFRB) mRNA, partial sequence | 453 bp linear mRNA | |
| HQ142028.1 | *Ipomoea aquatica* DFRB (DFRB) mRNA, partial sequence | 486 bp linear mRNA | |
| HQ142017.1 | *Ipomoea aquatica* chalcone synthase (CHS-E) mRNA, partial sequence | 808 bp linear mRNA | |
| HQ141992.1 | *Ipomoea aquatica* chalcone synthase (CHS-D) mRNA, partial sequence | 854 bp linear mRNA | |
| HQ141973.1 | *Ipomoea aquatica* anthocyanidin synthase (ANS) mRNA, partial sequence | 873 bp linear mRNA | |
| HQ141953.1 | *Ipomoea aquatica* acetolactate synthase (ALS) mRNA, partial sequence | 738 bp linear mRNA | |
| FJ795794.1 | *Ipomoea aquatica* maturase K (matK) gene, chloroplast partial sequence | 819 bp linear DNA | Tugume A K et al. Submitted (25-FEB-2009) Department of Applied Biology, University of Helsinki, P.O. Box 27 (Latokartanonkaari 7), Helsinki FIN-00014, Finland |
| KU182875.1 | *Ipomoea aquatica* internal transcribed spacer 2, partial sequence | 222 bp linear DNA | Li YB and Wu B Submitted (23-NOV-2015) Research Center of Natural Resources of Chinese Medicinal Materials and Ethnic Medicine, Jiangxi University of Chinese Medicine, Xingwan Road of Wanli District, Nanchang,Jiangxi 330000, China. |

(continued)

**Table 5.2** (continued)

| Accession No. | Gene/Protein name | Features | Source |
|---|---|---|---|
| KP261915.1 | Ipomoea aquatica voucher FRI 70037 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence | 530 bp linear DNA | Simoes AR, Culham A and Carine M Bot J Linn Soc 179 (3):374–387 (2015) |
| KR025049.1 | Ipomoea aquatica voucher FRI 70037 trnK-rps16 intergenic spacer, partial sequence; and ribosomal protein S16 (rps16) gene, partial cds; chloroplast | 674 bp linear DNA | |
| KR024912.1 | Ipomoea aquatica voucher FRI 70037 maturase K (matK) gene, chloroplast partial sequence | 749 bp linear DNA | |
| AY101067.1 | Ipomoea aquatica tRNA-Leu (trnL) gene, partial sequence; trnL-trnF intergenic spacer, complete sequence; and tRNA-Phe (trnF) gene, partial sequence; chloroplast genes for chloroplast products | 809 bp linear DNA | Stefanovic S, Krueger L and Olmstead RG Am J Bot 89 (9):1510–1522 (2002) |
| HM367065.1 | Ipomoea aquatica clone Q5 metallothionein type 3-like protein mRNA, complete sequence | 201 bp linear mRNA | Huang B et al. J Agri Food Chem 57 (19):8950–8962 (2009) |
| AF110919.1 | Ipomoea aquatica internal transcribed spacer 1, 5.8S ribosomal RNAgene, and internal transcribed spacer 2, complete sequence | 598 bp linear DNA | Miller RE, Rausher MD and Manos PS Submitted (04-DEC-1998) Department of Botany, Duke University, Box90338, Durham, NC 27708, USA |

Source: Sources of the sequences are mentioned properly in the source column. All the sequences have been taken from National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/) with proper citation of authors names and affiliation. The majority of the data have been directly submitted in the database and those sequence data presented in journals are listed in the references section

specifically for creating pollution-safe, nutritionally-rich genotypes. Differential gene expression analysis based on high throughput RNA sequencing can be done for different cultivars of *Ipomoea aquatica* (showing differences in pollutant accumulating or sequestering properties) for identification of novel genes and transcription factors which can be a step toward making a transgenic elite plant in future for the betterment of humankind. Although this plant is normally cultivated under tropical condition, due to its huge potential as an important food supplement around the world, developing new strategies for cultivation under temperate conditions represents a promising future research area.

## Appendices

### *Appendix I: Research Institutes Relevant to Water Spinach*

| Institution | Specialization and research activities | Contact information and website |
|---|---|---|
| State Key Laboratory for Biocontrol and School of Life Sciences | Pollution-safe cultivar through traditional breeding, transcriptomics | Sun Yat-sen University, Guangzhou,510275, China<br>Website: http://www.sysu.edu.cn |
| Research and Instructional Farm of Horticulture, Department of Vegetable Science | Traditional and molecular breeding, agronomy | Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India<br>Website: http://www.igau.edu.in/ |
| Laboratory of Aquatic Vegetables | Breeding | Yangzhou University, Yangzhou, 225009, P.R. China<br>Website: http://en.yzu.edu.cn/ |
| Fengshan Tropical Horticultural Experiment Station | Germplasm maintenance, breeding | Taiwan Agricultural Research Institute, Fengshan, Kaohsiung, Taiwan<br>Website: https://www.tari.gov.tw/english/ |
| Department of Botany | Phytochemistry | University of Allahabad, Allahabad, India<br>Website: http://www.allduniv.ac.in/ |
| School of Environment and Life Science | Agronomy | University of Salford, Salford M5 4WT, United Kingdom<br>Website: https://www.salford.ac.uk/ |
| College of Natural Resources | Plant breeding | University of California, Berkeley, CA 94720, USA<br>Website: https://www.berkeley.edu/ |
| Humboldt-University of Berlin | Molecular biology, breeding | Institute of Horticultural Sciences Lentzeallee 75, 14195 Berlin Germany<br>Website: https://www.hu-berlin.de/ |
| Department of Botany | Taxonomy | Dr. B. A. M. University, Aurangabad, (M.S.), India<br>Website: http://www.bamu.ac.in/ |
| Department of Safety and Environmental Engineering | Breeding and molecular biology | Hunan Institute of Technology, Hengyang 421002, China<br>Website: http://www.hnit.edu.cn/ |
| Key Laboratory of Tropical Agro-environment | Agronomy, nutrition | Ministry of Agriculture/South China Agricultural University, Guangzhou 510642, P.R.China<br>Website: http://english.scau.edu.cn/ |
| Resources and Environment College | Pollution-safe cultivar through traditional breeding | Qingdao Agricultural University, Qingdao 266109, China<br>Website: https://www.qau.edu.cn/ |

(continued)

| Institution | Specialization and research activities | Contact information and website |
|---|---|---|
| College of Agronomy | Pollution-safe cultivar through traditional breeding | Hunan Agricultural University, Changsha 410128, China<br>Website: http://english.hunau.edu.cn/ |
| Advanced Pharmacognosy Research Laboratory | Pharmacognosy and phytochemistry | Department of Pharmaceutical Technology, Jadavpur University, Kolkata, 700032, India<br>Website: http://www.jaduniv.edu.in/ |
| Department of Horticulture | Breeding, nutrition | Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal 741252<br>Website: https://www.bckv.edu.in/ |

## *Appendix II: Genetic Resources of Water Spinach*

| Cultivar | Important traits | Cultivation location | Source |
|---|---|---|---|
| *Ipomoea aquatica* - Variant I | Broad leaved | Southeast Asia | Austin (2007) |
| *I. aquatica* - Variant II | Narrow leaved | Southeast Asia | Kaiser Hamid et al. (2011) |
| Kankoong beeasa | Dark-green leaves and stems and purple flowers | Java | Cornelis et al. (1985) |
| Kankoong nagree | Yellowish-green leaves, yellowish stems and white flowers | Java | Cornelis et al. (1985) |
| Pak Quat | White stems | Hong Kong | Pritesh Pandey and Madan Jha (2019) |
| Ching Quat | Green stems | Hong Kong | Pritesh Pandey and Madan Jha (2019) |
| cv. QLQ | Low shoot Cd cultivar | China | Xin et al. (2010) |
| cv. T308 | High shoot Cd cultivar | China | Baifei Huang et al. (2014) |
| Red stem cultivar | Dryland cultivation | China | Austin (2007) |
| White stem cultivar | Wetland cultivation | China | Austin (2007) |

(continued)

| Cultivar | Important traits | Cultivation location | Source |
|---|---|---|---|
| Taiwan filiform-leaf *I. aquatica* | High phytoremediation potential | Taiwan | Quan-Ying Cai et al. (2008) |
| Hong Kong white-skin *I. aquatica* | Low phytoremediation potential | Hong Kong | Saikat Dewanjee et al. (2015) |
| cv. Taiwan 308 | Non-Cadmium-PSC | Taiwan, China | Wang et al. (2009) |
| Xianggangdaye, Sannongbaigeng, and Jieyangbaigeng | Non-Cadmium-PSC | China | Wang et al. (2009) |
| cv. Daxingbaigu, Huifengqing, Qiangkunbaigu, Qiangkunqinggu, Shenniuliuye, and Xingtianqinggu | Cadmium-PSCs | China | Wang et al. (2009) |
| Thaiqinggengliuye water spinach (Liuye) | Non- Arsenic-PSCs | China | Dua et al. (2015) |
| Hong Kong chunbaidaye water spinach (Daye) | Arsenic-PSCs | Hong Kong, China | Wang et al. (2009) |
| cv. YQ | Low-Cd-Pb | China | Junliang Xin et al. (2012) |
| cv. GDB | High-Cd-Pb | China | Baifei Huang et al. (2012) |
| Salween | Small bamboo-like leaves and Suitable for the hot rainy season | Northern Thailand, Vietnam, southern China | Grubben (2004) |
| Liao | Bamboo-like leaves for the dry season | Northern Thailand, Vietnam, southern China | Grubben (2004) |
| Chinwin | Branching cultivar | Northern Thailand, Vietnam, southern China | Cornelis et al. (1985) |

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