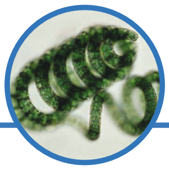
REVIEW

The place of halophytes in Pakistan's biofuel industry





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An unsustainable supply of fossil fuel necessitates the need to look for suitable alternatives. One solution lies in using plant biomass, which can be converted into a wide range of biofuels. To avoid conflict between feed and fuel, the crops available for human consumption being used presently as biofuel feedstock may be replaced with halophytes, which have the potential to thrive in saline lands and can be irrigated with brackish water; some can even tolerate seawater salinity. This approach will help in producing sustainable fuel without encroaching on the good quality land and water resources needed for food crops. A candidate species should preferably be perennial, having high yield in saline lands with minimum inputs. Other attributes include cellulose/hemicellulose >25–30%, lignin <10%, low salt load in foliage and a non-invasive nature. The unexplored aspects of agronomy of these wild plants need careful study, especially with regards to land degradation and ecological consequences, before large-scale cultivation.

The population across the world is increasing rapidly and has crossed the seven billion mark. This population is projected to climb to nine billion by the year 2050 with most of the growth occurring in poorer countries, particularly in Africa and south Asia, according to present demographic trends [1]. Consequently, the resources of arable lands and good quality water are under pressure. Modern agriculture methods have increased per unit area yield of food crops and have so far managed to meet the demands. This increased supply, whose sustainability is uncertain in the long run, has been at the cost of over-exploitation of soil and water resources. High doses of chemical fertilizers, particularly nitrogenous, and excess quantities of irrigation water, needed for optimal yields of 'improved varieties' such as those of wheat and rice of the 'green revolution' of the 1960s, is causing pollution of underground water and has resulted in salinization of prime agricultural land. Over 800 million ha of the world is presently affected by salinity [101] and 45 out of 230 million ha of irrigated agricultural land has become saline [2]. This necessitates some forward planning and the need to look beyond the traditional resources to satisfy our needs, as all our field crops are sensitive to salinity. One solution may lie in making use of halophytes; plants of saline habitats, some of which may thrive even with sea water irrigation, can be put to an array of uses [3].

It has been realized that fossil energy resources, which are the main drivers of our economy, are not sustainable [4]; consequently, it may be worthwhile to explore the potential of halophytes as a source of fuel for the benefit of mankind through mobilization of salty water and saline land – both are plentiful on planet Earth. This article aims to review work done so far in this field, especially with reference to selection criteria for suitability of particular species and the bottlenecks in achieving desirable goals. The possibilities of utilizing halophytes are immense and some species from the Pakistan coast have been identified as needing attention as biofuel feedstock candidates. Similar or even better species may, however, be found elsewhere.

Sustainability & environmental consideration

The use of plant biomass as fuel has been documented since early human history. Fossil fuels have gradually

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gained popularity due to their high abundance and denser energy, making them the preferred fuels in many regions. The history of use of plant biomass as fuel is as old as mankind itself. In most developing countries however, wood is still a major source of energy [102]. It has also been realized that the fossil fuel resources are not sustainable and are depleting. According to some projections, we will have utilized all the available gas and petroleum reserves in this century, and coal will be the only fossil fuel left for use in the next [4]. Consequently, there is a resurgence of interest in biomass energy, which is sustainable. Some additional carbon from what was captured during photosynthesis may be released during the production of biofuel from biomass than if mechanical methods were used; however, through enzymatic digestion this release can be minimized, making it almost carbon neutral. Burning the fossil fuel releases enormous quantities of CO_2, which may result in a relatively higher increase in ambient CO_2, climate change and acidification of oceans [5,6].

Key terms

Biotic and abiotic stress: Negative influence of living and nonliving factors, respectively, on another entity.

Hydrolysis: Decomposition of a chemical compound by reaction with water or acid (acid hydrolysis).

Pyrolysis: Thermochemical decomposition of organic biomass in the absence of oxygen.

Saccharification: Process of breaking a complex carbohydrate (as starch or cellulose) into its fermentable monosaccharide components.

Fermentation: Breakdown of sugar into an acid or alcohol.

Halophytes as an energy feedstock

The plant kingdom consists of about 400,000 flowering species [103], of which some 2600 are halophytic [7]. The halophytes have a diverse distribution but they are more common in arid/semi-arid regions of the tropics and subtropics [8]. For instance, a large area starting from Rajasthan in India and extending through to Pakistan, the Middle East, North and Central Africa all the way to Morocco, is home to a variety of halophytes [3]. Other areas where diverse populations of halophytes occur include parts of Australia, China, the USA and South America. Pakistan alone has approximately 410 halophytic species distributed on the Sindh/Balochistan coast, and inland from coast to mountains in the north including the Indus basin [9]. These halophytes belong to 58 families with the highest number of species (90) present in the Chenopodiaceae (now called Amaranthaceae) followed by Poaceae (68), Cyperaceae (30), Papilionaceae (29), Asteraceae (24) and Tamaricaceae (23), while other families are represented by less than 10 species [9].

The Institute of Sustainable Halophyte Utilization (University of Karachi, Pakistan), with the mandate of exploring the potential uses of halophytes, is in the process of surveying the coastal region of Pakistan and has so far identified species suitable for biofuels [10] in addition to animal fodder [11], edible oil [12] and medicines [13]. It is noteworthy that a halophytic grass has recently been used on a commercial scale to make turf [14] and the use of halophytic grasses as animal feed is fairly common as a sole source or as a supplement to regular feed [KHAN MA *ET AL.*, UNPUBLISHED DATA], but their usages have remained comparatively underexplored. This includes the use of halophytic biomass as a source of biofuel feedstock.

Selection criteria for energy crops

Various grains, such as corn, wheat, soybean and rapeseed, are currently major feedstocks for biofuel. According to a report, humans and livestock consume 48 and 35%, respectively, of the total grain produced annually, and approximately 17% is used for producing biofuels [15]. Faced with an increasing demand for energy and food, there is resistance against large-scale production of biofuel from food items, due to its impact on availability of food and inflation. The ideal feedstock for biofuel is hence outside the human food chain, such as the species of halophytes that do not infringe on prime agricultural land and good quality water resources. It is primarily important that a biofuel crop should be high yielding with few inputs, and have a wide environmental tolerance and ecological compatibility with desirable lignocellulosic content (discussed later). The species should be native to the area, have a fast growth rate and preferably be perennial to save cost of replanting. Further attributes include resistance to **biotic and abiotic stresses**, high water use efficiency and low fertilizer requirements. Integrating the quick check system [16] in the development of cash crop-halophytes may serve as a baseline study by facilitating data gathering on growth behavior and physiological/biochemical responses of plants under uniform salinity and controlled environmental conditions.

▪ Feedstock composition

Hydrolysis of lignocellulosic biomass and further conversion into sugars is affected by feedstock characteristics; cellulose/hemicellulose in excess of 25–30% each and <10% lignin are desirable and cost effective [10]. A further consideration is the elemental composition of plant biomass, which generally consists of oxides, silicates, carbonates, sulfates, chlorides and phosphates of Ca, Fe, K, Mg, Na and Si, together with trace amounts of S, P, Cl and Mn [17]. Studies on the catalytic effect of mineral matter on biomass **pyrolysis** through various methods showed that even a small amount of certain minerals like Ca, K, Na, Mg and Fe may affect pyrolysis adversely and cause problems in **saccharification** [18]. Halophytes, especially those from the family Amaranthaceae, accumulate large amounts of salt in their biomass but they are also capable of excreting excess salts through specialized salt glands, with halophytic grasses having the ability to block Na^+ and Cl^- at the root level. Information about the conversion process of biomass and optimization in saccharification of these halophytes is lacking to the best of our knowledge. The selection of the most suitable crops among halophytes should hence be done very carefully, keeping in mind not only the lignocellulosic composition but also their salt tolerance or avoidance strategies with special consideration of ion content.

▪ Stress tolerance

During growth, plants are exposed to a variety of biotic and abiotic stress, to which all conventional field crops are fairly sensitive. Salinity, being one of the more common hazards, attains special significance as it reduces yields considerably. Salinity tolerance ranges in halophytes widely with some species having tolerance to seawater or even higher salinity; for instance, *Arthrocnemum macrostachyum* survives in up to 1000 mM NaCl [19]. Many of these plants are not only salt tolerant but also possess osmo-tolerance because the primary effect of salinity is osmotic, which at higher salt concentrations also exerts ionic toxicity. These plants generally utilize the resources at their disposal efficiently (especially water) because they are programmed to live in hostile environments [20]. Depending on the quality of soil/water of a particular site, there is a wide array of halophytic plants to choose from for combining desirable attributes, such as high yields and suitable lignocellulose contents. It may be mentioned here that halophytes are generally not very susceptible to insect pests; however, that aspect needs attention in large-scale plantations.

▪ Life strategy & maintenance

Producing biomass becomes economical if the target crop is perennial. Further considerations include inputs, particularly fertilizers and water required for optimum yields. Obligate halophytes, *Suaeda fruticosa*, for example, require certain salt concentration for optimum growth; hence, those growing in the wild under specific conditions may produce large biomass. They are also mostly pest-free but these aspects require very careful monitoring for a wild plant that is being domesticated.

▪ Yield & growth rates

The potential of a crop, especially under stressful conditions, may be judged from seed germination, growth rates and height of the plants. These are therefore important criteria in deciding the suitability of a crop as a source of biofuel. In the absence of any systematic studies, information about exact yield and the potential of most halophytes is generally lacking; this will anyway be affected by the soil salinity and quality of irrigation water. However, it is noteworthy that the net primary productivity per unit area is reported to be highest in swamps and salt marshes where halophytes dominate, and where mangroves are the most productive plants growing submerged in seawater [21].

Bioethanol production from lignocellulosic biomass

Bioethanol has been recognized as a transportation fuel and can be used directly or blended with gasoline in various proportions [22,23]. The bulk of plant biomass contains varying amounts of lignocellulose (40–60% cellulose, 20–40% hemicellulose and 10–25% lignin) [24], which can be converted into a range of biofuels through various processing methods [5]. However, this is not as simple as it sounds because conversion of biomass into biofuel (bioethanol) involves hydrolysis of cellulose through highly specific cellulase enzymes and subsequent **fermentation** of the sugar formed by yeast or bacteria. Success in ongoing research in this direction would open up the opportunity of converting cellulosic material from any source, such as timber or agriculture waste, into sugar. The halophytes, however, still have the edge in biomass production over other sources because of the availability of vast tracts of saline lands lying barren worldwide, where normal agriculture or forestation is not feasible but these plants of saline habitat can be grown with salty water irrigation. In coastal areas, biomass production of some halophytes may even be possible with sea water irrigation.

Currently, large-scale industrial production of bioethanol comes from sugarcane, corn, soybean and cereals; this is easier but leads to direct conflict with the food sector [25]. The focus of research has consequently shifted to use lignocellulosic biomass from nonfood plants as feedstock for biofuel. One bottleneck in this process is the presence of lignin–hemicellulose networks in cellulose fibers, which interrupt the enzymatic hydrolysis of cellulose, an important step in bioethanol production. To maximize sugar yield, this network has to be hydrolyzed [26]. Therefore, plants that have low lignin contents should be chosen for bioethanol production, in order to minimize cost of additional steps required for separation of lignin.

The acid hydrolysis of cellulose and hemicelluloses results in the production of both C_5 and C_6 sugars. While the hexose sugars may be more readily amenable to fermentation, the pentoses are recalcitrant in this regard and pose a major bottleneck [25]. Hence, the resulting sugar mixture must first be separated (which often involves complicated techniques and adds to the cost) and then fermented by different microorganisms known to ferment either hexose or pentose sugars alone. Not many effective microbial catalysts have so far been found that can directly metabolize oligosaccharides to produce marketable products, exceptions being the production of xylanases by fungi (*Aspergillus niger* [27]) or actinomycetes [28].

Potential candidates for bioethanol

An initial study conducted by our research group on a number of halophytic species of our coastal area shows wide diversity in their lignocellulosic composition (Figure 1). These data exhibit a general trend of plant tissue composition and mineral contents which may vary with growth conditions and plant age. Characteristics of some promising species are presented in Table 1 and their other details are given below. The desirable features of these species include their salt tolerance, perennial growth, high water-use efficiency, fast growth rate and low energy input along with desirable lignocellulosic contents.

Phragmites karka (Retz.) Trin. ex Steud (Poaceae)

A perennial reed with creeping rhizomes that is commonly found in marshes and brackish water swamps. It is spread primarily by rhizomes and it produces numerous seeds that can germinate at 500 mM NaCl [29]; plants could subsequently grow under more saline conditions [Khan MA, Unpublished Data]. The height of *P. karka* is comparable with the already reported biofuel crop *Miscanthus* [30], with a high biomass yield of composition similar to *Cynodon dactylon* [31].

Halopyrum mucronatum (L.) Stapf. (Poaceae)

This is a perennial grass growing on sand dunes in the coastal areas of Pakistan. It grows well while inundated regularly with seawater and produces dimorphic seeds twice in a year [32]. Its biomass, having 37% cellulose, 28% hemicellulose and 5% lignin, makes it a better candidate for ethanol production than switchgrass (*Panicum virgatum*) [31]. The grass can additionally be grown with seawater irrigation for dunes stabilization, checking land erosion, for carbon sequestration and so on.

Desmostachya bipinnata (L) Stapf. (Poaceae)

This is a tall perennial grass distributed near coastal and inland areas of Sindh province. The seeds can germinate at >400 mM NaCl [33] and plants exhibit fairly good growth on saline and sodic soil where brackish water is available [Khan MA, Unpublished Data]. Its fairly high salt tolerance on light textured soils [34] and ability to resist metal contaminations [35] have also been reported. The composition of its biomass (26% cellulose, 24% hemicellulose and 7% lignin) makes it a good biofuel candidate.

Panicum turgidum Forssk (Poaceae)

A perennial halophytic grass generally used as a fodder, but its chemical composition (28% cellulose, 28% hemicellulose and 6% lignin) also makes it a good candidate for bioethanol production. Essentially a desert grass widely distributed in Pakistan, *P. turgidum* can tolerate 200 mM NaCl at germination stage [36] and grows in dense bushes through tillers, reaching up to 1 m in approximately 30 days [11]. The identification and development of a more salt-tolerant *P. turgidum* may help address the scarcity of good quality water in many arid agricultural regions of the world.

Typha domingensis Pers. (Poaceae)

Found near coastal areas of Pakistan, this is a rhizomatous perennial with the ability to tolerate flooding and moderate salinity. Where perpetual supply of water and nutrients are available, it becomes an aggressive invader. However, its biomass containing 26% cellulose, 38% hemicelluloses and 4% lignin may challenge any second-generation species for biofuel production; for instance, water-hyacinth (*Eichhornia crassipes*), which has 35–50% of hemicellulose [37] but high (12%) tannin, which is undesirable for fermentation [31].

Urochondra setulosa (Trin.) C.E. (Poaceace)

A dominant species on the sand dunes, saline flats and saltwater creeks in the Indus Delta and near Karachi coast [38]. *U. setulosa* produces a large number of seeds, which could germinate at approximately 1000 mM NaCl [39] and can adapt in drought situations, making the plant very important ecologically in saline/water-scarce areas [40]. With 25% cellulose, 25% hemicellulose and 6% lignin, it is a suitable candidate for bioethanol. Its very slow growth rate, however, requires agronomic interventions and genetic breeding to make it more acceptable.

Aeluropus lagopoides (Linn.) Trin. ex Thw. (Poaceae)

A rhizomatous perennial grass that dominates the seasonally inundated coast as well as inland salt flats of arid Pakistan [41]. It appears to be propagating mainly through rhizomes in monospecific stands or through seeds while colonizing open niches [42]. It is a good candidate for a biofuel feedstock because of its perennial habit [43] and lignocellulosic biomass (26.67% cellulose, 29.33% hemicellulose and 7.67% lignin); however, this species does not grow very tall, which means it does not have enough unit area/time yield to be considered as a biofuel crop.

Biodiesel production prospects

Rudolph Diesel first proposed the use of plant oil as automobile fuel in the 19th century [104]. Subsequently it has been realized that biodiesel has a high potential as an environmentally friendly and renewable energy source for the future [44]. Biodiesel is a mono-alkyl ester derived from oils, which has characteristics similar to petroleum-derived diesel. The conversion of edible or nonedible oil from 'oilseed plant species' or animal fats into biodiesel may be relatively easy as it involves only hydrolysis and separation of glycerol. It may serve as a substitute for conventional diesel and can be used in existing diesel engines without significant modification [45]. Currently, approximately 84% of the world's biodiesel requirement is met by rapeseed oil and the remaining is from sunflower (13%), palm (1%), soybean and some other oils (2%) [105]. Incidentally, all of them are edible and their use for producing biodiesel may have adverse consequences on the price of food commodities. If biodiesel production is further increased, the world food situation would be adversely affected [46]. In order to meet this challenge, biodiesel should be produced from low-cost feedstocks such as nonedible oils, used frying oils, animal fats, soap stocks and greases. However, the available quantities of these waste oils and animal fats are not enough to meet the current demands for diesel. Additionally, biodiesel needs to have lower environmental impacts and ensure the same level of performance as of existing fuels [47]. This problem could be dealt with by using nonconventional oil sources, without compromising food resources and sparing good quality water and land for conventional crop production for essential food items. Transition to a second-generation biofuel source, such as halophytes, can contribute to a reduction in land requirements due to their high energy yields per unit area, as well as to their nonrequirement of agricultural land.

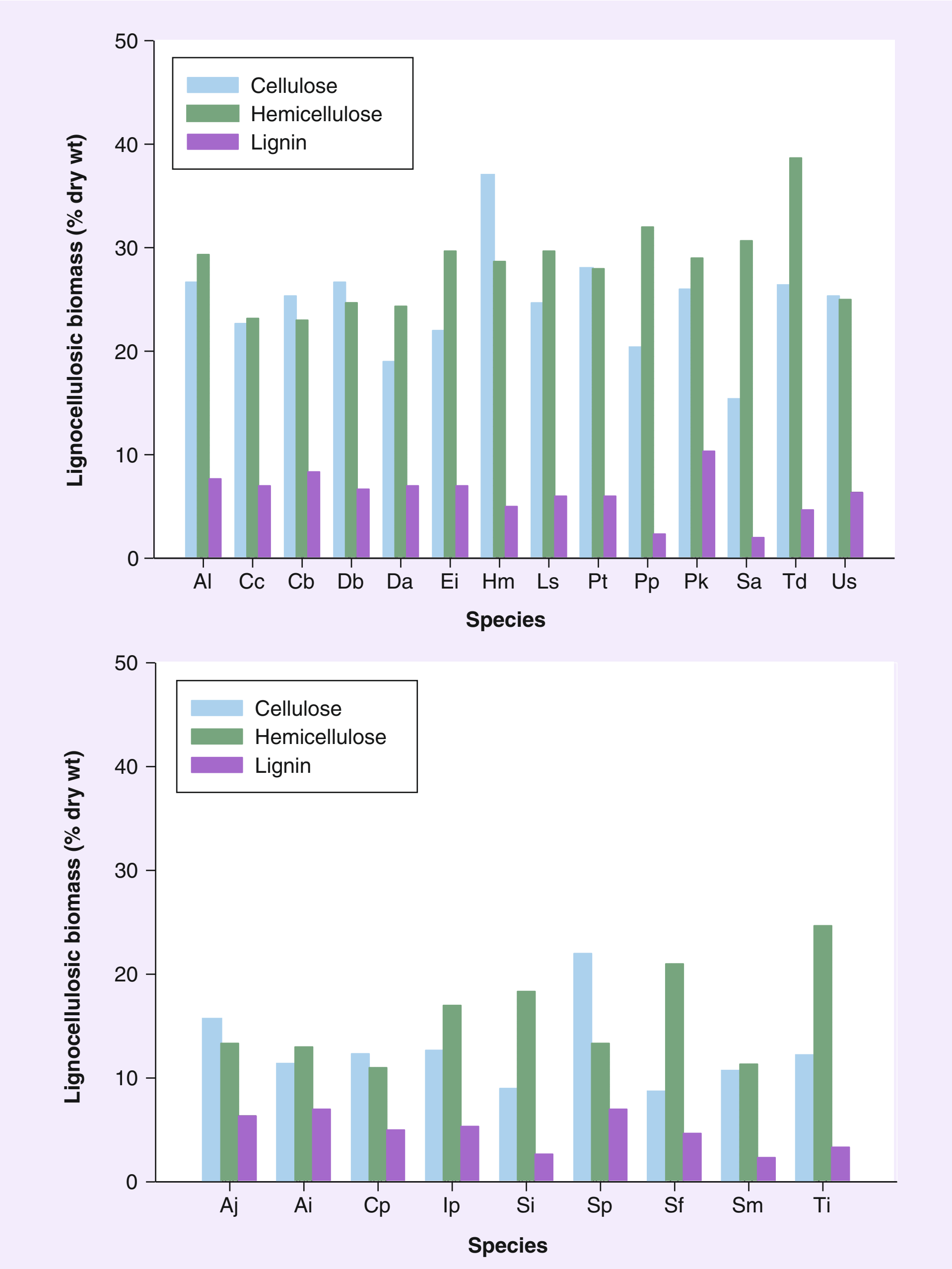

Figure 1. Lignocellulosic contents of common halophytic species of local flora. The method of the Association of Official Agricultural Chemists [62] was used, involving estimation of neutral detergent fiber (NDF), which accounts for cellulose, hemicelluloses and lignin contents. Acid detergent fiber was determined sequentially using the residue from NDF determination. Hemicellulose was calculated by subtracting acid detergent fiber from NDF [63]. Lignin was obtained by ashing the hydrolysed residue [10].

Ai: *Arthrocnemum indicum*; Aj: *Aerva javanica*; Al: *Aeluropus lagopoides*; Cb: *Chloris baraeta*; Cc: *Cenchrus ciliaris*; Cp: *Calotropis procera*; Da: *Dichanthium annulatum*; Db: *Desmostachya bipinnata*; Ei: *Eleusine indica*; Hm: *Halopyrum mucronatum*; Ip: *Ipomea pes-caprea*; Ls: *Lasiurus scindicus*; Pk: *Phragmites karka*; Pp: *Paspalum paspaloides*; Pt: *Panicum turgidum*; Sa: *Salsola imbricate*; Sf: *Sueda fruticosa*; Si: *Sporobolus ioclados*; Sm: *Sueda monoica*; Sp: *Salvadora persica*; Td: *Typha domingensis*; Ti: *Tamarix indica;* Us: *Urochondra setulosa*.

Modified with permission from [10].

▪ Potential candidates for biodiesel

Seeds of many halophytes contain appreciable amounts of oil [12,48] and may serve as a source of

Table 1. List of halophytes specie as potential sources of bioethanol.

Candidate species	Family	Site/province	Life form	C_3/C_4
Aeluropus lagopoides	Poaecae	Hawksbay/SIN	SFC	C_4
Desmostachya bipinnata	Poaceae	Abbas goth/BAL	NP	C_4
Halopyrum mucronatum	Poaceae	Manora/SIN	SFC	C_4
Panicum turgidum	Poaceae	Moosa Goth/BAL	NP	C_4
Phragmites karka	Poaceae	KU Campus/SIN	NP	C_3
Typha domingensis	Poaceae	Hawksbay/SIN	NP	C_3
Urochondra setulosa	Poaceae	Hawksbay/SIN	SFC	C_4

BAL: Balochistan, Pakistan; KU: University of Karachi; NP: Nano-phanerophyte; SFC: Sub-fruticose chamaephyte; SIN: Sindh.
Adapted with permission from [10].

biodiesel [Abidin Z, Khan MA, Unpublished Data]. Seeds of *Salicornia bigelovii* contain 30% oil [49], *S. fruticosa* and *A. macrostachyum* seeds contain about 25% oil, while those of *H. mucronatum, Cressa cretica*, *Haloxylon stocksii* and *Alhaji maurorum* contain 22.7, 23.3, 23.2 and 21.9% oil, respectively, with variable ash and sodium contents (Table 2). Initial screening has shown that the fatty acid methyl esters (FAME) of the oil of many of these halophytes are comparable to those of conventional oils presently used for biodiesel production [Khan MA, Unpublished Data]. Our research group is analyzing seeds of approximately 100 halophytes distributed along the Pakistan coast and on the basis of these data we will be able to select suitable candidates for a biodiesel crop. All these are wild species for which seed yield estimation does not exist, considering that we are trying to use saline land and brackish water that was regarded as waste. There are millions of the acres of saline land available for such production. It appears that in local conditions, these species produce numerous seeds twice a year and we expect them close to that of mustard oil seed production. These expectations are under natural conditions; however, when cultivated using proper irrigation and cultural practices it is expected that the yield will increase significantly [11]. This will be followed by experiments on the quality of biodiesel extracted from these oils for assessing their suitability for engines. Compositions of some promising species from our local flora are presented in Table 3, with other details given below.

Table 2. Seed composition of potential biofuel species.

Species	Oil (%)	Ash (%)	Na (ppm)
Alhagi maurorum	21.9	2	607
Arthrocnemum macrostachyum	25.0	39	3573
Cressa cretica	23.3	7	310
Halopyrum mucronatum	22.7	2	53
Haloxylon stocksii	22.7	20	3550
Salicornia bigelovii	30.0	7	
Suaeda fruticosa	25.0	7	1623

Adapted with permission from [12].

S. bigelovii

A leafless annual saltmarsh plant with green-jointed and succulent stems that ultimately form terminal fruiting spikes in which seeds are borne [50]. It is being evaluated as oilseed crop for brackish and seawater-irrigated agriculture in the coastal deserts of Mexico [51]. It yields 2 t/ha of seed containing 28% oil and 31% protein, similar to soybean yield and seed quality [52]. It hence appears to be a potentially valuable new biodiesel seed crop for the coastal deserts; however, the cost of production may increase due to the need of annual planting.

H. stocksii (Boiss.) Benth & Hook (Amaranthaceae)

A stem-succulent perennial distributed from southern Sindh and Balochistan up to the Northern Himalayan mountain valley of Chitral, Pakistan [12], in association with *S. fruticosa, Salsola stocksii* and *Sporobolus arabicus* [53,54]. *H. stocksii* tolerates 500 mM NaCl concentration at the germination level [54,55]. The seeds contain 23% oil, making it a good candidate for biodiesel production.

S. fruticosa (L.) Forssk. (Amaranthaceae)

A perennial halophyte of inland and coastal salt marshes and salt deserts of Pakistan [56]. Seed germination occurs in up to 400 mM NaCl [55]. It is used locally as fodder for livestock and is a source of washing soda [57]. Seeds contain 25% oil, which is edible [12] and may be used for making biodiesel. The utility of this leaf-succulent species in maintaining soil salinity levels by removing excess salts from soil has been demonstrated recently [11].

C. cretica Linn. (Convolvulaceae)

One of the dominant halophytic perennial plants, widely distributed in pure stands along the coastal salt marshes of Karachi, Pakistan, and whose seeds can germinate in up to 850 mM NaCI. [58], *C. cretica* has also been used to relieve asthma and coughs by the rural people of India [59]. The sufficient amount of oil in their large numbers of seed can be used for biodiesel conversion.

Table 3. List of halophytes species as potential sources of biodiesel.

Candidate species	Family	Site/province	Life form	C_3/C_4
Arthrocnemum macrostachyum	Amaranthaceae	Clifton/SIN	NP	C_3
Haloxylon stocksii	Amaranthaceae	Hawksbay/SIN	NP	C_4
Salicornia bigelovii	Amaranthaceae	Manora/SIN	NP	C_4
Suaeda fruticosa	Amaranthaceae	KU Campus/SIN	NP	C_4
Cressa cretica	Convolvulaceae	KU Campus/SIN	SFC	C_3
Alhagi maurorum	Papilionaceae	KU Campus/SIN	NP	C_3

BAL: Balochistan, Pakistan; KU: University of Karachi; NP: Nano-phanerophyte; SFC: Sub-fruticose chamaephyte; SIN: Sindh.
Adapted with permission from [10].

A. macrostachyum (Moric) C Koch (Amaranthacea)

A perennial, stem-succulent halophyte found in the intertidal zone of the Arabian Sea at Karachi, Pakistan [19]. The species is characterized by articular stems with carnose segments, reduced and stem-united leaves [60]. Seed germination of *A. macrostachyum* was only inhibited at the highest salinity (4% NaCl, 5% $MgCl_2$) but the ungerminated seeds showed a high recovery in distilled water (81 and 83%, respectively) [61].

A. maurorum Medic. (Papilionaceae)

A spiny, deciduous, perennial, saltmarsh under-shrub, native from Eurasia, commonly found in both inland and coastal areas of Karachi, Pakistan [12]. The perennial plant grows from a massive rhizome system, which may extend over 1.8 m into the ground. It has a wide soil tolerance, thriving on saline, sandy, rocky and dry soils. It does best when growing next to a source of water, such as an irrigation ditch. It is unpalatable to animals and irritating when it invades forage and grazing land. Seeds of *A. maurorum* may be used as a source of oil for biodiesel.

Pitfalls & precautions

Circumstantial and experimental evidence proves the superiority of halophytes over the present-day agricultural plants in tolerating salinity stress; however, detailed information concerning their growth behavior is largely missing compared with conventional crops, which are backed by thousands of years of efforts to domesticate them, with the process still ongoing. First and foremost is the availability of planting material, whether it be the seed or rootstock. The seeds of halophytes, which may be recalcitrant to germination, are generally of very small size and their availability is restricted to short specific periods of the year making it difficult to collect in large quantities.

Reliable estimates of seed or biomass yields of halophytes are not available, which require systematic cultivation of the target species in big plots with suitable cultural practices on aspects such as spacing, fertilizer doses, irrigation schedule and harvesting interval. Our research group has some information about the biomass yield of *P. turgidum* [Khan MA, Unpublished Data], but individual candidate species need to be similarly assessed. These studies are crucial before embarking on any commercial application. It is, however, noteworthy that because these are untested species of the wild, it is difficult to forecast the time and efforts needed for desirable outcome from the agricultural practices. As in any new venture, known and unforeseen problems may be encountered and will need to be solved over time. This was also the case with conventional agricultural crops, which now have greatly improved yields, with mechanization taking over manual harvesting and making the task much easier.

If rootstocks are being used as planting material, their availability, collection and transportation to planting site may also be problematic and costly. For commercial-scale planting, very large nurseries are needed requiring suitable infrastructure and involving expenses. Tissue culture techniques, if employed to meet the demand, will require technical know-how and prior assessment of economic feasibility.

For reasons mentioned earlier, a low salt load in biomass is desirable for efficient pyrolysis and saccharification. Halophytic grasses thus become preferable because they restrict ion uptake at the root level and reduce salt buildup in foliage. This advantage, however, carries the hazard of increasing soil salinity, especially when soil is already saline and being irrigated with brackish water. One remedy may lie in combining a salt excluder (a grass) for use as biofuel, and a salt accumulator, *S. fruticosa* for instance, in the same land at suitable intervals and separately harvested [11]. This, however, needs further study.

The species considered suitable are predominantly C_4 type (**Tables 1 & 3**) with the potential of becoming aggressive invaders, harming the biodiversity of the area. This needs close observation in large-scale plantations, with care being taken to restrict species to their natural habitats. For instance, *P. karka* is a fast growing and invasive species among our research group's studied plants with water-logging tolerance; therefore, we should target it to be cultivated in similar conditions where it can flourish and produce the lignocellulosic biomass, without harming the ecosystem.

Concluding remarks

Biofuel from plant biomass has the advantage of being sustainable. However, considering the energy demand and the quantity of biomass likely to be available for conversion, it would be very optimistic to suggest that the biofuels will fully meet our future needs. These second-generation fuels can, at best, be expected to serve as a supplement to fossil fuel, such as the current blending of ethanol with petrol and thus helping in making the fossil fuel last longer than predicted. Cultivating halophytes in saline lands will spare fresh water and good agricultural lands for food, and provide lignocellulosic biomass of desirable quality for conversion as biofuel. Perennial grasses appear as better feedstock due to their high yield and low salt load in foliage; however, the salt buildup in soil needs close observation.

Future perspective

Keeping in view the current pace of land degradation and competition between food and fuel crops, diversification through halophyte utilization to suit particular soil conditions seems a viable alternative. A number of halophytes can be found that have desirable characteristics for biofuel production. Saline culture of halophytes is not a large challenge, but care is needed to use native plants to avoid any hazardous ecological consequences and restrict such planting to areas where soils are already saline and the irrigation water is brackish. The knowledge of some of the mechanisms adopted by plants to cope with salinity and the methods to deal with salt buildup in root zone are available and more information is accumulating. There is a need to explore the possibility of optimizing growth of candidate species through rigorous agronomic trials and to produce lignocellulosic biomass of suitable composition in quantities higher than that mentioned above. A better understanding of ecophysiological and agronomical responses of these species will help in efficient management and manipulation of plant growth characteristics suited to the prevailing conditions.

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Executive summary

Sustainability & environmental consideration

- Fossil fuels are depleting fast and require a more sustainable alternative, such as biofuel from halophytic lignocellulosic biomass. This approach is environmentally sound and addresses the food versus fuel debate.

Halophytes as an energy feedstock

- Although halophytes are a small fraction of the plant kingdom, their abundance in saline soils and tolerance to salty water irrigation, both of which are abundant worldwide, warrants investigation of their potential use as a source of biofuel.

Selection criteria for energy crop

- A candidate species should preferably be perennial, high yielding, requiring low inputs and cost effective in conversion into biofuel. It should have low lignocellulosic recalcitrance and be amenable to fermentation.

Bioethanol production from lignocellulosic biomass

- It is theoretically possible to convert cellulose from any source into sugar, which can be fermented into ethanol, if low-cost methods are available for this purpose.

Biodiesel production prospects

- Converting seed oil into biodiesel is relatively straightforward as it involves only hydrolysis of oil and separation of glycerol.

Pitfalls & precautions

- Collecting large quantities of feedstock, seed or biomass, will require small-scale field trials for growth maximization before embarking on large-scale cultivation. Large nurseries and tissue culture techniques may meet the demand of commercial-scale planting if technical know how for low-cost production is available.

Conclusion

- Research is needed to yield maximization of candidate species through agronomic trials and breeding for desirable traits such as low lignin and high cellulose content.
- Replacing fossil fuel completely with biofuel is highly unlikely in the foreseeable future. Therefore, developing a mix of techniques to use solar, wind and water as an energy source, along with fossil and biofuels, may provide a viable solution to meet demands.

References

Papers of special note have been highlighted as:

- ■ of interest
- ■■ of considerable interest

1 McDonald RI, Green P, Balk D *et al.* Urban growth, climate change, and fresh water availability. *Proc. Natl Acad. Sci.* 108, 6312–6317 (2011).

2 Munns R, Tester M. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* 59, 651–681 (2008).

3 Khan MA, Ansari R, Gul B, Qadir M. Crop diversification through halophyte production on salt prone land resources. *CAB Rev. Perspect. Agric. Vet. Sci. Nutri. Nat. Resour.* 1(48), 1–8 (2006).

4 Shafiee S, Topal S. When will fossil fuel reserves be diminished? *Energy Policy* 37, 181–189 (2009).

5 Abbasi T, Abbasi M. Biomass energy and the environmental impacts associated with its production and utilization. *Renew. Sust. Energy Rev.* 14, 919–937 (2010).

■■ **Discusses potential of converting biomass to biofuels ranging from gas to liquids, and the associated environmental implications.**

6 Doney SC, Fabry VJ, Feely RA, Kleypas JA. Ocean acidification: the other CO_2 problem. *Ann. Rev. Mar. Sci.* 1, 169–192 (2009).

7 Menzel U, Lieth H. Halophyte database V. 2.0 update. In: *Cash Crop Halophytes.* Lieth H, Mochtchenko M (Eds). (CD-ROM) Kluwer Academic Publishers, Dordrecht, The Netherlands (2003).

8 Arzani A. Improving salinity tolerance in crop plants: a biotechnological view. *In Vitro Cell. Dev. Biol. Plant* 44, 373–383 (2008).

9 Khan MA, Qaiser M. Halophytes of Pakistan: characteristics, distribution and potential economic usages. In: *Sabkha Ecosystem Volume II: West and Central Asia.* Khan MA, Boer B, Kust GC, Barth HJ (Eds). Springer, Berlin, Germany, 129–153 (2006).

■■ **Detailed information about the diversity of halophytes found in Pakistan and their possible utilization.**

10 Abideen Z, Ansari R, Khan MA. Halophyte: potential source of lignocellulosic biomass for ethanol production. *Biomass Bioenergy* 35, 1818–1822 (2011).

■■ **Provides information on the screening of some local halophytes for lignocellulosic contents to demonstrate the huge potential of halophytes as biofuel feedstocks.**

11 Khan MA, Ansari R, Ali H, Gul B, Nielsen BL. *Panicum turgidum*, a potentially sustainable cattle feed alternative to maize for saline areas. *Agric. Ecosyst. Environ.* 129, 542–546 (2009).

■ **Prospect and problems of initial experimental production of halophytic grass in new areas before full-scale cultivation in the field experiment.**

12 Weber DJ, Ansari R, Gul B, Khan MA. Potential of halophytes as source of edible oil. *J. Arid Environ.* 68, 315–321 (2007).

■■ **Provides initial information about the oil content and fatty acid profiles of the local halophytes, which may help in their utilizations for human consumption.**

13 Qasim M, Gulzar S, Shinwari ZK, Aziz I, Khan MA. Traditional ethnobotanical uses of halophytes from Hub Baluchistan. *Pak. J. Bot.* 42, 1543–1551 (2010).

14 DePew MW, Tilman PH. Commercial application of halophytic turfs for golf and landscape developments using hyper-saline irrigation. In: *Ecophysiology of High Salinity Tolerant Plants.* Khan MA, Weber DJ (Eds). Springer, Berlin, Germany, 255–278 (2006).

15 Worldwatch. *Grain Harvest Sets Record, but Supplies Still Tight. Vital Signs 2007–2008.* Worldwatch Institute, Washington DC, USA (2008).

16 Koyro HW. Study of potential cash crop halophytes in a quick check system. In: *Task for Vegetation Science, Cash Crop Halophytes: Recent Studies – 38.* Lieth H, Mochtchenko M (Eds). Kluwer Academic Publishers, Dordrecht, The Netherlands, 5–17 (2003).

17 Zevenhoven-Onderwater M, Backman R, Skrifvars BJ, Hupa M. The ash chemistry in fluidised bed gasification of biomass fuels. Part I: predicting the chemistry of melting ashes and ash–bed material interaction. *Fuel* 80, 1489–1502 (2001).

18 Yang H, Yan R, Chen H, Zheng C, Lee DH, Liang DT. Influence of mineral matter on pyrolysis of palm oil wastes. *Combustion Flame* 146, 605–611 (2006).

■ **Provides information about the influence of mineral matter on pyrolysis of biomass.**

19 Khan MA, Ungar IA, Showalter AM. Salt stimulation and tolerance in an intertidal stem-succulent halophyte. *J. Plant Nutri.* 28, 1365–1374 (2005).

20 Koyro HW, Khan MA, Lieth H. Halophytic crops: a resource for the future to reduce the water crisis? *Emir. J. Food Agric.* 23, 1–16 (2011).

■ **Describes the higher water efficiency of halophytes and its significance to reduce water shortage.**

21 Smith RL. *Ecology and Field Biology (5th Edition).* Addison-Wesley Educational Publishers, Inc. USA, 175 (1996).

22 Demirbas MF, Balat M, Balat H. Biowastes-to-biofuels. *Energy Convers. Manage.* 52, 1815–1828 (2011).

23 Malc-aa J, Freire F. Renewability and life-cycle energy efficiency of bioethanol and bio-ethyl tertiary butyl ether (bioETBE): assessing the implications of allocation. *Energy* 31, 3362–3380 (2006).

24 Mathew GM, Sukumaran RK, Singhania RP, Pandey A. Progress in research on fungal cellulases for lignocellulose degradation. *J. Sci. Ind. Res.* 67, 898–907 (2008).

25 Del Campo I, Alegria I, Zazpe M, Echeverria M, Echverria I. Diluted acid hydrolysis pretreatment of agri-food wastes for bioethanol production. *Indust. Crops Prod.* 24, 214–221 (2006).

26 Foyle T, Jennings L, Mulcahy P. Compositional analysis of lignocellulosic materials: evaluation of methods used for sugar analysis of waste paper and straw. *Bioresour. Technol.* 98, 3026–3036 (2007).

27 Bjerre AB, Olesen AB, Fernqvist T, Ploger A, Schmidt AS. Pretreatment of wheat straw using combined wet oxidation and alkaline hydrolysis resulting in convertible cellulose and hemicelluloses. *Biotechnol. Bioeng.* 49, 568–577 (1996).

28 Nascimento RP, Coelho RRR, Marques S *et al.* Production and partial characterisation of xylanase from *Streptomyces* sp. strain AMT-3 isolated from Brazilian cerrado soil. *Enzyme Microbiol. Technol.* 31, 549–555 (2002).

29 Zehra A, Khan MA. Comparative effect of NaCl and sea salt on germination of halophytic grass *Phragmites karka* at different temperature regimes. *Pak. J. Bot.* 39, 1681–1694 (2007).

30 Sorensen A, Teller PJ, Hilstrom T, Ahring BK. Hydrolysis of miscanthus for bioethanol production using dilute acid presoaking combined with wet explosion pre-treatment and enzymatic treatment. *Bioresour. Technol.* 99, 6602–6607 (2008).

31 Sun Y, Cheng J. Hydrolyis of lignocellulosic materials for ethanol production. *Bioresour. Technol.* 83, 1–11 (2002).

32 Siddiqui ZS, Khan MA. The role of enzyme amylase in two germinating seed morphs of *Halopyrum mucronatum* (L.) stapf in saline and non-saline environment. *Acta Physiol. Plant.* 33, 1185–1197 (2011).

33 Gulzar S, Khan MA, Xiaojing L. Seed germination strategies of *Desmostachya*

bipinnata: a fodder crop for saline soils. *Rangeland Ecol. Manage.* 60, 401–407 (2007).

34 Fakhireh A, Shahriaria R, Mansouri SH, Nouri S, Pahlavanravi A. An autecology study of *Desmostachya bipinnata* in sistan plain. *Rangeland Spring* 4, 60–71 (2010).

35 Ali M, Ahmed T, Rashid A. Phyto-sociological synthesis as inferred from soil analysis of some industrial areas of Punjab. *Asian J. Plant Sci.* 3, 320–324 (2004).

36 Al-Khateeb SA. Effect of salinity and temperature on germination, growth and ion relations of *Panicum turgidum* Forssk. *Bioresour. Technol.* 97, 292–298 (2006).

37 Kumar A, Singh LK, Ghosh S. Bioconversion of lignocellulosic fraction of water hyacinth (*Eichhornia crassipes*) hemicellulose acid hydrolysate to ethanol by *Pichia stipitis*. *Bioresour. Technol.* 100, 3293–3297 (2009).

38 Shaikh F, Gul B, Li WQ, Liu XJ, Khan MA. Effect of calcium and light on the germination of *Urochondra setulosa* under different salts. *J. Zhejiang Univ. Sci. B* 8, 20–26 (2007).

39 Zehra A, Gul B, Ansari R, Khan MA. Role of calcium in alleviating effect of salinity on germination of *Phragmites karka* seeds. *S. Afr. J. Bot.* 78, 122–128 (2011).

40 Ishnava K, Ramarao V, Mohan JSS, Kothari IL. Ecologically important and life supporting plants of little Rann of Kachchh. *Gujarat J. Ecol. Nat. Environ.* 3, 33–38 (2011).

41 Khan MA, Gulzar S. Light, salinity and temperature effects on the seed germination of perennial grasses. *Am. J. Bot.* 90, 131–134 (2003).

42 Ahmed MZ, Gilani SA, Kikuchi A, Gulzar S, Khan MA, Watanabe KN. Population diversity of *Aeluropus lagopoides*: a potential cash crop for saline land. *Pak. J. Bot.* 43, 595–605 (2011).

43 Torbatinejad N, Maghsoodlowrad H, Gharabash AM. Nutritive value of *Aeluropus littoralis* and *Aeluropus lagopoides* in sheep. *J. Agr. Sci. Nat. Res.* 7, 31–45 (2000).

44 Peterson CL. Vegetable oil as a diesel fuel: status and research priorities. *Trans. Am. Soc. Agric. Engineers* 1413–1422 (1986).

45 Murugesan A, Umarani C, Subramanian R, Nedunchezhian N. Bio-diesel as an alternative fuel for diesel engines – a review. *Renew. Sustain. Energy Rev.* 13, 653–662 (2009).

46 Kumar A, Sharma S. Potential non-edible oil resources as biodiesel feedstock: an Indian perspective. *Renew. Sustain. Ener. Rev.* 15, 1791–1800 (2011).

47 Reinhardt G, Rettenmaier N, Koppen S. How sustainable are biofuels for transportation? In: *Bioenergy: Challenges and Opportunities*. International Conference and Exhibition on Bioenergy. Guimarães, Portugal, 6–9 April 2008.

48 Glenn EP, O'Leary JW, Watson MC, Thompson TL, Kuehl RO. *Salicornia bigelovii* Torr: an oilseed halophyte for seawater irrigation. *Science* 251, 1065–1067 (1991).

49 Glenn EP, Brown JJ, O'Leary JW. Irrigating crops with seawater. *Sci. Am.* 279, 76–81 (1998).

50 Zerai DB, Glenn EP, Chatervedi R *et al.* Potential for the improvement of *Salicornia bigelovii* through selective breeding. *Ecol. Eng.* 36, 730–739 (2010).

51 Rueda-Puente EO, Garcia-Hernandez JL, Preciado-Rangel P *et al.* Germination of *Salicornia bigelovii* ecotypes under stressing conditions of temperature and salinity and ameliorative effects of plant growth-promoting bacteria. *J. Agron. Crop Sci.* 193, 167–176 (2007).

52 Glenn EP, Brown JJ. Salt tolerance and crop potential of halophytes. *Crit. Rev. Plant Sci.* 18, 227–255 (1999).

53 Khan MA. Studies on germination of *Cressa cretica* L. seeds. *Pak. J. Weed Sci. Res.* 4, 89–98 (1991).

54 Khan MA, Ungar IA. Influence of salinity and temperature on the germination of *Haloxylon recurvum*. *Ann. Bot.* 78, 547–551 (1996).

55 Khan MA, Ungar IA. Germination of the salt tolerant shrub *Suaeda fruticosa* from Pakistan: salinity and temperature responses. *Seed Sci. Technol.* 26, 657–667 (1998).

56 Hameed A, Zaheer MA, Gulzar S, Khan MA. Effect of disinfectant in improving seed germination of *Suaeda fruticosa* under saline conditions. *Pak. J. Bot.* 41, 2639–2644 (2009).

57 Freitag H, Hedge IC, Jaffri SMH, Kothe HG, Omer S, Uotila P. *Flora of Pakistan. No. 204. Chenopodiaceae.* Department of Botany, University of Karachi, Karachi, Pakistan (2001).

58 Aziz S, Khan MA. Seed bank dynamics of a semi-arid coastal shrub community in Pakistan. *J. Arid Environ.* 34, 81–87 (1996).

59 Sunita P, Jha S, Pattanayak SP. *In-vivo* antitussive activity of *Cressa cretica* Linn using cough model in rodents. *Phcog. Res.* 1, 157–161 (2009).

60 Redondo-Gomez S, Wharmby C, Moreno FJ *et al.* Presence of an internal photosynthetic cylinder surrounding the stele in stems of the tribe Salicornieae (Chenopodiaceae) from SW Iberian Peninsula. *Photosynthetica* 43, 157–159 (2005).

61 Vicente MJ, Conesa E, Alvarez-Rogel J *et al.* Relationships between salt type and seed germination in three plant species growing in salt marsh soils of semi-arid Mediterranean environments. *Arid Land Res. Manage.* 23, 103–114 (2009).

62 Association of Official Agricultural Chemists. *Official Methods of Analysis (18th Edition)*. Association of Official Agricultural Chemists, Gaithersburg, MA. USA, 47 (2005).

63 Jung HJ, Vogel KP. Lignification of switch grass (*Panicum virgatum*) and big blue stem (*Andropogon gerardii*) plant part during maturation and its effect on fiber digestibility. *J. Sci. Food Agric.* 59, 166–176 (1992).

▪ Websites

101 FAO. FAO land and plant nutrition management service. www.fao.org/ag/agl/agll/spush

102 MNRE. Ministry of new and renewable energy booklets on renewable energy www.Mnes.nic.in

103 Edwards L. Estimate of flowering plant species to be cut by 600,000. www.physorg.com/news204438872.html

104 Knothe G. Historical perspectives on vegetable oil-based diesel fuels. www.biodiesel.org/resources/reportsdatabase/reports/gen/20011101_gen-346.pdf

105 FAO, Commodities and Trade Division. Biofuels and commodity markets – palm oil focus. www.oilpalmworld.com/biofuels.pdf