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Halophytes: Potential source of ligno-cellulosic biomass for ethanol production

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ABSTRACT

World reserves of petroleum are being consumed rapidly and expected to exhaust by the middle of this century. This realization has led to the introduction of various grades of ethanol supplemented fuel. However, ethanol demands met from sources used for food may cause food shortage. This necessitates exploiting saline lands to produce non-food ligno-cellulosic biomass which, may be converted into ethanol without compromising human food production. Halophytes which produce plenty of biomass using saline resources (water and soil) may be an important alternative. This study shows that species like *Halopyrum mucronatum*, *Desmostachya bipinnata*, *Phragmites karka*, *Typha domingensis* and *Panicum turgidum* found in the coastal region of Pakistan, have potential as bio-ethanol crops. These perennial grasses are salt tolerant with high growth rates to produce ligno-cellulosic biomass of good quality (26–37% cellulose, 24–38% hemi-cellulose and <10% lignin) for ethanol production.

1. Introduction

Fossil energy resources (oil, gas and coal) are the main fuel of our industrial economies and consumer's societies but these are also responsible for the adverse effect of greenhouse gas emission on the environment. In addition, these resources are not sustainable and are depleting fast. Petroleum which provides about 44% of the energy requirement [1] along with gas at their current rate of consumption will last for about 35 years and coal is likely to be the only available source of energy for the next century [2]. These projections may not be very accurate but they ring alarm bells demanding remedial measures to offset a catastrophe looming on the horizon.

Supplementing petrol with ethanol has been practiced for some time but its production from species otherwise used for food by direct fermentation of sugar or by hydrolysis and subsequent fermentation of starch, generates debate on competition between food and fuel. There are adverse consequences, particularly for the poor, in terms of escalating prices for food in a world where population growth continues unabated. However, there are sources of material other than food crops having potential for conversion into ethanol. For instance, in most grasses and other species, cells containing secondary cell walls constitute the bulk of the support tissues and account for dominant fractions of lignin, hemi-cellulose and cellulose in the biomass [3]. Among these, lignin, because of association with phenolic acids (p-coumaric and ferulic acids), is not easily biodegradable and hinders in releasing sugars for subsequent ethanol fermentation [4]. This has prompted research to minimize lignin contents through genetic modifications [5]; selecting plants which are outside the human/animal food chain and already have low lignin may however, be an easier and a more desirable option.

Halophytes are distributed in inland and coastal salt marshes and deserts are reported as one of the most

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productive sources in terms of ligno-cellulosic biomass [6]. This invites particular attention in view of the facts that about 43% of earth land mass is arid or semi arid and 98% of its water is saline [7], over 800 million hectare of the world is affected by salinity [8], 45 million out of 230 million hectare of prime irrigated agricultural land has become saline and the menace is creeping into arable lands [9]. Cultivation of halophytes on these vast degraded saline lands by using huge resource of under-ground brackish water or even seawater in some cases would spare arable land and fresh water for conventional agriculture. If the candidate species are perennial, they have long canopy duration and carry further saving on planting cost required for the annuals [10]. Perennial trees and grasses are hence a better choice for this purpose [11]. Pakistan has about 410 halophyte species, both annuals and perennials [12] many of which could be used for ligno-cellulosic biomass production as source of material for biofuel. We report here some of these species from our seacoast which could be suitable for this purpose.

2. Materials and methods

2.1. *Sample collection*

Ligno-cellulosic biomass compositions of 23 halophytic species were examined (Table 1). These species were collected from different coastal and nearby saline areas of Karachi metropolitan (Sindh province) and adjoining Balochistan province of southern Pakistan to determine their potential to produce bio-ethanol. The plant material (stems and leaves) was air dried ground and stored in airtight plastic bags for subsequent analysis.

2.2. *Ligno-cellulosic analysis*

The ligno-cellulosic biomass analysis is related to plant fiber estimation. We used the method of AOAC [13] involving multifunction process for the separation of cellulose, hemi-cellulose and lignin from the other constituents of ligno-cellulosic biomass. The method involves estimation of Neutral Detergent Fiber (NDF) which accounts for the cellulose, hemi-cellulose and lignin content and represents most of the fiber or cell wall fractions in biomass. Acid Detergent Fiber (ADF) was determined sequentially using the residue left from NDF determination. The hemi-cellulose was calculated by subtracting ADF from NDF [14]. The NDF and ADF treated plant material was then hydrolyzed with 72% H_2SO_4 to determine cellulose. Lignin was obtained by ashing of hydrolyzed residue.

3. Results

Chemical testing was conducted on the species which were largely perennials (except for two annuals, *Cenchrus ciliaris* and *Dichanthium annulatum*) distributed in a 100 km radius around Karachi. The majority (13) of the species belonged to the Poaceae, five to Amaranthaceae and one each to Convolvulaceae, Ascelpiadaceae, Salvadoraceae, Tamaricaceae and Typhaceae. A wide variation in their height was recorded (Table 1). The quantity of cellulose, hemi-cellulose, and lignin also varied among the species (Table 2). *Suaeda fruticosa*, *Suaeda monoica*, *Arthrocnemum indicum* and *Salsola imbricata* contained low (around 8–11%) cellulose followed by *Calotropis procera*, *Tamarix indica*, *Ipomea pescaprea* and *Aerva javanica* (12–15%), while the rest contained >20% cellulose and the highest amount (37%) was found in *Halopyrum mucronatum*.

Table 1 – Potential of halophytic energy plants on the basis of surveys.

Species	Family	Distribution	Province	Habit	Life strategy	Height (cm)
1. *Aeluropus lagopoides*	Poaceae	Hawks bay	Sindh	Grass	Perennial	10–15
2. *Aerva javanica*	Amaranthaceae	University campus	Sindh	Shrub	Perennial	130–150
3. *Arthrocnemum indicum*	Amaranthaceae	Hawks bay	Sindh	Shrub	Perennial	50–100
4. *Cenchrus ciliaris*	Poaceae	Hab river road	Baluchistan	Grass	Annual	3–25
5. *Chloris barbata*	Poaceae	Hab river	Baluchistan	Grass	Perennial	100
6. *Calotropis procera*	Asclepiadaceae	University campus	Sindh	Shrub	Perennial	300
7. *Desmostachya bipinnata*	Poaceae	Abbas goth	Baluchistan	Grass	Perennial	200
8. *Dichanthium annulatum*	Poaceae	Hab river road	Baluchistan	Grass	Annual	15–85
9. *Eleusine indica*	Poaceae	Hab river road	Baluchistan	Grass	Perennial	15–85
10. *Halopyrum mucronatum*	Poaceae	Manora	Sindh	Grass	Perennial	100
11. *Ipomea pes-caprae*	Convolvulaceae	Hawks bay	Sindh	Climber	Perennial	100
12. *Lasiurus scindicus*	Poaceae	Hub river road	Baluchistan	Grass	Perennial	90
13. *Panicum turgidum*	Poaceae	Moosa goth	Baluchistan	Grass	Perennial	40–200
14. *Paspalum paspaloides*	Poaceae	Hub river road	Baluchistan	Grass	Perennial	5–30
15. *Phragmites karka*	Poaceae	University campus	Sindh	Grass	Perennial	500–700
16. *Salsola imbricata*	Amaranthaceae	University campus	Sindh	Shrub	Perennial	100–200
17. *Salvadora persica*	Salvadoraceae	Makran coastal highway	Baluchistan	Shrub	Perennial	15–90
18. *Suaeda fruticosa*	Amaranthaceae	University campus	Sindh	Shrub	Perennial	160–200
19. *Suaeda monoica*	Amaranthaceae	Makran coastal highway	Baluchistan	Shrub	Perennial	100
20. *Sporobolus ioclados*	Poaceae	Hawks bay	Sindh	Grass	Perennial	15–60
21. *Tamarix indica*	Tamaricaceae	Hawks bay	Sindh	Shrub	Perennial	200–600
22. *Typha domingensis*	Typhaceae	Hawks bay	Sindh	Grass	Perennial	200–400
23. *Urochondra setulosa*	Poaceae	Hawks bay	Sindh	Grass	Perennial	100–200

Table 2 – Ligno-cellulosic contents of halophytic biomass (% dry weight).

Name of species	Cellulose	Hemi-cellulose	Lignin
1. *Aeluropus lagopoides*	26.67	29.33	7.67
2. *Aerva javanica*	15.67	13.33	6.33
3. *Arthrocnemum indicum*	11.33	13.00	7.00
4. *Calotropis procera*	12.33	11.00	5.00
5. *Cenchrus ciliaris*	22.67	23.17	7.00
6. *Chloris barbata*	25.33	23.00	8.33
7. *Desmostachya bipinnata*	26.67	24.68	6.67
8. *Dichanthium annulatum*	19.00	24.33	7.00
9. *Eleusine indica*	22.00	29.67	7.00
10. *Halopyrum mucronatum*	37.00	28.67	5.00
11. *Ipomea pes-caprae*	12.67	17.00	5.33
12. *Lasiurus scindicus*	24.67	29.67	6.00
13. *Panicum turgidum*	28.00	27.97	6.00
14. *Paspalum paspaloides*	20.33	32.00	2.33
15. *Phragmites karka*	26.00	29.00	10.33
16. *Salsola imbricata*	9.00	18.33	2.67
17. *Salvadora persica*	22.00	13.33	7.00
18. *Sporobolus ioclados*	15.33	30.67	2.00
19. *Suaeda fruticosa*	8.67	21.00	4.67
20. *Suaeda monoica*	10.67	11.33	2.33
21. *Tamarix indica*	12.17	24.67	3.33
22. *Typha domingensis*	26.33	38.67	4.67
23. *Urochondra setulosa*	25.33	25.00	6.33

Hemi-cellulose was low (11–13%) in *A. javanica*, *A. indicum*, *C. procera*, *Salvadora persica*, and *S. monoica* while others had >20% hemi-cellulose with *Typha domingensis* containing exceptionally high (38.67%) hemi-cellulose. The lignin content in all the species was generally less than 10%. *H. mucronatum*, *Desmostachya bipinnata*, *Phragmites karka*, *T. domingensis* and *Panicum turgidum* appear promising potential source of ligno-cellulosic biomass which could be converted into ethanol. The detailed description of these plants follows.

P. karka (Retz.) Steud, a salt tolerant perennial halophytic grass, is one of the dominant species distributed in marshy habitats around Karachi attaining height up to 5–6 m similar to already reported biofuel crop *Miscanthus* which can grow up to 4–5 m height [15]. *P. karka* grows in stagnant water and is tolerant of flooding as well as salt. It is capable of producing more biomass for bio-ethanol production than most other conventional grasses of this area because of its fast growth rate. The seeds can germinate at 500 mM NaCl [16] and plant could subsequently grow under more saline conditions (Khan, unpublished data). With 26% cellulose, 29% hemi-cellulose and 10% lignin contents, it compares well with Bermuda coastal grass (*Cynodon dactylon*) which has 25% cellulose, 35.7% hemi-cellulose and 6.4% lignin [17].

H. mucronatum (L.) Stapf. is a perennial grass growing on sand dunes in the coastal areas of Pakistan where it attains a height of 1–2 m. *H. mucronatum* grows well while inundated regularly with seawater and produces seed twice a year [18], but the growth is optimum at 90 mM NaCl [19]. The biomass contains 37% cellulose, 28% hemi-cellulose and 5% lignin; these characteristics make it a better candidate for ethanol production than Switch grass (*Panicum virgatum*) which contains 45% cellulose, 31.4% hemi-cellulose and 12% lignin [17].

D. bipinnata (L.) Stapf. a tall perennial halophytic clonal grass with stolons sending culms up to 2 m height, is distributed around near coastal and inland areas of Sindh province. Its' seeds can germinate at >400 mM NaCl [20] and plants exhibit fairly good growth on saline and sodic soil where brackish water is available (Khan, unpublished data). *D. bipinnata* could produce ligno-cellulosic biomass containing 26% cellulose, 24% hemi-cellulose and 7% lignin making it a good biofuel candidate.

P. turgidum Forssk, a perennial halophytic grass is generally used as a good fodder but its chemical composition (28% cellulose, 28% hemi-cellulose and 6% lignin) also makes it a candidate for bio-ethanol production. Basically a desert grass widely distributed in Pakistan, *P. turgidum* is a versatile plant which can grow and thrive in variable weather conditions. It can tolerate 200 mM NaCl at germination stage [21] and grows in dense bushes through tillers, reaching up to 1 m in about 30 days. The capacity to produce biomass is high due to multiple harvests in a season.

T. domingensis Pers., found near coastal areas of Pakistan is a rhizomatous perennial with long, tender green stalks and brown flowers. It can tolerate perennial flooding, reduced soil aeration conditions and moderate salinity, therefore regarded as an indicator wetland plant. The conditions where perpetual supply of water and nutrients are available, *T. domingensis* becomes an aggressive invader in both saline and non-saline wetlands. Its' lush green leaves and tall fibrous stems produce biomass containing 26% cellulose, 38% hemi-celluloses and 4% lignin which may challenge any second generation species for biofuel production, for instance water-hyacinth (*Eichhornia crassipes*) which has 35–50% of hemi-cellulose [22] but with high (12%) tannin [23] which is undesirable for fermentation.

4. Discussion

The unstable but generally inflationary trends of world oil prices, driven by forces of demand and supply, have a crippling influence on the economies of many countries; Pakistan, for instance, spends a staggering US$ 3.1 billion annually on importing petroleum products. Efforts have lately been made to reduce the cost by blending petrol with suitable low-cost additives like ethanol [24] but this has created a dilemma of choosing between food and fuel as ethanol is conveniently produced by fermenting sugar. This puts undue pressure on the use of sugar producing crops; should they be used for food or fuel? Despite the skepticism shown by certain researchers about the limitations regarding the resource efficiency of producing ethanol from wheat/sugarcane/corn and biodiesel from rape seed [25]; bio-ethanol production from maize in USA has exceeded that of sugarcane in Brazil and steps are underway in the UK to build refineries which can use wheat grain for the same purpose [26]. It is noteworthy that improving efficiency of agricultural systems through better management may have the potential of reducing the area of land required for food production by as much as 72% by the year 2050 [27] and make resources available for other usages such as biofuel but population growth demands that food production must double if food security is to be maintained. There is also no denying that present day agriculture is under stress; crop yields are limited by an increasing variability in weather due to greenhouse effect that can exacerbate the hazardous effects of soil salinity and shortage of good quality irrigation water.

The feasibility of converting ligno-cellulosic vegetative biomass of plants into sugar, which is subsequently fermented to ethanol, opens new venues to tackle the problem of ‘food or fuel’ because the grain is spared for food in the process. Halophytes grow under conditions where both available water and soil are saline. Therefore use of halophytes as biofuel crop is advantageous because they do not compete with conventional crops for high quality soil and water and hence do not encroach on the resources needed for food crops [7]. United States alone has the potential to produce enough ligno-cellulosic biomass to supply 60 billion gallons of ethanol annually without displacing agricultural crops [5]. Halophytes may have several unique features ranging from distribution and growth habitat to aspects of composition that make them a potentially interesting bioresource for biofuels. It is evident (Tables 1 and 2) that some halophytes like *P. karka*, *P. turgidum*, *H. mucronatum*, *T. domingensis* and *D. bipinnata* which possess characteristics like high biomass with high cellulose/hemi-cellulose and low lignin contents, have the potential to replace the newly suggested ligno-cellulosic bioresource for producing bio-ethanol like *Buddleja davidii* which, contains 30% lignin, 35% cellulose, and 34% hemi-celluloses [28].

Bio-ethanol from ligno-cellulosic biomass is widely recognized as an environmental friendly and acceptable substitute for gasoline or as an additive to gasoline because it releases only that much CO_2 which it absorbed during photosynthesis. Selecting suitable species from non-food sources does away with the food vs. fuel dilemma to a great extent. Although not common as human diet, halophytes are a valuable alternate of cattle feed. *P. turgidum* for instance when fed green, has been proved to be as good as maize and *Panicum* hay can effectively replace wheat straw in cattle feed [29]. Similarly, *D. bipinnata* can supplement up to 75% wheat straw in cattle feed (Khan et al., unpublished data). Another advantage of using halophytes is their natural abundance in saline habitats; they are hence already well adapted to prevailing conditions and there is no danger of any adverse ecological consequences likely in introducing an alien species. The species of this study have several desirable attributes like being perennial not requiring fresh sowing every year which saves expenses. These species need minimum maintenance because they are hardy, generally free from pests and are highly productive in saline soils irrigated with salty water, some even with seawater. Little work has been reported on yield maximization of these perennial salt tolerant grasses and there is likely to be a room for improving their yield through proper agronomic management. The yield of *P. turgidum* could be $>$50 tons/acre/year [29] and it may further be improved through conventional breeding or more advanced molecular methods.

The conversion of ligno-cellulosic material into ethanol involves hydrolysis of cellulose through cellulase enzyme and fermentation of the sugar formed by yeast or bacteria. The cellulase enzymes are highly specific and a crucial step in the process of conversion. Lignin can be a limiting factor which can resist hydrolysis of biomass by saccharification and increases the cost of production. There is need to improve these processes to increase efficiency of conversion and decrease production costs [30]. The high cost of production could be offset by exploring the possibility of extracting other suitable high value chemicals like proteins which are present in abundance in leaves of many plants [31]. Our target species have desirable cellulose/hemi-cellulose and low lignin contents (Table 2) which can lead to more sugar yield and subsequently more ethanol production through fermentation. The species found promising in this study closely resemble coastal Bermuda grass (*C. dactylon*), Switch grass (*P. virgatum*) [23] and *Miscanthus x giganteus* hybrid [26]. However, it is noteworthy that Switch grass has undesirably high (12%) tannin while the *Miscanthus* hybrid is a non-halophyte and would hence compete with food crops for resources.

5. Conclusion

This research suggests that halophytes can compete favorably with other conventional sources for biofuel production. It provides an option of selecting perennial, high biomass plants that contain suitable ligno-cellulosic material for conversion into ethanol and can be grown without encroaching upon arable land and fresh water. These plants are abundant in nature, are outside the human food chain and require low maintenance which makes them relatively inexpensive to grow. Many interrelated aspects need investigation but information on ligno-cellulosic biomass potential of a few species on a particular regional site opens opportunity of selecting future bio-ethanol candidates. There is a need to investigate the possibility of breeding plants having desirable characters especially low lignin contents to minimize recalcitrance to bioconversion along with increasing biomass yields. Improvements in genetics, agronomy and the conversion process will undoubtedly help in the development of a feasible biofuel production system from halophytes which can enhance and improve the feedstock availability and efficiency of biofuel production.

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