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Testing of Some Halophytic Plants for Forage, Biofuel Production

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and Soil Bioremediation

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### Abstract

Rapid population growth in the developing countries of arid and semiarid regions and concomitant decline in productivity of agricultural lands due to the negative impact of climate changes, shortage of good-quality irrigation water and increasing soil salinity, are exerting enormous pressure on the dwindling supplies of human consumption for forage, food and fuels. Biosaline agriculture is a proper solution in this saline environment. It can facilitate the adaptation to the increasing salinization and decreasing availability of fresh water. To achieve the aforementioned objectives, a Field trial was carried out in salt affected soil around the Coast of Qaron Lake to evaluate the impact of irrigation with diluted saline lake water (12.5, 25, 37.5, 50, 62.5, 75, 87.5 and 100%) in addition to Fresh water, on total fresh productivity, chlorophyll a + b, proline, soluble carbohydrates, succulence, osmotic potential, nutritional value and the content of cellulose and hemi-cellulose of these halophytic forage plants for biofuel production as well as its role in bioremediation of the salt affected soil. All tested plants tolerated harvesting eight times per year and were capable of recovering and maintaining a fresh productive biomass up to 10.11 ton fed<sup>-1</sup>year<sup>-1</sup>. The value of crude protein varied between 11.03 to 11.45 %. It also contains cellulose and hemicelluloses varied between 21.65 to 28.64%, these cellulosic biomass can use for ethanol production. Successive cuttings of these halophytic plants improve soil quality. Leptochloa fusca followed with Sparina patents were more effective for soil bioreclaimation. In conclusion we can call these halophytic plants (Environmentally Smart Crops) because it did not compete with conventional food crops resources (arable land valid food crops production, fresh water) and produce new crops valid to be used as forage or fuel in salt affected habitats.

Key words: Halophytic plants, saline habitats, forage production, biofuel, soil bioremediation.

### **1 - Introduction**

The world's population is expected to reach 9 billion in 2050 [1]. This increase, together with accelerating urbanization, water scarcity, desertification and the negative impact of climate changes on fresh water resources, will exert upwards pressure on food, forage and fuel demand and critically undermine efforts for sustainable development [2]. With current climate warming and increased evapotranspiration, global salinization will steadily continue [3]. Moreover, currently at least 97% of the global water are seawater, 20 % of the world's irrigated land is salt affected and/or irrigated with waters containing elevated levels of salts [4]. Therefore, an integrated approach for solutions is required through economic, social and environmentally sustainable developmental opportunities [5]. Cultivation of halophytic plants seems to be an ideal management practice of such soil types, when fresh water is not sufficient [6]. Halophytic forage plants such as Leptochloa fusca, Sporobolus virginicus, and Spartina patens are highly salt tolerant halophytic forage plants grown well in coastal salt marsh [7]. It has a special place in newly emerging farming systems, especially in coastal areas and where freshwater resources are not available or in short supply. It environmentally smart crops because it can ensure food security, contribute to energy security, guarantee environmental sustainability, tolerate the impacts of climate change (water stress, salt stress and high temperatures), increase livelihood options, sequester CO2 and bioremediate salt affected soil [8]. Growing these plants can increase sustainable productivity, strengthen farmers' resilience, reduce agriculture's greenhouse gas emissions and cause transformation of agriculture, in the way we grow food, feed and biofuel and treat the environment. Thus freeing fresh water and high quality soil for food and feed and bringing poor land into production [9]. A wealth of halophytic flora exists which can be exploited for an array of uses like food, forage, fodder, fuel wood, oilseed, medicines, chemicals, landscaping, ornamentals, and environment conservation through carbon sequestration [10].

Bioenergy crops are crops capable of producing renewable energy from materials derived from biological sources. Many different perennial and annual halophytic plants can be included under this heading, including oil producing crops and crops as sources of lignocellulosic biomass [11]. However, a drawback to conventional biofuel crops is that they require the diversion of farmland, pastures, and rangelands from food to fuel production. Thus, a major issue in producing bioenergy crops is the interaction between food producing or energy production because there is a limited amount of arable

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land. That is why saline agriculture, an undeveloped source of both food and fuel, is so interesting [12]. A promising avenue is the production of biofuels from halophyte crops [13], as they can be produced on land that is not suitable for conventional agriculture.

The Egyptian flora comprises about 2300 species of which 80 are halophytic, belonging to 32 genera and 17 families [14]. There are some perennial grasses from these halophytic plants that are recommended as forage, fodder and biofuel crops using saline resources. These Halophytic plants are abundant in nature, are outside the human food chain and require low maintenance which makes them relatively inexpensive to grow. Flowers and Yeo (1995) [15] have advocated that it is perhaps cheaper, easier and may be more successful to domesticate a wild salt-tolerant species than modify an existing crop to get gainful returns from a saline environment. The aim of this paper is to domesticate these halophytic plants and to evaluate their potentiality of for producing biomass for forage, fuel and soil bioremediation in salt affected habitats.

### 2- Materials & Methods

Field trial was conducted during 2012-2013 seasons on salt affected farm on the Coast of Qaroun Lake to study the effect of saline water irrigation on fresh biomass production, some physiological aspects, nutritional values, cellulose, hemicelluloses and lignin of three halophytic plants namely: Leptochloa fusca, Sporobolus virginicus and Spartina patens, as well as the effect of the successive cuttings of these plants on soil bioremediation. Nine salinity levels of diluted salty water from the Lake (12.5%, 25.0%, 37.5, 50%, 62.5%, 75%, 87.5% and 100%) in addition to fresh water, were used in irrigation. Irrigation was carried out every 7 days with the specified treatment by mixing Lake water with fresh water in one cubic meter plastic tanks (irrigation with fresh water was applied for all treatments every 45 days for leaching). Chemical characteristics of irrigation water are presented in Table (1). Rhizomes of Leptochloa fusca, *Sporobolus virginicus* and *Spartina patens* were transplanted on April 7<sup>th</sup> at 2012. Each plant type was grown in three plots. Each plot was 4 m<sup>2</sup> and subjected to its specific salinity treatment. The mechanical and chemical analysis of the soil was carried before the experiment and after one year from transplanting using the standard method described by Klute (1986) [16], Table (2). Eight equal doses of calcium superphosphate  $(15.5\% P_2O_5)$ ,  $(48.0 \% K_2O)$  and urea (46.5% N) at the rate of 32 kg. P<sub>2</sub>0<sub>5</sub>/fed., 24 kg. K<sub>2</sub>O/fed. and 105 kg N/fed., respectively were added after each cutting. Eight cuttings were taken at 45 days intervals. Three replicates were taken for each treatment to determine fresh weight (Ton/fed). The following physiochemical as measurements were determined in the fresh harvested shoot of the fourth cutting: chlorophyll a+b (mg/g fresh weight) according to Von Wettstein (1957) [17], proline (µg/g) according to Bates et al., (1979), [18], Osmotic potential were then obtained from the corresponding values of cell sap concentration tables given by Gusev (1960) [19]. Then the harvested shoots were then dried to constant weight at 70° and the values of succulence (ratio of fresh weight/dry weight) were calculated according to Tiku (1975) [20]. Soluble carbohydrates content was also determined by the method described by Dubois et al., (1956) [21]. Crude protein (CP), crude fiber (CF), ether extracts (EE) and ash by standard analytical methods after AOAC (2005) [22]. Nitrogen free extract (NFE) was calculated by the following formula: % NFE = 100 -(%CP + %CF + %EE + %ash). ADF and NDF were determined as Komarek, (1993) [23]. The ligno-cellulosic biomass analysis is related to plant fiber estimation. We used the method of AOAC (2005) [22] involving multifunction process for the separation of cellulose, hemi-cellulose and lignin from the other constituents of ligno-cellulosic biomass. The obtained results were subjected to statistical analysis of variance according to Snedecor and Cochran (1982) [24].

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Table (1)	): C	nemical	analysis	of alluted	saline water	irrigation	of Qaroor	Lake
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Characters	Fresh water	12.5%	25.0%	37.5%	50.0%	62.5%	75.0%	87.5%	100.0%
pH	7.69	7.89	8.13	8.22	8.42	8.43	8.55	8.65	8.69
EC (ds/m)	0.75	6.99	13.69	19.58	25.87	32.01	39.02	45.98	53.65
Na (mg/L)	55.39	1625.36	3265.36	4885.36	6752.14	8235.65	10005.5	11528.4	13411.3
K (mg/L)	2.15	55.36	85.36	125.36	152.36	188.39	212.36	234.45	375.14
Cl (mg/L)	128.36	2455.56	4236.12	6655.25	8256.26	10600.4	12358.5	14705.4	16412.4
Ca (mg/L)	85.36	100.36	112.03	125.69	131.88	155.56	186.34	248.36	263.76
Mg (mg/L)	10.36	31.26	52.45	75.36	113.56	125.36	161.23	188.92	227.12

### **3 Results & Discussions**

#### 3.1 Role of halophytic plants on soil bioremediation

Soil analyses befor transplantion and one year after growing L fusca. S virgincus and S, patens are shown in (Tables 2), date show that values of Mg, K, organic C and percentage of silt were slightly decreased after one year of growing the three halophytic plants. However, L fusca recorded the highest values for the previous characters as compared with the other species. Whereas, Ec (electrical conductivity), HCO<sub>3</sub>, SO<sub>4</sub>, Cl, Ca, Na and percentage of sand increased by the end of the experiment with superiority to L fusca, However, pH values and percentage of clay were not affected. These results are in agreement with those obtained by Tawfik et al., (2013) [9]. In this concern, Zaharan et al., (1982) [25] observed that Juncus rigidus decreased the soil EC from 33 to 22 dS m<sup>-1</sup>. Singh et al., (1989) [26] also conducted a longterm field study on an alkaline soil in order to improve fusca. They concluded that the soil EC decreased from 2.20 to 0.42 dS m<sup>-1</sup>. Akhter et al., (2004) [27] stated that kallar grass (Leptochloa fusca) accomplished the best removal of salts but had very little beneficial effect on pH and SAR. Numerous suggestions have been advanced to remediate the effects of salts in the soil by some halophytic plant species by their ability to mitigate salts in soil solution by plant uptake as the most environmentally sustainable method in dealing with the saline-sodic condition. In this concern, Ravindran et al., (2007) [28] hypothesized that beneficial effects of plants in reclamation are not well understood but appear to be related to the physical action of the plant roots, addition of organic matter, increase in dissolution of CaCO<sub>3</sub> and mobilization of calcium that help reclaim soil sodicity and crop uptake of salts. They added that Suaeda maritima and Sesuvium portulacastrumexhibited greater accumulation of salts in their tissues as well as higher

such soils by growing Prosopis julifera and Leptochloa

reduction of salts in the soil medium. Furthermore, Ahmed (2010) [29] stated that, *Leptochloa fusca* behaved as a typical halophyte having both accumulating and excreting properties. He added that, the efficient salt excretion from the shoot makes it a useful plant to deplete excessive salt from the root – zone and to provide a better root–environment for the growth of other plants. Growing this plant increase air exchange, organic matter and hydraulic conductivity, decrease rhizosphere pH, stimulate biological activity, dissolve native CaCO<sub>3</sub>, enhance leaching of salts, lower the water table of waterlogged soils, release plant nutrients and the shoot

foliage can increase organic matter, humus and soil mulching, decrease surface evaporation and progressively improve soil physical properties. In our experiment, the halophytic grass *L. fusca*, *S. virginicus* and *S. patens* are very useful on salt-affected soils with superiority to *L fusca* as They can improve saline and alkaline conditions, they good biological method for the reclamation of salt affected soils so that many commercial and forage crops can be grown. They excrete salts through specialized glands and are therefore reasonably palatable to farm animals.

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Soil	Before transplantation		After one year of growing <i>L fusca</i>		After one year of growing <i>S virginicus</i>		After one year o f growing S patens	
abaractors								
characters	0-30 cm	30-60 cm	0-30 cm	30-60 cm	0-30 cm	30-60 cm	0-30 cm	30-60 cm
EC (m mohs/cm)	15.36	9.68	10.12	5.68	11.25	6.35	12.65	9.68
HCO3%	13.25	12.58	12.55	11.58	12.87	11.65	11.98	10.68
SO4%	80.47	60.25	71.65	55.36	73.68	56.84	72.65	54.98
Cl%	199.54	178.59	179.58	161.50	182.47	163.74	181.58	162.14
Ca (ppm)	78.58	75.94	73.65	70.85	74.54	71.58	73.69	71.09
Mg (ppm)	27.69	25.47	28.69	27.54	28.30	27.65	27.79	26.87
K (ppm)	1.89	1.77	2.02	1.89	1.99	1.87	1.91	1.79
Na (ppm)	299.68	221.58	266.36	180.98	271.68	187.69	270.31	185.48
pH	7.45	7.12	7.41	7.02	7.48	7.15	7.52	7.12
Organic C	2.15	2.01	2.56	2.41	2.66	2.51	2.44	2.30
Sand	22.36	23.48	21.25	22.35	21.58	22.47	21.45	22.48
Silt	16.25	15.35	17.21	16.35	17.02	16.14	16.89	15.87
Clay	61.39	61.17	61.54	61.30	61.40	61.39	61.66	61.65

#### 3.2 Effect of saline irrigation on total fresh productivity.

Data presented in fig (1) show that L fusca, S virginicus and S. patens behave like a true halophytes, highly tolerant of salinity. Growth performance seemed to appear significant tolerance to saline irrigation. However increasing saline irrigation concentration significantly increased fresh total productivity ( up to 25% in L fusca), (37.5% in S virginicus) and (50% in S patens). However, higher saline irrigation levels adversely affect the previous character. Similar results were obtained by Akhter et al., (2004) [27] who reported that low NaCl concentrations stimulate growth of some halophytic species. Such stimulatory effect of moderate salinity on growth of some halophytic plants may be attributed to improved shoot osmotic status as a result of increased ions uptake metabolism (Naidoo et al., 1995) [30]. Abdal (2009) [31], reported production of S. bigelovii of 11 Ton/ha based on seawater irrigation in sandy soils of coastal areas in Kuwait over a similar cycle duration. On the other hand, the reduction in growth and yield under high salinity levels could be attributed to the reduction in photosynthesis, disturbance in mineral uptake, protein synthesis or carbohydrate metabolism (Al-Garni, 2006) [32]. He added that in most halophytic species growth decreases gradually with the increase of salt rate in the culture medium above a critical threshold specific to each species. In addition, Ashour et al.(2004) [33] attributed the reduction in growth at higher salinity level to reduced turgor and high energy cost of massive salt secretion and osmoregulation. Similar results were obtained by Tawfik et al., (2011) [7] who reported that low NaCl concentrations stimulate biomass productivity of Sporobolus virginicus. Much literature has pointed out the potential of halophytes to be used as crops cultivated under saline conditions (Rozema and Flowers, 2008) [3]. Halophytic species may show enhanced growth at moderate salinity levels around 50-100 mM NaCl root zone salinity and are able to grow at salinity levels around half or even full strength seawater of around 500 mM NaCl (Flowers and Colmer, 2008) [34]. It has been reported that, regardless of the salt concentration used, salt stress had different degrees of inhibition on the growth of plants (Sevengor et al., 2011) [35]. In the present study, medium concentrations of saline irrigation promoted fresh productivity. Similar performances were also found by (Qi et al., 2005 and Li et al., 2006) [36 & 37] who found that the growth of Suaeda salsa increased significantly with NaCl concentrations when exposed to hyposaline environment. As for the inhibition of high salt stress on growth of halophytes, it could be attributed to decreases in cell metabolism and the toxicity of Na<sup>+</sup> that caused irreversible damage due to prolonged exposure to high concentrations of NaCl (Shabala et al., 2009) [38]. Chookhampaeng (2011) [39] stated that, high Na<sup>+</sup> and Cl- ions concentrations in the external solution of plant cells will produce a variety of negative consequences which lead to ionic imbalance, a continual damage on function and structure of cell membrane and leading to membrane dysfunction and cells death.



## 3.3 Effect of saline irrigation on biochemical composition and some physiological aspects.

Saline irrigation affects the studied parameters in different ways. Data presented in Fig (2 - 6) show that raising irrigation salinity levels significantly increase the content of soluble carbohydrates, proline and osmotic

potential values in the tested halophytic plants. On the other hand moderate saline irrigation (37.5 and 50% saline irrigation) generally increased succulence values. High levels of saline irrigation decreased the content of chl. a+b in L. fusca and S. patens. Similar results were obtained by Youssef (2009) [40]. In this concern, Munns, (2002) [41] stated that, reduction of photosynthesis under high levels of salinity can be due to biochemical and destructive reactions becauses the ion accumulation in different parts of the plant exerts toxic effects on physiological processes in plant. Moreover, Youssef and Al-Fredan (2008) [42] suggested that, under high salt stress plant cells decrease their osmotic potential by accumulation of some solutes such as proline and soluble sugars. Sugars, in addition to the role of regulating osmotic balance, also act as the metabolic signals in the stress conditions (Munns and Tester 2008) [43]. Proline shows an indirect protective function due to its antioxidant properties in addition to the direct effect to stabilize the macro-molecules and their hydration layers (Bohnert et al., 2004) [44]. Murphy et al., (2003) [45] suggested that both proline and soluble carbohydrates act as compatible solutes under high salinity levels. Kusaka et al., (2005) [46] added that, the observed increase in the osmotic potential might be due to the accumulation of inorganic solutes, several organic components such as sucrose, glucose, quaternary ammonium compounds, and amino acids including proline. Furthermore, Munns (2003) [47] proved that, higher concentrations of carbohydrates in response to salinity are probably due to reduced growth. He added that, Excessive sodium ions at the root surface disrupt plant potassium nutrition. Because of the similar chemical nature of sodium and potassium ions, sodium has a strong inhibitory effect on potassium uptake by the root. Potassium deficiency inevitably leads to growth inhibition because potassium, as the most abundant cellular cation, plays a critical role in maintaining cell turgor, membrane potential and enzyme activities. In our study proline and soluble sugars content increased significantly in the leaves of all cultivars as the salt concentration increased. Osmotic adjustment by accumulation of osmolytes is an important adaptation of halophytes to counter physiological drought imposed by salinity (Flowers and Colmer, 2008) [34]. Halophytes are reported to accumulate organic osmolytes such as proline, glycinebetaine and sugars mainly in cytoplasm for osmotic adjustment without impairing metabolic activities (Debez et al., 2010) [48]. Accumulation of these organic osmolytes in cytoplasm compartmentalization in the vacuole contributes significantly in overall water relations of halophytes to obtain water from saline soils.





(µg.g<sup>-1</sup>) LSD 5%: 29.23



carbohydrates % LSD 5%: 2.88



5%: 0.13



## 3.4 Nutritional values of different plant species grown under 50% Lake water irrigation

Current knowledge of the nutritive value of halophytic plants in Egypt is limited. Halophytic plant species vary considerably in their chemical composition and nutritive value. Climate factors, i.e. temperature, humidity precipitation and light intensity play an important role in controlling the nutrient contents and nutritive value of plants as they affect assimilation, photosynthesis and metabolism. Data in Table (3) showed that *L fusca, S virginicus* and *S patens* contained

moderate amounts of crude protein (CP) which it seems fair enough to cover the nitrogen requirements of grazing animals. They also contained high levels of fiber and ash contents, which could limit intake, and digestibility of such forages. Similar results were obtained by (El Shaer *et al.*,2004) [49]. Ether extract (EE), nitrogen free extract (NFE), acid detergent fiber (ADF) and neutral detergent fiber (NDF) varied considerably among the different species. Table (3) also shows that the highest values for ash, NDF, EE and NFE were recorded in *L fusca*, while the highest values CP was recorded in *S virginicus*. On the other hand, the highest values for CF and ADF were recorded in *S patens*. It is reported that fibrous materials and ash contents of halophytic feed materials are higher and increase while gross energy and protein contents are low and decrease with advancing maturity (Kandil and El Shaer, 1988) [50]. Based on data in Table (3), it seems that these halophytes could cover the essential nutrients for maintenance requirements of small ruminants according to the recommended nutritional requirements of livestock in Egypt as indicated by Kearl (1982) [51]. Halophyte plants spreading in Egyptian desert and coastal areas are considered as a source of forage and fodder because of their moderate content of protein (El Shaer *et al.*, 2005) [52].

Table 3: Nutritiona	l values in diffe	rent plant spec	cies under 50%	Lake water irrigation

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Plant species	CP%	CF%	Ash%	ADF%	NDF%	EE	NFE
Leptochloa fusca	11.23	29.36	29.64	23.65	13.69	2.56	27.21
Sporobolus virginicus	11.45	29.56	29.58	23.68	13.36	2.44	26.97
Spartina patens	11.03	31.02	28.36	24.36	13.23	2.42	27.17
LSD 5%	0.55	1.21	1.82	1.76	0.864	0.11	2.55

# 3.5 Lignocellulosic biomass in different plant species grown under 50% lake water irrigation.

L fusca, S virginicus and  $\overline{S}$  patens have desirable cellulose/hemi-cellulose and low lignin contents ( Table, 4) which can lead to more sugar yield and subsequently more ethanol production through fermentation. It is evident from Tables (4) that the highest values for cellulose amounted to (28.64%) and hemi-cellulose amounted to (24.87%) were recorded in S virginicus, while the highest value of lignin amounted to (10.36%) was recorded in L fusca.. Similar results were obtained by Xianzhao et al., (2012) [53], who stated that, bioethanol is probably the most ideal approach to solve energy crisis in the recent future. At present bioethanol is mostly produced from carbohydrate-rich plants.In this regard, Xianzhao et al., (2013) [13] mentioned that in coastal zone, there are some halophytes such as Helianthus tuberosus, Tamarix chinensis, Achnatherum splendens, Phragmites australis and so on, which can be irrigated directly with seawater and have great potential to produce fuel alcohol. They added, the widely coastal tidal flat can offer excellent environmental conditions for fully utilizing and cultivating halophytic energy plants at a large spatial scale, which will have a great potential of supplying bio-energy. The feasibility of converting lignocellulosic vegetative biomass of halophytic plants into sugar, which is subsequently fermented to ethanol, opens new venues to tackle the problem of 'food or fuel. 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 (Rozema and Flowers, 2008) [3]. 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. The conversion of lignocellulosic material into ethanol involves hydrolysis of cellulose through cellulase enzyme and fermentation of the sugar formed by yeast or bacteria. 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. In this concern, Abideen *et al.*, (2011) [54] stated that, Bio-ethanol from lignocellulosic 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. Ling (2010) [55] added that, Most of energy halophytic species with the properties of drought tolerant, saline-resistant and high net productivity are found to be suitable for growing in salt affected soil.

Table 4: Content of cellulose, hemi-cellulose and lignin in different plant species (Under irrigation with 50% soling water in the fourth cutting)

Plant species	Cellulose %	Hemi-cellulose %	Lignin %
Leptochloa fusca	27.32	23.65	10.36
Sporobolus virginicus	28.64	24.87	9.36
Spartina patens	24.69	21.65	10.02
LSD 5%	1.98	1.78	0.65

## 4 Conclusions

There is no denying the fact that salt-affected lands and water are precious resources available to mankind for beneficial exploitation. Saline agriculture can facilitate the adaptation to the increasing salinization and decreasing availability of fresh water. So that, many salt affected soils considered to be unsuitable for agriculture, can be turned into productive agricultural areas. These plants do not compete for good quality water and productive farmlands. They can be potentially used to produce huge amounts of biomass while grown with brackish water on saline land, without competing with conventional agriculture. In conclusion we can call this halophytic plant (Environmentally Smart Crops). Cultivation of halophytes on these vast coastal saline lands by using huge amounts of seawater in some cases would spare arable land and fresh water for conventional agriculture.

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