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Review Paper

Study of some economically important under-utilized crops for cultivation on wastelands and biotechnology approaches for propagation and gene cloning

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Abstract

With an ever increasing population, there is a very rapid depletion of natural resources. Degradation of land, which is a non-renewable resource, often occurs under conditions of rapid growth of human population. Consequently, land available for primary production of biomass is getting more scarce. Therefore, it has become very necessary to explore some plant resources which can be cultivated on wastelands and tackle the problem of land degradation. Focus should be on some under-utilized but potential industrial crops like Jojoba *(Simmondsia chinensis (Link) Schneider),* Jatropha *(Jatropha curcas Linn.),* Colocynth *(Citrullus colocynthis (L.) Schrad.),* Guayule *(Parthenium argentatum Gray),* Paradise tree *(Simarouba glauca DC.),* which are lesser known species in terms of trade and research but highly economically useful and also well adapted to stress conditions. These crops being desert shrub and semi-xerophytic in nature, require less water and can tolerate saline as well as alkaline soils. Such crops are very useful for sustainable development of wastelands as these can be cultivated at large-scale on degraded lands. In these economically important crops, biotechnological approaches can be very useful for their mass propagation and cloning of genes coding for economic important traits.

Keywords: Sustainable development, degraded lands, micropropagation, gene cloning, semi-xerophytes

Introduction

It has been anticipated that the requirement of food grains will be around 250 million tonnes by the year of 2020, which means an extra 72 million tonnes of food grains are to be produced [1]. However scarcity of cultivating land due to rapid human population growth has raised fears regarding starvation and malnutrition. Currently, around 25% of all land is highly degraded, 36% is moderately degraded and only 10% is improving [2] (Figure 1). Therefore, focus should be on agriculture under stress situations like marginal lands. Wastelands are the degraded, under-utilized lands which are ecologically unstable with complete loss of top soil and are generally not suitable for cultivation. Wastelands include areas affected by salinity, water logging, ravine, sheet and gully erosion, riverine lands, alkalinity, shifting cultivation, shifting and sand dunes, wind erosion, extreme moisture deficiency, coastal sand dunes etc. There is declination in the productivity of wastelands as plants are encountered by abiotic stresses like water stress (draught and flood), temperature stress (high and low temperature), salt stress, nutrient stress, heavy metal contamination etc. However, when properly managed, these wastelands can be utilized and remediated to solve the problems of hunger and malnutrition.

Vegetation effects

To prevent erosion or soil loss, vegetation cover or green manure is used, as presence of vegetation can encourage infiltration of water into soil. It has also been shown that the rate of erosion and runoff decreases exponentially with increased vegetation cover. Of these fertility restoring plants, the Leguminous plants which extract nitrogen from the air and fixes it in the soil, and food crops/trees as grains, barley, beans and dates are the most important. However, green manures should also provide better opportunities such as food, fodder, income etc. as farmers want to plant something that not only fertilizes the soil but also be used as cash crop.

There are some under-utilized but potential industrial crops like Guayule (for rubber), Jojoba, Colocynth, *Jatropha* (for industrial oil), Paradise tree (for edible oil) which can be cultivated on wastelands as they are well adapted to stress conditions, require less water, can tolerate saline and alkaline soils. Biotechnological approaches like micropropagation, gene cloning open great possibilities for cultivation and utilization of these plants. Economic important traits, traits related to stress tolerance can be further enhanced by genetic engineering techniques. Several biotechnological studies related to micropropagation, gene cloning and stress tolerance in these plants are shown in table 2 & 3.

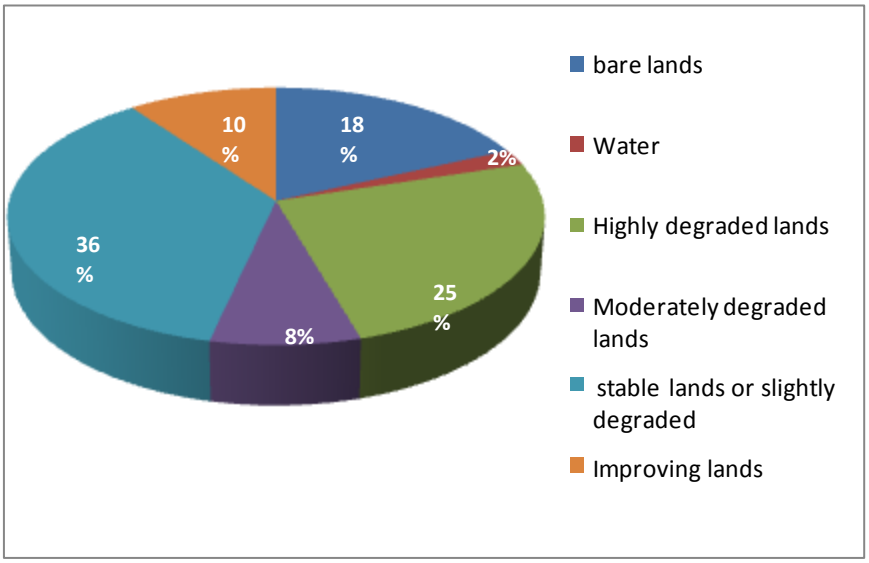

Figure 1: Status of global land degradation [2]

Jojoba (*Simmondsia chinensis* (Link) Schneider)

Jojoba (*Simmondsia chinensis* (Link) Schneider), also known as bucknut, jojove, goat nut, deer nut, pignut, wild hazel, quinine nut, coffee berry, gray box bush, lemonleaf [3,4] is the sole species of family Simmondsiaceae, placed in the order Caryophyllales. This plant is native to the Sonoran Desert of Arizona, Northern Mexico, now grown commercially in Australia, Argentina, Chile, Peru, Egypt and Israel. It is a dioecious, perennial, evergreen, arid xerophytic shrub which can tolerate saline, alkaline soils and drought conditions. It is an environment friendly crop, needs minimal agricultural practices especially pesticides treatments. As this plant is with a deep root system and low water requirement, it can be easily adapted to many types of arid regions in the world and can be grown on highways, roadsides, hedges. Unlike most of the xerophytic plants, jojoba has larger and broader leaves, which it usually retains year round and seeds have thin coat.

Uses of jojoba

Jojoba is considered to tolerate fairly high levels of salinity, water stress and it can help in sustainable development of wastelands. However, this plant is mainly grown for the characteristic liquid wax (also known as jojoba oil) present in its seeds which have various potential uses like substitute to sperm whale oil, high temperature lubricants for high-speed machinery, for extreme pressure lubricants

(sulfurized jojoba oil), treatment of leather, factice for rubber, in varnishes, as polishing wax (by hydrogenation into hard wax), in fruit coating, in carbon paper, as candles, in soap making, numerous pharmaceutical uses, in dietectic salad oil, in cosmetics, hair oils, resins, plasticizers, evaporation retardants, softening agents for textiles, linoleum or chewing gum, sources of straight chain alcohols and acids which are produced as intermediates in the production of several other products. Unlike, other seed oils which are triglycerides, jojoba oil is different, made up of liquid wax ester composed of a long alcohol chain to which one molecule of fatty acid is attached [5].

Table 1: Biotechnological studies related to micropropagation, gene cloning

1. Jojoba

Micropropagation of Jojoba by *in vitro* seedling culture and by shoot regeneration *via* organogenesis	Llorente et al. 1998 [15], Roussos et al. 1999 [10], Agrawal et al. 2002 [11], Singh et al. 2008 [16]
Production of liquid wax by zygotic and somatic embryos of Jojoba with promising results was reported	Wang and Janick 1986 [17]
Cloning of a cDNA coding for KCS from developing embryos of Jojoba, involved in microsomal fatty acid elongation was reported	Lassner et al. 1996 [18]
The soil born *Agrobacterium rhizogenes* bacteria was utilized to induce root formation	Benavides and Radice 1998 [19]
Complete plantlets with 1-2 roots each were obtained from *in vitro* shoots cultured on MS media supplemented with NAA and BA	Sardana and Batra 1998 [20]
Introduction of KCS genes cloned from *S. chinensis* or *B. napus* into rapeseed mutants with low erucic acid content showed complementation of the Canola fatty acid elongation mutation (*fae*) leading to the restoration of erucic acid synthesis in transgenic rapeseed	Luhs et al. 1998 [21]
Jojoba WS was cloned from developing embryos of Jojoba in combination with jojoba fatty acyl-coA reductase (FAR) and a KCS from *Lunaria annua* (a plant that accumulates large amounts of very-long-chain fatty acids in its seed oil), was expressed with high levels of wax in transgenic Arabidopsis seeds	Lardizabal et al. 2000 [22]
Efficient micropropagation protocols were developed for elite male and female genotypes of *Simmondsia chinensis* using nodal segments	Tyagi and Prakash 2004 [23]
Heterologous wax ester biosynthesis was established in a recombinant *E. coli* strain by coexpression of a fatty alcohol-producing bifunctional acyl-coenzyme A reductase from jojoba and a bacterial wax ester synthase from *Acinetobacter baylyi* strain ADP1, catalyzing the esterification of fatty alcohols and coenzyme A thioesters of fatty acids	Kalscheuer et al. 2006 [24]
The factors influencing rooting of *in vitro*-derived shoots of selected jojoba clones were studied	Bashir et al. 2007 [25]
Influence of plant growth regulators, sucrose, genotype of the donor explants on induction of somatic embryogenesis as well as factors involved in germination	Aly et al. 2008 [26]
The effect of substrate, medium composition, irradiance and ventilation on jojoba plantlets at the rooting stage of micropropagation were described	Mills et al. 2009 [27]

About 90% of world jojoba production is used for cosmetic products like hair, skin care products, bath oils, soaps etc. Jojoba wax is very useful in alleviating minor skin irritations, in diets to decrease cholesterol level, stimulating hair growth, rejuvenation, as oxidant due to alpha tocopherol found in

the leaves. Jojoba oil contains Omega-3 fatty acids which are essential fatty acids (as human body cannot synthesize them but highly required for good health), provide vitality and reduce cholesterol levels in humans, may play an important role in the treatment of Alzheimer's disease, depression, eczema, high blood pressure, attention deficit hyperactivity disorder (ADHA). Jojoba meal is the by-product of seed oil extraction, rich in protein and has potential as a feed for live stock [6].

Simmondsins, a glycoside, cyano-methylene-cyclohexyl glycoside are anti-nutritional components, present in jojoba oil, cake resulting from the oil extraction process and it functions as an appetite suppressant rather than a toxin. Jojoba oil is also registered as a biopesticide and is very effective against white flies on all the crops and powdery mildew on grapes and ornamentals as it forms a physical barrier between the insect pest and the leaf surface. Jojoba oil is safe for environment and non-target organisms when used directly. Jojoba oil does not break down under high pressure or temperature, can replace diesel oil as their physical and chemical properties are similar. However, jojoba oil is biodegradable, nontoxic, environment friendly as it contains less carbon than fuels like diesel, consequently lower emissions of CO_2, CO gases. Unlike diesel, jojoba oil contains no sulphur which eliminates harmful sulphur oxides, corrosive sulphuric acid leading to longer engine life. Also jojoba oil is safer for use as they have higher flash point than that of diesel oil.

Biotechnology approaches for propagation, gene cloning

The major problem in the seed production of jojoba is that being a dioecious crop, its sex is not determined prior to flowering (3-4 years from cultivation). Clonal propagation of elite individuals of known sexuality is necessary to ensure that the plants in commercial plots will be productive [7]. Asexual propagules in commercial jojoba plantations are very advantageous as plant growth, yield is predictable and uniform [8]. Vegetative propagation can be achieved by rooting semi-hardwood cuttings, but the maximum number of possible propagules is limited by plant size and time of planting [9]. Selective breeding of jojoba aims mainly at developing plants that have high oil content, large seed, flowers at every node, seeds in cluster, early flowering to escape frost damage, starting of early seed production. Tissue culture techniques have been applied only to a limited extent in Jojoba [10,11].

2. Jatropha

Plant regeneration in *J. curcas* has been accomplished through organogenesis from various explants, including: mature leaf petiole and hypocotyls axillary node via somatic embryogenesis from mature leaf explants	Sujatha et al. 2005 [28], Deore and Johnson 2008 [29] Sujatha and Mukta 1996 [30] Sujatha et al. 2005 [28], Shrivastava and Banerjee 2008 [31] Jha et al. 2007 [32]
Agrobacterium-mediated transformation of *Jatropha* using cotyledonary leaf explants was reported	Li et al. 2007 [33]
A full-length cDNA of the carboxyltransferase (accA) gene of acetyl-coenzym A (acetyl- CoA) carboxylase from *Jatropha curcas* was cloned and sequenced	Xie et al. 2009 [34]
A method for rapid and efficient plant regeneration from shoot apices, and generation of transgenic plants by direct DNA delivery to mature seed-derived shoot apices of *Jatropha* has been developed	Purkayastha et al. 2010 [35]
Effect of age and orientation of the explant on callus induction and de novo shoot regeneration from cotyledonary leaf segments of *J. curcas* has been studied	Mazumdar et al. 2010 [36]
An efficient Agrobacterium-mediated genetic transformation and plant regeneration protocol for *J. curcas* using leaf explants has been reported	Mazumdar et al. 2010 [36], Kumar et al. 2010 [37]
A *Jatropha curcas* casbene synthase homolog (JcCSH) with high sequence similarity to casbene synthases from *Ricinus communis, Euphorbia esula* & *Sapium sebiferum* was cloned from *Jatropha* leaf tissue.	Nakano et al. 2012 [38]

However, Plant regeneration via tissue culture is an important tool in mass propagation, mutant selection and genetic transformation as growth of jojoba plants from tissue culture is more vigorous than seedling and rooted cuttings and also mass production of pathogen free clones can be obtained for commercial plantations [12]. Biochemical and molecular based techniques are very helpful in identification of sexuality of plant at early seedling stage also in selection of plants and cell lines characterized with high quality/ quantity fattyacids. Regarding biotechnological studies related to stress tolerance, impact of treated wastewater on growth and physiology of Jojoba seedlings has been observed by Hussain et al. (2011) [13]. The role of carbohydrates to salinity tolerance has also been investigated by Roussos et al. (2005) [14]. Several other biotechnological studies related to Jojoba are shown in table 1 & 2.

3. Guayule

Development of tissue culture techniques for asexual propagation of guayule	Radin et al. 1982 [39], Lovelace et al. 1982 [40]
The major protein of guayule rubber particles is a cytochrome P450. Characterization based on cDNA cloning and spectroscopic analysis of the solubilized enzyme and its reaction products.	Pan et al. 1995 [41]
Cloning, characterization, and heterologous expression of cDNAs for farnesyl diphosphate synthase from the guayule rubber plant which reveals that this prenyl transferase occurs in rubber particles.	Pan et al. 1996 [42]
Several genes encoding enzymes and proteins associated with rubber synthesis have been cloned. This includes the major guayule rubber particle protein (RPP) gene, and a 24 kDa protein tightly associated with the so-called small rubber particle protein (SRPP)	Backhaus and Pan 1997 [43] Kim et al. 2004 [44]
A simple, efficient protocol for *in vitro* micropropagation of guayule is reported.	Castillon and Cornish 2000 [45]
Various allylic diphosphate synthetase genes were introduced in tissue-culture generated transgenic guayule plants of the USDA lines AZ 101, AZ-2 and N6-5	Veatch et al. 2005 [46]
A new method for guayule tissue culture, using low light and ammonium has been developed	Dong et al. 2006 [47]
Overexpression of 3-hydroxy-3-methylglutaryl coenzyme A reductase in guayule has been reported	Dong et al. 2013 [48]

4. Paradise tree

In vitro shoot multiplication of *Simarouba glauca*	Rout and Das 1994 a & b [49,50], Rout et al. 1999 [51]
Callus induction in *Simarouba glauca*	Hadke et al. 2008 [52]
Determination of sex in *Simarouba glauca* through molecular marker for improved production	Prasanthi et al. 2011 [53]
Study of *in vitro* multiplication system in *Simarouba glauca*	Dudhare et al. 2014 [54]

5. Colocynth

Efficient plant regeneration via organogenesis	Ntui et al. 2009 [55]
Higher content of total cucurbitacins and cucurbitacin-E have been attained in colocynth callus culture as a result of the impact of different combinations of growth regulators	Hegazy et al. 2010 [56]
Callus formation, phenolics content and related antioxidant activities in tissue culture of *Citrullus colocynthis*	El-baz et al. 2010 [57]
Appraisal of secondary metabolites in *in vitro* cultures of *Citrullus colocynthis*	Tanveer et al. 2012 [58]
In vitro plant regeneration and assessment of genetic fidelity using ISSR and RAPD primers	Verma et al. 2012 [59]
High frequency plant regeneration from shoot tip explants of *Citrullus colocynthis* (Linn.) Schrad.	Meena et al. 2014 [60]

Table 2: Biotechnological approaches related to stress tolerance

1. Jojoba

A fragment of 837 bp cDNA designated as ScRab encoding a full length 200 amino acid long polypeptide (homologous to the Rab subfamily of small GTP binding proteins) was isolated from shoot cultures of the salt tolerant jojoba, and cloned in *E. coli*, where the protein was expressed. The same group isolated a cDNA fragment from salt stressed jojoba shoots chloroplasts post-transcription RNA. They reported that salinity stress inhibited the post-transcriptional processing of chloroplast 16S rRNA	Mizrahi-Aviv et al. 2002 [61]
The role of carbohydrates on the salt tolerance of jojoba explants *in vitro* was reported	Roussos et al. 2005 [14]
Evaluation of Jojoba Seedling Growth and Physiological Response to treated wastewater Regime	Alrababah et al. 2011 [62]
Impact of brackish water on growth of jojoba	Hussain et al. 2011 [13]
Growth and biochemical responses of jojoba explants cultured under mannitol-simulated drought stress *in vitro*	Roussos et al. 2013 [63]
Effect of water stress on vegetative growth and some physiological aspects of Jojoba [*Simmondsia chinensis* (Link) Schneider] in newly reclaimed sandy soil	Hussein et al. 2013 [64]

2. Jatropha

Role of Aquaporin JcPIP2 in drought responses in *Jatropha curcas*	Zhang et al. 2007 [65]
Development of transgenic plants in *Jatropha* with drought tolerance. The first one overexpresses the *PPAT* gene, which encodes an enzyme that catalyzes the CoA biosynthetic pathway, the second overexpresses the *NF-YB* gene, which encodes a subunit of the NF-Y transcription factor, and the last overexpresses the *GSMT* and *DMT* genes, which encode enzymes that catalyze production of glycine betaine.	Tsuchimoto et al. 2012 [66]

1. Guayule

Stress induced proteins in *Parthenium argentatum* leaves were reported	Sundar et al. 2003 [67]

2. Colocynthm

Cloning and expression analysis of rboh gene encoding respiratory burst oxidase in *Citrullus colocynthis*	Si et al. 2008 [68]

***Jatropha* (*Jatropha curcas* Linn.)**

Jatropha curcas Linn., also known as Barbados Nut, Purging Nut, Physic Nut is a perennial, drought tolerant shrub or small tree of family Euphorbiaceae, which is native of tropical South America and Africa but later on distributed to other parts of the world by the Portuguese settlers [69] via the Cape Verde Islands and Guinea [70]. *Jatropha* exhibits a wide tolerance to different soils, climates and it can

be cultivated on marginal and degraded lands or on any type of soil (gravelly, sandy or saline, stony soils, rock cerevices). Being drought tolerant plant, *Jatropha* can be cultivated with success in areas with scanty rainfall [69] and it can be used to reclaim degraded areas [71,72,73]. The drought tolerance is due to well developed much branched secondary root system which often penetrate deeply to take maximum advantage of soil moisture. *J. curcas* is an economically important species and adds to the capital stock of the community, for sustainable generation of income and employment [74].

Uses of *Jatropha*

Jatropha curcas is suitable for cultivation on degraded lands in the arid and semi-arid regions. Its seeds contain, moisture 6.62%, protein 18.2%, fat 38.0%, carbohydrates 17.3%, fibre 15.50%, and ash 4.5% [69]. In seeds, the oil content is 35 to 40% whereas in the kernel, the oil content is 50 to 60% [69] and the oil contains 21% saturated fatty acids and 79% unsaturated fatty acids [69]. Non-edible oil which is present in the seeds is used in making soap, candles, varnish and as lubricant, hydraulic oil etc [75,76]. *Jatropha* oil is environmentally safe, cost-effective, renewable source of non-conventional energy and a good substitute for kerosene, diesel and other fuel oils [77]. The oil cake which is left behind after the extraction of oil from the seeds is an excellent organic manure as rich in nitrogen, phosphorous and potassium and can also be used in Bio-Gas production. Various parts of this plant are of medicinal value i.e. its latex contains an alkaloid known as “jatrophine” which is believed to have anti-cancerous properties. It is also used as an external application for piles. Its roots are used as an antidote for snakebites. The leaves are used for fumigating houses against bed bugs [78]. Technologies are now available, whereby it could be possible to convert *Jatropha* oil into edible oil which could prove to be a boon for developing countries [69]. In general, the oil is reported to be mixed with groundnut oil for adulteration. This indicates the possibilities of obtaining edible oil from *Jatropha* oil base [69].

Biotechnology approaches for propagation, gene cloning

With the help of tissue culture techniques, various plant tissues and organs including leaves, embryos, anthers, ovary, ovules, pollen and other tissues can be cultured *in vitro.* These techniques are also very helpful in breeding processes in *Jatropha*. By using *in vitro* cloning techniques, uniform performance among parental lines can be maintained. Micropropagation of *J. curcas* by shoot multiplication and regeneration from different tissues has been reported by various authors [79,30,80,29]. For large scale propagation of elite genotypes of *J. curcas*, somatic embryogenesis has also been demonstrated [32]. Transgenic plants in *J.curcas* containing genes for aquaporin (JcPIP2), betaine aldehyde dehydrogenase (JcBDI) which play important role in its rapid growth under dry and saline conditions has also been produced using Genetic Engineering. For the first time, establishment of high efficient method for genetic transformation via *Agrobacterium tumefaciens* infection of cotyledon disc was done by Li et al. (2006) [81]. Biotechnological approaches related to stress tolerance in this plant are shown in table 1, 2.

Guayule (*Parthenium argentatum* Gray)

Guayule (*Parthenium argentatum* Gray) is a small, low branching perennial desert shrub in the family Asteraceae. It is native to the deserts of the South Western United States, Northern Mexico and it can also be found in the US states of New Mexico, Texas and the Mexican states of Zacatecas, Coahuila, Chihuahua, San Luis Potosi, Nuevo Leon and Tamaulipas. This low water requiring plant is well adapted to hot desert environment and it can also grow well on waste lands, marginal areas of semi-arid regions. From this industrial crop, natural rubber, latex, ethanol, non-toxic adhesives and other chemicals are extracted. This plant is resistant to many pests and diseases as it produces terpene resins which are natural pesticides. Products obtained from Guayule are bio-degradable and are substitutes for many synthetic, petroleum based products which are harmful to the environment and also expensive to dispose of. Guayule is an economically viable bio-fuel crop and has benefit over food crops as bio-fuel as it can be grown in areas where other food crops would fail. Natural rubber latex is the first commercial product made from Guayule which is safe for people who are sensitized or allergic to natural latex from tropical sources as it is free of tropical proteins.

Uses of Guayule

Guayule is a perennial plant that can be cultivated in semi-arid regions [82] with maximum productivity of 2,000 kg ha-1 year-1 has been reported [83]. This plant can be grown well on wastelands and marginal lands with relatively low nutrient concentration. With the development of new clean technologies it is possible to extract various important products like natural rubber, latex, ethanol, non-toxic adhesives. Guayule is the only species other than *Hevea Brasiliensis,* which has been used for latex production on a commercial scale. However natural rubber latex obtained from Guyule, is free from tropical proteins (super sensitizing proteins), so safe for people who are allergic to natural latex from tropical sources. Mature guayule plants produce high quality natural rubber in bark parenchyma tissue, mainly during winter when night temperatures are moderately cold, between 6 and 15 C [84-89]. The high quality of guayule rubber makes it amenable for industrial applications and is comparable to that produced by the rubber tree, *Hevea brasiliensis* [90].

Biotechnology approaches for propagation, gene cloning

Guayule is being developed as a new industrial crop suitable for cultivation in arid and semiarid regions [91]. Guayule is propagated through seeds but germination is typically low and variable and efforts are being made to improve seed quality and germination [91]. Osmo-priming has been used to increase the germination percentage and uniformity [91]. Guayule was first established in tissue culture by Bonner in 1950 to study the effects of various chemicals and extracts on rubber production. The use of tissue culture may be important to maintain the genetic stocks of selected guayule germplasms and cultivars, because the identity of selected lines cannot be maintained in successive generations due to lack of control over sexual reproduction and apomixis. In an attempt to stimulate rubber production in guayule, Yokoyama et al. (1977) [92] sprayed juvenile plants with 2-(3,4-dichlorophenoxy) triethylamine (TEA derivative). This treatment resulted in increased isoprenoid levels in the plant tissue. A new method for Guayule tissue culture, using low light and ammonium has also been published [47]. In genetic manipulation experiments, a resistance gene to the herbicide ammonium-glufosinate and various allylic diphosphate synthetase genes were also introduced in tissue-culture generated transgenic guayule plants [46]. Stress induced proteins has also been reported in this plant [67]. Some other biotechnological studies related to Guayule are shown in table 1 & 2.

Paradise tree (*Simarouba glauca* DC.)

Paradise tree (*Simarouba glauca* DC.), also known as King Oil Seed Tree, Laxmi taru, Aceituno, Bitterwood, is a member of family Simarubaceae. It is a multipurpose evergreen tree, native to Florida in the United States, southern Florida, South America, and the Lesser Antilles. It can grow well on marginal lands or wastelands with degraded soils. It can adapt a wide range of temperature and can grow under a wide range of agro climatic conditions like warm, humid and tropical regions. It has got well developed root system thus efficiently checks the soil erosion and improves ground water level. During summer season, it also prevents overheating of the soil surface in the wastelands. These plants are polygamodioecious having variations in floral characteristic, with a male to female ratio of 3:2, about 5% of the population produces exclusively staminate flowers and 40-50% of the population produces mainly male flowers and a few bisexual flowers (andromonoecious) while the remaining 40-50% of the population produces only the pistillate flowers. This plant has the potentiality to produce 2000-2500 kg seed/ha/year [93].

Uses of Paradise tree

This tree facilitates wasteland reclamation, as it forms a well-developed root system that efficiently checks soil erosion, supports soil microbial life, and improves ground water position, also checks overheating of the soil surface all through the year and particularly during summer. It has many medicinal properties like its leaf and bark contain glaucarubin, a chemical useful in the treatment of amoebiasis, diarrhea, provide resistance against malaria. Its seed contain 60-75 % oil, which is edible and used in the manufacture of vegetable fat (Vanaspati), margarine and hence are economically very important. Its oil is used in making of soaps, detergents, lubricants, paints etc, however refining of oil is essential to improve oil quality and making the oil suitable for human consumption, storage, bio-fuel production. Its dry seeds contain 30-40 % protein with 59-62% unsaturated fatty acids. Some

major products obtained from this tree are bio-diesel from seeds, ethanol from fruit pulps, bio-gas from fruit pulp, oil cake, leaf litter, thermal power from leaf litters, shell, unwanted branches etc.

Biotechnology approaches for propagation, gene cloning

This plant is conventionally propagated by seeds but the problem is short viability of its seeds (2-3 months) and also the polygamodioecious nature. About 60% of the seedlings are males, remaining are females and among them only few are high bearers [94]. Therefore, method of softwood grafting using productive females is used. However, this method has several limitations as it is season dependent, requires intensive labor, large number of root stocks. Biochemical and molecular markers for polygamodioecious nature of this plant has been worked out [95]. Several studies on *in vitro* culture of this plant have also been reported using leaf and nodal explants [96,97], somatic embryogenesis from callus cultures of cotyledons [49,50,98] and role of peroxidase in rooting of micro-shoots has also been worked out [51].Direct shoot regeneration without an intervening callus stage is preferred as direct shoot regeneration from explants would maintain genotype fidelity [99] where as extensive callus formation and long term callus culture can lead to somaclonal variations [100]. Several other biotechnological studies related to paradise tree are shown in table 1.

Colocynth (*Citrullus colocynthis* (L.) Schrad.)

Colocynth (*Citrullus colocynthis* (L.) Schrad.), commonly known as tumba, bitter apple, bitter cucumber, desert gourd, egusi or vine of Sodom, is a primitive type of water melon belonging to Cucurbitaceae family. This plant is small, perennial, creeping herb and retains soil binding capacity of considerable significance. This multi-purpose desert plant is originated from Tropical Asia and Africa, now has been widely distributed in the Saharo-Arabian phytogeographic region in Africa and the Mediteranean region. This plant can grow on marginal lands and may improve soil quality when intercropping is done [101]. Colocynth is drought tolerant plant, can grow well in arid environment as this plant can maintain its water content without any wilting of leaves or desiccation even under severe drought conditions [102]. This plant accumulates citrulline under drought conditions which functions as efficient radical scavenger and thus contributing to oxidative stress- tolerance [103]. Its seeds may be useful in biofuel production and oil obtained from the seeds (53%) is used for medicinal and soap production.

Uses of Colocynth

Colocynth is a fruit bearing, drought resistant plant that is used for various purposes like it can either be eaten or used in medicine or used as energy source, e.g. oilseed and biofuel. This plant has many medicinal properties as it is used in the treatment of rheumatism, tuberculosis, analgesic and stimulates the immune system [104]. This plant is also known for its potency as a hydragogue (causes water accumulation in the colon) and catharsis (induces defecation) producer in humans when consumed at more than two grams dry fruit weight [105]. Oral ingestion of its fruits at doses lower than 300-800 mg daily is prescribed in some of the middle eastern locations for the treatment of diabetes to avoid the intestinal side-effects [105]. Its fruit pulp is dried to form a powder which is used as a bitter medicine and drastic purgative. Its fruits are also used for constipation, edema, bacterial infections, cancer treatment, fever, amenorrhea, jaundice, leukemia, rheumatism, vermifuse, insect repellant [106,107]. Various secondary metabolites have also been reported in this plant like cucurbitacins, flavonoids, caffeic acid derivatives, terpenoides, flavonoid glycosides, cucurbitacin glucosides [108,109].

Biotechnology approaches for propagation, gene cloning

This plant is cultivated for its seeds, which contain oil (53%), protein (28%) [110], good source of vitamins (A, B1, B2 and C), minerals such as S, K, P, Ca, Mg, Fe and Zn, also known to eliminate tape worm and serve as a purifier of internal organs. Due to excessive and destructive exploitation of this plant, it is getting depleted fastly [111]. This plant can be propagated both by sexual and vegetative means, however vegetative propagation is more preferred as seed germination is poor due to extreme xeric conditions. *In-vitro* plant regeneration protocol for this plant has also been reported using shoot buds, nodal explants [112] and using cotyledonary explants [55] to induce morphogenesis at high frequency. Colocynth callus culture has also been done to attain higher content of total cucurbitacins, cucurbitacin-E using different combinations of growth regulators [56]. Regarding drought tolerance, two genes (*CcrbohD* and *CcrbohF*) which encode for respiratory burst oxidase proteins

have been cloned [68]. Several other biotechnological studies related to colocynth are shown in table 1 & 2.

Conclusion

Waste lands are degraded lands, but are capable of producing forage, fuel, fodder, medicines, essential oils, vegetation cover to prevent further soil degradation. Creating vegetation cover on the wastelands is an ideal way to prevent extension of wastelands, stoppage of further soil erosion. Large scale cultivation of desert plants discussed in this paper (Jojoba, *Jatropha*, Colocynth, Guayule, Paradise tree) is an ideal way to sustainable development of waste lands. These plants are under-utilized but potential industrial crops and they can tolerate drought conditions, saline conditions, wide range of temperatures, desert habitat conditions. Biotechnical approaches in these plants open great possibilities for their cultivation, utilization. As human population is increasing very rapidly, biotechnological studies are very helpful in developing ways of making these plants better suited to environmental challenges like drought, salinity or extreme temperatures. Cloning of genes related to economic importance, stress tolerance is considered crucial for agriculture on marginal lands consequently securing the world's food supply.

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