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Seaweed Biotechnology to Combat Desertification

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Abstract

This paper presents biotechnological laboratory work on the successful isolation of protoplasts from the green seaweed species *Ulva lactuca,* an intertidal seaweed species from the moderate North Atlantic well known for its algae blooms and tremendous biomass production. We suggest a model to combat desertification and create new arable area by first -based on seaweed biotechnological techniques- create a special "*Ulva*-Desert" High-Temperature-Tolerance (HTT)-strain. This strain which is suitable for desert aquaculture could be obtained by protoplast fusion with the tropical heat resistant *Ulva reticulata* for which protoplasts with a temperature tolerance of 30°±1°C were recently isolated by the research group of Gupta et al. Seaweeds of the genus *Ulva* are well-known for their tremendous oceanic "seaweed-blooms" or "green-tides" of green biomass for which we hypothesize they will create appropriate sulfur gasses with main emphasis on volatile dimethyl sulfide [$(CH_3)_2S$] (DMS) and its precursor β-dimethylsulfonium propionate [$(CH_3)_2\ S^+CH_2CH_2COO^-$] (DMSP) which will following the sulfur cycle stimulate at the oceans cloud formation resulting in rainfall in the deserts and climate cooling. In this way seaweed biotechnology combined with natural geosphere-biosphere processes might reverse the trend of global warming, combat desertification, contribute to climate change mitigation, at intermediate terms (≈decades) and increase the global potential of renewable arable area.

Keywords: Blooms; Climate Mitigation; Combat Desertification; Cloud Albedo; Dimethyl Sulfide (DMS); Global Warming; β-Dimethylsulfonium Propionate (DMSP); Seaweed Biotechnology; Protoplast Isolation; Seaweed Desert Aquaculture; *Ulva lactuca*

Introduction

FAO [1], recently in 2013 shifted its policy making agriculture energy-smart and climate-smart as being two strategies being part of a new larger paradigm which recognizes that to ensure global food security "we will have to do more with less". This demands an integral international effort to implement innovative energy-smart solutions which are cost effective and also reach the remote areas of our planet were primarily the underdeveloped countries are located and which are the regions were an unfettered growth of the world population will take place [2-4]. In addition, increasing desertification with decreasing yields in the (semi)arid area tremendously increases due to global warming. Especially in these regions stroke by famine and hunger catastrophes, like the African continent which also contains the largest hot desert of our globe the Sahara with a surface area of 9,100,000 km^2. The primary challenge in combating desertification is to cope with its enormous complexity, a myriad of numerous global environmental related processes and their linkages. FAO [1] published in 2013 a "Policy Brief" where it is stipulated an interdisciplinary "Nexus" approach making agriculture in nearby future energy-smart and climate-smart [1]. In combination with the [5] manuscript -supporting the perception that "Science & Technology" are vital tools in the fight against desertification- we will in this manuscript suggest a model by a combination of smart innovative techniques for which some are very recently developed research areas. It is essential for Europe to perform biotechnological research with seaweeds because in other parts of the world this is already at extreme high scientific level like the protoplast isolation technique for seaweeds of Prof. Dr. C.R.K. Reddy [6,7]. Marine algae produce during blooms (or when environmentally stressed or dying), excrete Dimethyl Sulfonium Propionate (DMSP), which has an osmoregulation function but may also by bacterial conversion processes towards volatile Dimethyl Sulfide (DMS) affect the Sulfur cycle. In this manuscript, seaweed biotechnology, the sulfur-cycle in combination with smart blue-green technologies based on seaweed culture in our tropical deserts will jointly be combined in order to combat desertification 5] and form an opposing "Power" to "Global Warming" [8]. [9] Were the first who suggested that the biological production of

DMS by phytoplankton in the ocean could affect low-level cloud albedo over the oceans, and thus influence the Earth's heat budget (Figure 1).

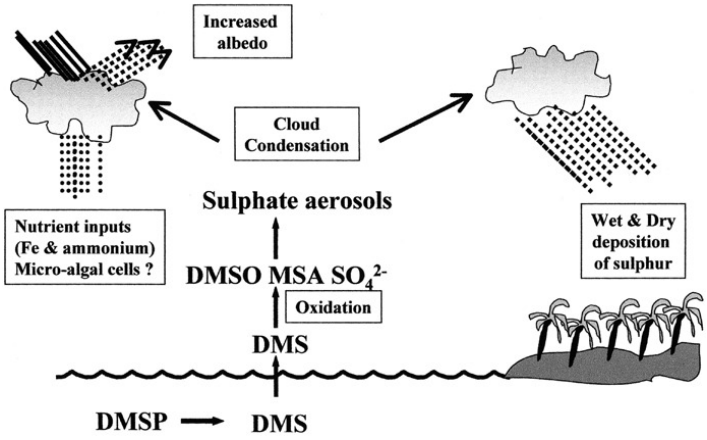

Figure 1: At first DMS will result in more oceanic clouds which are composed largely of water vapor that has condensed on submicroscopic particles, called Cloud Condensation Nuclei (CNN). CNN are composed of sub-micrometer particles, many of which are largely composed of sulfate salts of which the most important is the by *Ulva* seaweed biomass produced DMS. With increased amount of clouds and CNN at first the more solar irradiance will be scattered and reflected resulting in a cooling effect on climate. Second, the clouds will produce a mild form of acid rain as precipitate originating from the increased amounts of oceanic clouds. In this way it is suggested the process of desertification can be combatted including the process of global warming. Source [10].

The pelagic seaweed *Ulva lactuca* is well known for its tremendous biomass production of around 40-50 MT (metric tons) dry weight/ha/year (≈ 365 days) in an Ulva Bioreactor just like we proved in Lelystad, (the Netherlands) in 2016 [3] and our colleagues in Denmark earlier in 2011 [11].

The innovative aspect of this study is that we suggest to use our deserts for land-based seaweed aquaculture of a by biotechnological engineering created seaweed "*Ulva*-Desert" strain with high temperature tolerance suitable for desert aquaculture at our tropical deserts nearby the oceanic water like the Sahara desert. In this way at our at present unexploited tropical deserts in the remote areas of our planet Earth large amounts of rotting "*Ulva*-Desert" strain can be cultured at low labor and infrastructure costs which also in a similar way as our phytoplankton blooms in the ocean produces via DMS. This compound has via the Sulphur cycle an effect on cloud formation at nearby oceans and by in this way induced local rainfall at our Sahara Desert in combination with it cooling effect on climate global warming. We hypothesize, in this way increased desertification can be combatted and in the long term over a period of 3 decades tropical desert can be converted to renewable arable area as outlined by [12] and depicted in Figure 2.

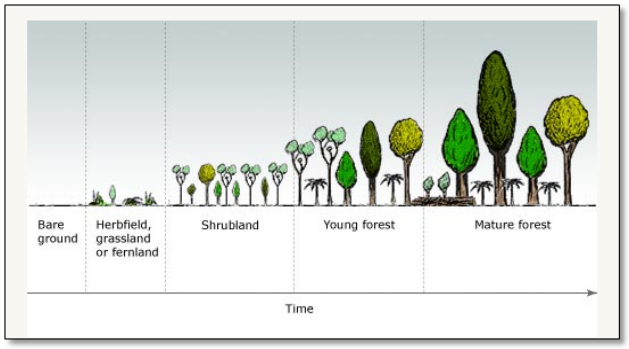

Figure 2: Due to increased atmospheric levels Dimethyl Sulfide (DMS) produced by tremendous amount of rotting green biomass of our special by biotechnology produced "*Ulva*-Desert Strain" produced by desert seaweed aquaculture local climate will be affected in two ways. First a cooling effect via the "Cloud Condensation Nuclei" (see figure 1 sulfur cycle). With increased amount of clouds and CNN at first the more solar irradiance will be scattered and reflected resulting in a cooling effect on climate. Secondly rain via the sulfur cycle resulting even in rain. This strategy is a long-term strategy estimated to last ≈3 decades before tropical desert via intermediate stages can be transformed to tropical forest or renewable arable area which can provide terrestrial agriculture of renewable culture land resources suitable for food production [Source modified: 12].

In order to create the "*Ulva*-Desert" High-Temperature-Tolerance (HTT)-strain the most successful genetic manipulation method that until this moment has been applied for seaweed strain improvement relies on the fusion of protoplasts of different lines (or related species) [13]. Protoplasts are living plant cells without cell walls, or "naked cells" without a cell wall that offer a unique uniform single cell system that facilitates several aspects of modern biotechnology. Fusion of protoplasts leads to the addition of two different genomes [14]. In all cases this leads to the addition of two different genomes. This biotechnological technique of protoplast fusion has a few decades ago already successfully been applied for the red seaweed species *Gracilaria,* famous for its commercial important agar production. Protoplast fusion of different seaweed strains of *Gracilaria* seaweed species has resulted to transfer of these genetic characteristics from a cold-water strain to a warm water strain with a higher temperature tolerance so that in this way agar production in a *Gracilaria* strain other than the temperate climate zone successfully could be applied [13].

In the research manuscript we will describe extensively our successfully laboratory approach in order to isolate vital protoplast of the seaweed species *Ulva lactuca* of the temperate North-East Atlantic waters.

Material & Methods

Method of Protoplast Isolation

Seaweed material of *Ulva lactuca*

Fronds of the green (Chlorophyta) seaweed species *Ulva lactuca* were collected one day before the experimental laboratory day from the upper- and mid-littoral zone at the location "Katse Heule", Eastern Scheldt, the Netherlands. Approximate coordinates: 51°32'39 N and 3°52' E. The fresh collected seaweed material was stored in one m^3 tanks with fresh oceanic Eastern Scheldt water for several weeks. For the protoplast isolation 0.3 g of the outer plant material -where the meristem is located- (Figure 3) were cut into small pieces (fragments) using a sharp knife.

Original method

For the original method the procedures of [15] were followed with some modifications in enzyme mixture solutions according to [15-17]. Our first major adjustments which was made in the whole method for isolation of vital protoplasts was usage of fresh young growth seaweed fronds obtained from our seaweed hatchery (see Figure 3). It seems logical, but is often overlooked to work with fresh material, which is a prerequisite for success.

Figure 3: For *Ulva lactuca* a single seaweed consists of the thallus (or body) with around its center structures known as blades or seaweed fronds. Blades originate from elongated stem-like structures, the stipes. The holdfast, a brown root-like structure, anchors the kelp to the substrate of the ocean. Growth occurs at the base of the meristem, where the blades and stipe meet. For the isolation of protoplasts, it is important to use fresh grown material at seaweed fronds (top photo).

Enzyme mixture preparation

The enzyme mixture solution was made from 2% Macero enzyme R-10 (Serva), 2% Cellulase Onzuka R-10 (Serva), 0.5% Dextran sulfate (Pharmacia Biotech), 1mM $CaCl_2$ (Duchefa), 3% NaCl (Duchefa), 0.6% Mannitol (Serva) and 50mM MES (Duchefa). All of the compounds were dissolved in 50ml autoclaved seawater (32‰) and stirred for 10 min. The pH was adjusted to 7.0. The temperature during the preparation was kept at 4°C. The largest part of the enzyme solution was used immediately. The rest were stored at -20°C in 2ml tubes pending further procedure.

Isolation

For the isolation the seaweed frond fragments were roughed up with Carborundum and rinsed 5 times using wash buffer (0,2M Mannitol, 20mM HEPES; sea water pH 7). As second step the fragments were immersed in 6ml enzyme solution. Samples were incubated for 3h; 4h and 5h on a slowly horizontal shaking platform (80rpm) under dark conditions at 28°C.

Purification

After incubation the samples were filtered through a steel sieve (100µm) to remove cell wall debris and undigested material. Hereafter, the residue was rinsed in ECS- wash buffer (10mM KCl, 100mM NaCl, 10mM $CaCl_2$, 10mM $MgCl_2$ 10mM HEPES, sea water; 0,1M Mannitol; pH8.1) and centrifuged at 3.000rpm for 5min. Protoplasts were spotted by using a Nikon fluorescence microscope (Nikon optiphot/ Japan 21186; Camera: Kappa Type DX 20L-FW).

Adjustments to the original method

As basis compounds for the enzyme solution 0,4M Mannitol and 50mM MES were dissolved in tap-water. The pH was adjusted to 6.0. Hereafter the enzyme abalone (0.05 %) (SIGMA) and the enzyme cellysine (0.2 %) (SIGMA) were added under 4°C to the basic solution. The solution was capped at 4°C during use and stored at -20°C in 2 ml tubes pending laboratory protoplast isolation techniques with *Ulva lactua*. It should be emphasized that the abalone enzyme is derived from the abalone snail (Haliotidae), a natural grazer on Ulva seaweed species [18] that probably contains the enzymes to dissolve the strong cell wall of this seaweed.

Results & Discussion

In this research manuscript we demonstrated we were able to produce protoplasts ("Naked Cells") or cells of this seaweed species in which the cell wall has been removed, but the plasma membrane is intact. In our case we were able to produce protoplasts of the seaweed *Ulva lactuca* mainly based on two new findings added to the existing protocols for protoplast isolation for seaweed species: a). The usage of fresh meristem material from the seaweed frond of *Ulva lactuca*; b). Adding abalone powder to the conventional enzyme extract. Probably because this snail is a natural grazer for *Ulva lactuca* and contains the enzymes to dissolve the hard seaweed wall. The production of these "Naked" seaweed cells opens the option of genetic transformation protoplast fusion techniques. A similar protoplast isolation procedure is suggested to be developed for an Ulva seaweed species with a high temperature tolerance e.g. an Ulva seaweed species from the tropical oceans. As by lucky chance, recently from the tropical seaweed species Ulva reticulate (collected at the west coast of India), also successful protoplasts were isolated [6,7]. Because desertification is a globally urgent compelling problem as a result

of global warming, we have decided to publish our results with regard to protoplast isolation of *Ulva lactuca* at this stage in order to give an impetus to this biotechnological kind of research with seaweeds. We are aware that the technique of protoplast fusion -just as in the case of *Gracilaria* for a heat resistant tropical strain [13]- has not yet been developed in our laboratories so grants for an international cooperation with e.g. of Prof. Dr. C.R.K. Reddy [6,7] are clearly warranted.

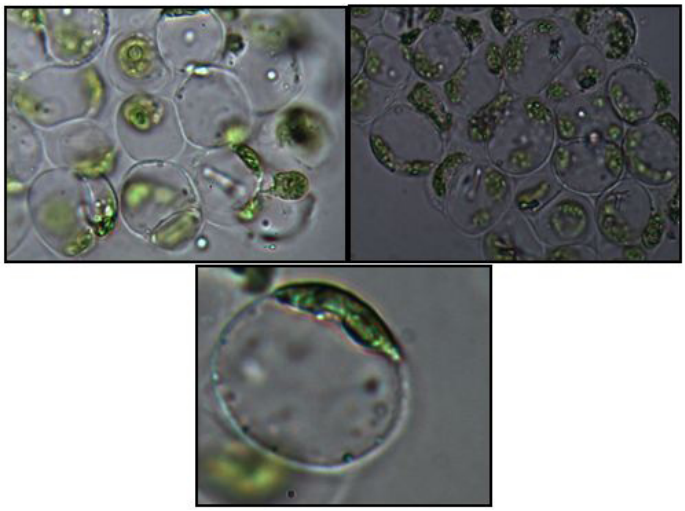

Figure 4: Protoplasts of *Ulva lactuca* with a size of the protoplasts of around 1 µ.

Whatever application seaweeds are used for "Normal Terrestrial Agriculture" it has taught us that optimal application requires efficient cultivation techniques as well as crop lines with the most desirable traits. Since the early start of "Terrestrial" agriculture, breeding technology of crops has primarily been based on sexual hybridization. In this case two lines are crossed followed by several backcrosses. For example, an agricultural cultivar is crossed with a wild variety. In most cases it is aimed to transfer a single trait to such cultivar; for example, a pathogen resistance gene. By several backcrosses of such hybrid with the cultivar near isogenic lines are obtained which have a genome composition that in principle is equal to the cultivar, except for a small "Intro-Gressed" region encoding the desired new trait. In this way it is avoided that less favorable traits of the wild variety would replace those of the cultivar. At this moment, sexual hybridization has hardly been used to improve the quality of seaweed-species *casu quo* -strains, for certain selected production traits. In the case of red seaweeds this is for example caused by the fact that it appears to be difficult to identify reproductive male and female plants [13]. Via this approach the best lines are simply selected from the biodiversity that nature has provided us. In this respect it is important to remark that it is generally assumed that for marine animal and/or oceanic plant species like seaweeds it is assumed they have a high genetic variability, low level of differentiation and a low signal signal/noise ratio [20]. So, because of this high genetic variability, specific attention has to be given to this selection procedure for seaweed lines with the most desirable production traits. Improvement of seaweed strains based on sexual hybridization would be very important for a further rapid development of marine culture with improved seaweed-species strains in a continent like e.g. Europe, were genetic engineering is not accepted in agricultural practices. In contrast, such sexual hybridization techniques are generally accepted in terrestrial agriculture in Europe [21] and therefore we expect that also in marine culture in the European oceans -these techniques with seaweed lines- will widely publically be accepted. This in contrast to genetic engineering (see below). However, on a global scale genetic engineering of seaweed species cannot be excluded. For seaweeds at present in other regions of the world than Europe already two genetic engineering techniques are mainly applied. In these studies seaweed strain improvement of seaweeds is currently especially based on technologies like: a). Mutagenesis and b). Protoplast fusion, respectively [13]. In addition, mutagenesis has been applied and has for example resulted in lines with a modified water temperature tolerance. However, mutagenesis leads in most cases to a loss of function phenotypes, whereas improvement of strains frequently would require gain of functions. For this reason, it seems probable that mutagenesis has only limited potential to contribute to strain improvement. The most successful genetic manipulation method that has been applied for seaweed species concerns the fusion of protoplasts of different lines (or related species), but also spores have been fused to protoplasts of somatic cells and even cells have been fused [7]. In all cases this leads to the addition of two different genomes. This addition of different genomes has in practice successfully been applied for seaweeds in several cases. For example, agar production from a red *Gracilaria* seaweed species has been transferred from a cold-water strain to a warm water strain [13]. In order to keep in pace with other biotechnological techniques in other regions at our globe outside Europe we decided to publish this research manuscript with our results on protoplasts isolation of *Ulva lactuca*. So, in principle a similar protocol for improving the temperature tolerance range for *Ulva lactuca* can be applied by fusing it with the protoplast of the tropical *Ulva reticulata* [6,7] as has successfully has been applied for the red *Gracilaria* seaweed [13]. This approach would make land-based desert seaweed tank-culture more feasible and would make optimal usage of our terrestrial unexploited deserts which still cover 1/5 of the available terrestrial surface area (Figure 5). True deserts, which have less than 50 mm rain each year, cover about 14% of the world's land area, or about 20,800,000 square ki-

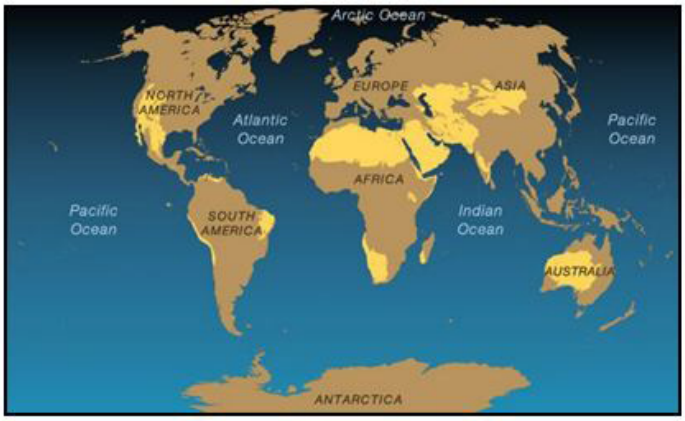

Figure 5: True deserts, which have less than 50 mm rain each year, cover about 14% of the world's land area, or about 20,800,000 km^2 [22] and their global dispersal (in yellow). The Sahara Desert at the Africa continent is the largest (tropical) desert of around 9,100,000 km^2 which amounts 43.75% of the total global desert area and has a western coastline of ≈ 1,110 km amounting 0.312% of the total length of coastline in the world ≈ 356,000 km.

Intertidal seaweeds from the moderate North Atlantic regions have a temperature stress tolerance in the range of 12.27-26.48˚C [19]. The average temperature for a tropical desert like the Sahara is 30˚C and can exceed during the hottest months of the year temperature in the range of 50˚C with extremes up to 58˚C [23]. So, land-based aquaculture of *Ulva lactuca* in our tropical deserts like the Sahara -which has a western coastline of ≈1,110 km amounting 0.312% of the total length of coastline in the world ≈ 356,000 km- is no option unless seaweed biotechnology can be applied.

Conflicts of Interest: None

References

1. FAO (2013) Climate-Smart Agriculture Sourcebook. 570.
2. UN (2008) UNFPA: United Nations (2008) State of World Population 2008. UN, New York, 108 pp. Reaching Common Ground: Culture, Gender and Human Rights, United Nations Population Fund, Technical Notes, 220 East 42nd Street, New York, NY 10017, Thorarya Ahmed Obaid, Executive Director, 100.
3. van Ginneken VJT (2011) Epidemiology of "Hunger in the World", the "Hunger-Obesity Paradox"; its Physiological and Endocrine mechanisms; Chapter 4 page 187-223. In: Biology of Starvation in Humans and other organisms. Editor: Todd C. Merkin, Nova Science Publishers, Inc.
4. van Ginneken V, de Vries E (2016) Towards a Seaweed Based Economy: The Global Ten Billion People Issue at the Midst of the 21st Century. Journal of Fisheries Sciences 10: 1-11.
5. UN/COP (2008) United Nations: "Conference of the Parties" on scientific and technological matters relevant to combating desertification and mitigating the effects of drought. An introduction to the United Nations to Combat Desertification. 16 Fact Sheets.
6. Gupta V, Bijo AJ, Kumar M, Reddy CKR, Jha B (2012) Detection of Epigenetic variations in the Protoplast-Derived Germlings of *Ulva reticulata* using Methylation Sensitive Amplification Polymorphism (MSAP). Mar Biotech 14: 692-700.
7. Gupta V, Kumari P, Reddy C (2015) Development and Characterization of Somatic Hybrids of *Ulva reticulata* Forsskål (×) *Monostroma oxyspermum* (Kutz.) Doty. Frontiers in Plant Science 6: 3.
8. Oreskens N (2004) The Scientific Consensus on Global Warming. Science 306: 1686.
9. Charlson RJ, Lovelock J, Andreae MO, Warren SG (1987) Oceanic phytoplankton, atmospheric sulfur, cloud albedo and climate. Nature 326: 655-661.
10. Welsh DT (2000) Ecological significance of compatible solute accumulation by micro-organisms: from single cells to global climate: FEMS Microbiology Reviews [FEMS Microbiol Rev] 24: 263-290.
11. Bruhn A, Dahl J, Nielsen HB, Nikolaisen L, Rasmussen MB, et al. (2011) Bioenergy potential of *Ulva lactuca*: biomass yield, methane production and combustion. Bioresour Technol. 102: 2595-2604.
12. Westbroek P (1991) Life as a Geological Force: Dynamics of the Earth. 240.
13. Cheney DP (1999) Strain improvement of seaweeds through genetic manipulation: current status. World Aquaculture 30: 55-65.
14. Bajaj YPS, Nagata T (1994) "Somatic Hybridization in Crop Improvement": VI Published by Springer.
15. Srinivasa U, Yuji F (2002) A simple method for mass isolation of protoplasts from species of *Monostroma, Enteromorpha* and *Ulva* (Chlorophyta, Ulvales). Journal of Applied Phycology 14: 165-168.
16. Reddy CRK, Dipakkore S, Kumar RG, Jha, Cheney DP, Fujita Y (2006) An improved enzyme preparation for rapid mass production of protoplasts as seed stock for aquaculture of macrophytic marine green algae. Aquaculture 260: 290-297.
17. Shenghao L, Xiaohang H, Chenlin L, Bailin C, Guang H (2008) Isolation of protoplasts and ion channel (Chlorophyta). J Appl Phycol 20: 29-34.
18. Troella M, Robertson-Andersson D, Andersonc RJ, Boltonb JJ, Maneveldtd G, et al. (2006) Abalone farming in South Africa: an overview with perspectives on kelp resources, abalone feed, potential for on-farm seaweed production and socio-economic importance. Aquaculture 257: 266-281.
19. Pizzola P (2008) *Ulva lactuca*: Sea lettuce; Marine Life Information Network: Biology and Sensitivity Key Information Program; Plymouth: Marine Biological Association of the United Kingdom; Encyclopedia of Life (EOL).
20. Waples RS (1998) Separating the Wheat from the Chaff: Patterns of Genetic differentiation in High Gene Flow Species. Journal of Heredity 81: 267-276.
21. Negrutiu I, Hinnisdaels S, Mouras A, Gills BS, Gharti-Chhetri GB, et al. (1989) Somatic versus sexual hybridization: features, facts and future. Acta Bot Need 38: 253-272.
22. Peel MC, Finlayson BL, Mcmahon TA (2007) Updated world map of the Köppen-Geiger climate classification. Hydrology and Earth System Sciences Discussions, European Geosciences Union 11: 1633-1644.
23. McGinnies WG, Goldman BJ, Paylore P (1984) Desert of the World (Univ. of Arizona Press, Tucson, 1968); J. L. Cloudsley-Thompson, Sahara Desert (Pergamon, New York, 1984); J. Swift, The Sahara (Time-Life Books, New York, 1975).