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# Exploiting the Essence of the Microbial Diversity of the Rann of Kutch for Devising Strategies for Salinity and Drought Tolerance

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It is predicted that by the year 2050, the challenge of providing food security to around 9.6 billion world population will be acute. In a rapidly changing climate scenario, there will be increase in frequency and intensity of stresses, particularly drought, indicating likely shift of vast arable land further to the kitty of already existing 40% of global land under arid and semi-arid areas. The extent of salinity affected area is also likely to increase further from the existing 20% of irrigated lands of the world. Thus, to bridge the demand and supply gap of food grains for feeding nearly 9.6 billion projected world population by 2050, we need to produce more in arid-, semi-arid and rain-fed systems and the areas affected by salinity need to be brought under cultivation. Rain-fed agriculture has further setbacks owing to recurrent and prolonged droughts forcing it to become over-dependent on groundwater for irrigation thereby depleting the aquifers and soil moisture. At the same time with predicted increase in the mean sea level due to global warming, more area in the coastal areas is likely to be engrossed by sea water rendering them unfit for cultivation.

It is known that for one gram of carbon gain, plants need to transpire 1.7 l of water. In a hypothetical situation, if the total biomass (yield and plant biomass) of a target crop is predicted to be around 7 tonnes/ha, there will be likely requirement of around 6800000 l of water to meet the transpirational demand of the plants. This luxury is unlikely to be available during acute drought and salinity stress. Therefore, strategies would have to be thought off as to how the carbon gain can be maximised even when there is drastic reduction in the transpirational loss of water by regulating the stomatal opening.

To cope with these situations, genetic enhancement is considered to be a major option, yet spectacular progress in developing truly drought- and salinity-tolerant cultivars have not been made. The bottleneck of such efforts, probably, has been their inability to provide a solution to the reduced photosynthesis during drought- and salinity-stress, and compensate them suitably, when plants resort to minimize stomatal opening or even shut them down during daytime to reduce transpirational losses or outflow of water resulting in drastic reduction in photosynthesis and carbon gain. If the situation persists for a long period, unless alternative methods are made available for carbon gain, the plants will eventually die due to lack of sufficient reserves to overcome the situation.

Under saline situation, if a crop is continuously irrigated with 4 EC (Electrical Conductivity) saline irrigation water, an estimated amount of 6000 kg of Na will be accumulated inside plant tissues/ha, if no control mechanisms exist in plants to reduce the entry of Na or detoxify it. This would make almost all the plants susceptible to salinity. But that does not happen. To reduce the inflow of Na inside the plant tissues, the plants resort to minimizing the transpiration by regulating the stomatal behaviour. This happens at the cost of drastically reducing the photosynthesis and carbon gain and enhancing photorespiration, especially in $C_3$ crops, reducing the net carbon gain further. Moreover, plants will also reduce the influx of Na by activating the sodium pumps in the root so that most of the Na is removed from the incoming solute. Still whatever amount of Na will enter the plant tissues will be good enough to impair the cellular machineries.

Therefore, the question remains as to how the concerns of salinity and drought can be addressed so that more area can be brought under cultivation to sustain agricultural growth. This needs stable signatures/traits that might have transferred evolutionarily across domains of life which can impart reasonable level of salinity and drought stress tolerance. Such 'treasure trove' might be present in a system which would have evolved and witnessed all events of evolution and has transferred the stable signatures/traits into new and newer forms of life in each step of evolution. As salinity tolerance

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**Author for Correspondence: Email- kkpal9426476749@gmail.com* is almost synonymous to drought tolerance, clues can be obtained from strains of extreme halophilic bacilli and their derived genera, archaea, and fungi that survive the extremities of salinity as prevalent in the otherwise undisturbed ecosystem of the Rann of Kutch in Gujarat with the history of stable evolutionary lineages. While studying the diversity of the extreme halophiles, we found that these groups of organisms can survive and perpetuate their races at upto saturated levels of NaCl concentration, the most inhospitable level of possible salinity.

The sequencing of the genomes and metagenomes of the representative organisms and samples of the Rann of Kutch and their diversity provided the much needed information about the strategies required to be adopted to circumvent such level of stresses and the ways and means of gaining valuable carbon required for growth and multiplication when there is very limited availability of nutrients and carbon sources in the growth environment. Invocation of the Darwinian 'survival of the fittest' has much relevance today than ever before to understand the mechanisms of osmo- and desiccation-tolerance.

Genes reported to have capability of imparting osmotolerance could not be detected in metagenomes and many of the genomes of the organisms obtained from the Little and Great Rann of Kutch. Then how did all these organisms survive in such inhospitable salinity? Critical evaluation of the sequence data of the metagenomes revealed that there are more than 20,000 species of organisms that can survive and multiply in more than 35% of salt concentration. When the salt concentration reaches around 50% level, the species richness reduced to around 15,000 species with predominance of extreme halophilic archaea. Contrary to the reported involvement of methyl aspartyl pathway in helping *Haloarcula marismortui* to thrive in the extreme salty settings prevalent in the Dead Sea, we found possible involvement of serine-glyoxylate, acetyl CoA, methyl malonyl- and ethyl malonyl-CoA pathways in a novel haloarchaeon and metagenomes in imparting salinity tolerance by helping the organisms to gain carbon and channelizing the intermediates to other anabolic reactions resulting in net positive carbon balance. This begins with the uptake of dissolved $CO_2$ as $HCO_{3-}$ which is then converted into phosphoenol pyruvate→ oxaloacetate→ malate--→ pyruvate/ phosphoenolpyruvate---→ glyoxylate----→serine-- →--→ by key enzymes like phosphoenolpyruvate carboxylase, malate dehydrogenase, isocitrate lyase, malate synthase, decarboxylases, serine hydroxymethyl transferase, etc. in a series of reactions to capture carbon from the environment and also to utilise respiratory $CO_2$ and eventually diverted them for synthesis of key intermediates required for sustaining life processes.

What is important is to have the intact network of the pathways and the expression of all the necessary enzymes in coordination. In addition to carbon gain, the organisms expressed Na/K/H pumps to remove most of the Na trying to enter into cytoplasm with solute. All the machineries required for such expression are present in bacilli and fungi (archaea-eubacteria-eukaryote pipeline) obtained from the Rann of Kutch. The big question is whether such pathways have been acquired by plants while transition from aquatic to terrestrial ecosystems during the process of acclimatization and evolution and divergence? If yes, whether it would be feasible to identify all these footprints or orthologs in plants so that crop plants truly tolerant to salinity can be identified which can address the issue of reduced photosynthesis during salinity stress by ensuring steady supply of carbon through alternative route/mechanisms to anabolic reactions to occur leading to net positive carbon gain.

The blue green algae like *Synechocystis*, a marine alga, has unique feature of concentrating $CO_2$ often called Carbon Concentrating Mechanism (CCM) to ensure continuous and steady supply of $CO_2$ around RUBISCO to ensure unabated photosynthesis during entirety of daytime for maximizing fixation of carbon required for rapid growth and development. Evolutionarily, $C_4$ and Crassulacean Acid Metabolism (CAM) are other CCMs wherein photosynthetic metabolism have evolved from $C_3$ background during the process of evolution and acclimatization to environmental extremities and are reported to be water-use efficient and drought tolerant as compared to $C_3$ crops. Taking clues from the CCMs from the photosynthetic prokaryotic background, of late, scientists are thinking of transferring the entire photosynthetic machinery of CAM into $C_3$ background which is being thought of as a solution for improving the much-needed water-use efficiency (WUE) of carbon assimilation and for improving drought-tolerance, exploiting the nocturnal $CO_2$ fixation and daytime $CO_2$ re-assimilation by inverse opening/closing of stomata. This will ensure reasonable photosynthesis behind closed stomata, during daytime, in drought-stress condition. However, there are enormous difficulties in stably transferring genes of all the modules of CAM into $C_3$ background because of little understanding about stomatal control and mesophyll succulence.

Though the mechanisms of net carbon gain in photo- and non-photosynthetic prokaryotic, lower group of non-photosynthetic eukaryotes, and photosynthetic plants would be different, evolutionary clues obtained from the photosynthetic prokaryotes would suffice the future strategies for ensuring drought and salinity tolerance in plants by ensuring steady supply of carbon through alternative means to ensure photosynthesis to continue even behind closed stomata during daytime in acute stress.