Moss: Little Things Which Can Fill the Huge Glitch in Agriculture

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The problem of drought, famine and farmer suicides:

It's a known fact that India is a largely agricultural country with a wide variety of crops grown not only for domestic use but also for export. It is also a fact that farming in India is mostly unorganized and dependent on natural weather cycles rather than usage of modern technologies. Farmers depend heavily on the rain gods and unpredictable weather cycles often cause largescale crop failures due to drought leading to bouts of famine. Add to this, other stress factors such as pests and crop diseases puts tremendous pressure on farmers and their families. In the recent past, this has also led to extreme measures among farmers including suicides and the situation does not seem to be improving.

India is the second largest producer of cereal grains in the world with a recorded production of 259.32 million tons in 2011-12 and it is roughly hypothesized that consumption will increase to 377 million tons by 2050. Besides feeding its own citizens, India is an exporter of cereals for many countries such as Iran, Saudi Arabia, Bangladesh, etc. The gap in supply and demand is likely to affect the economy of the country as well as the living condition of its citizens with an added speculation of a fall in agricultural production due to drought and pests. There is a dire need to identify new traits for developing resistant varieties and introduction of these for commercialization.

Agriculture in India is flawed as it involves the cultivation of varieties of wheat, rice and pulses (cereal crops) which are mostly susceptible to the adverse biotic and abiotic conditions of

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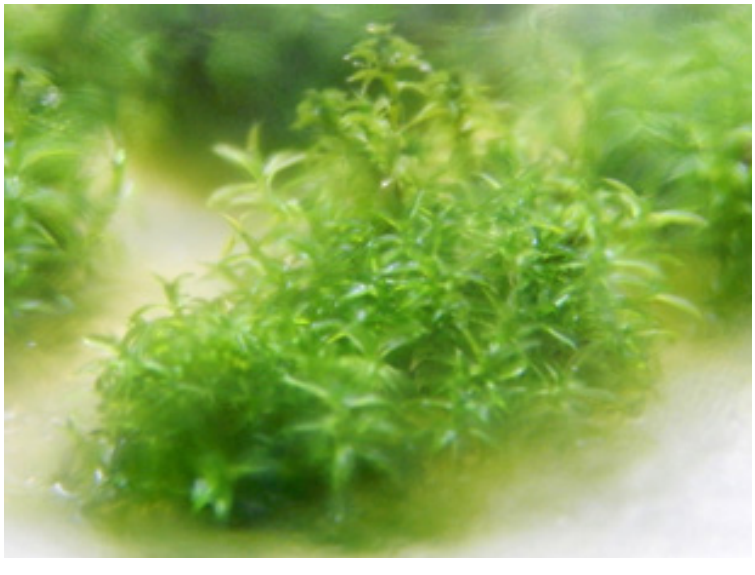
the environment while the tolerant varieties of crops remain neglected in the wild. Genetically modified crops(GMOs), with integrated traits of resistance, do not find government approval for commercial cultivation except for field trials, due to health concerns of citizens. After Bt Cotton there has not been a release of any new GM crop, all of which remain in the developmental stages. GMO versions of mustard named as Dhara Mustard Hybrid 11 (DMH-11) is the next enlisted to be released and has found clearance from GEAC (Genetic Engineering Approval Committee). In comparison to this data, there are already 10 GMO crops cultivated in U.S. including apple, canola, potato, soybean, sugarbeet, alfalfa, papaya, corn, cotton and squash. Standard American Diet (SDA) includes a high percentage of potato and corn which they seem to have already secured against stress conditions. India should not fall behind specially in terms of agriculture.

Moss to the rescue – A probable solution:

There are approximately 126 research institutes involved in the field of Biological and Agricultural Sciences (DST, Govt. of India)in India with numerous laboratories continuously identifying genes which confer tolerance traits.

Our laboratory at the University of Calcutta has been involved with the identification and analysis of dehydrins (drought responsive proteins) for its potential in drought tolerance. We chose *Physcomitrella patens*, a moss, for our molecular analysis as it would mark the evolutionary pattern from lower to higher group of organisms for the protein group under study. *Physcomitrella* is a well-known dehydration tolerant plant and its analysis has been restricted to only a small group of researchers in crop improvement. Dehydrins have been widely analyzed since its first report in



Invitro culture of gametophyte of Physcomitrella patens, on tissue culture media plates, under standard growth conditions

1996 (Close et al.), and it is fairly distributed from moss to angiosperms. Yet, its precise mechanism of action in drought tolerance remains to be unravelled.

The research:

With the research in our lab from (2012-2018), we were able to simulate the conditions of growth for *Physcomitrella* in a growth chamber and propagate it by tissue culture. We tried to derive a comparative analysis of three dehydrins (DHNs) reported from the moss namely DHNA, DHN B and DHN C. DHN A has been reported to provide enhanced tolerance to osmotic stress. All the dehydrins were cloned, sequenced and their proteins confirmed on SDS-PAGE protein gel. The proteins isolated were characterized for enzyme protection. Kinetic analysis with lactate dehydrogenase showed the effectivity of DHN A in retaining enzyme function under extremes of temperature conditions.

In order to define its mechanism of action, post translational modifications of the proteins were studied in detail. DHN A was found to undergo phosphorylation and a shift in protein gel assay due to change in molecular weight. The phosphorylation event was confirmed with phospho-specific staining with ProQ Diamond. This phosphorylation may be an important trigger for its enhanced activity under stress.

Besides, it is well reported that dehydrins have three conserved segments K, S and Y. With our analysis, we could identify another highly conserved segment in DHN A which was present in conjunction with Y segment in definite repeats throughout the protein. Biochemical analysis of the segments showed that they could form amphipathic alpha helices. Several deletion mutants of the protein with different permutations and combinations were generated to identify the function of each segment and the reason why so many repeats of a segment were present. Further, amino acids of the repeats were shuffled to make a mutant protein with the same amino acid composition but a shuffled position to break the structure of the protein. Such, in depth molecular analysis coupled with biophysical analysis was undertaken to provide an insight into the exact mechanism of its action of the protein so that it could be extracted for its use in bioengineering and manipulation of crop plants in a similar way.

Killing two problems with one Dehydrin an unexpected positive side effect:

The drought tolerant property of the DHN A was assessed by overexpression of the protein in tobacco. Identifying a protein from moss and its over-expression and stress tolerance in dicot showed its potential for field trials in crop plants in the future. Tobacco transgenics of the deletion mutants were also generated to define their role in stress.It was not in our agenda but as a byproduct of our experiments, we also identified DHN C to have antibacterial properties. It was effective against *Bacillus subtilis*, a common laboratory bacterial strain and *Rhodococcusfascians*, another bacterial strain. Spectrophotometric and SEM analysis revealed that the protein may be able to cause plasmolysis of the bacterial cells and target its membrane for disruption. This is the first

report of an antibacterial dehydrin from moss and only one or two dehydrins have been reported earlier from rice and wheat to have antibacterial properties.

Publications so far:

A part of these findings has been reported in Springer Journal *Planta* and continued experiments have been ongoing for further molecular studies.

Conclusion:

A comprehensive knowledge of the action of dehydrins would be beneficial in making it to the top of the list as a putative gene for biotechnological manipulation of crop plants. Efforts to develop tailored protein with different segments of the dehydrin and obtain a super-protein with all the resistance powers in a single molecule may be successful. This, when introduced into crop plants, would give rise to crops with superpowers of stress resilience.

Answers to sustenance of mankind can only be found when we travel the untrodden depths of wilderness and scientific achievements made by unearthing the solutions that are embedded in the expanse of nature.