Escherichia coli as an Eco-friendly Agent for Sustainable Development

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Abstract- Farmers are now facing a tremendous reduction in agricultural crop yield, due to the infertility of soils, various abiotic stresses and poor farming. The application of chemical fertilizers distresses soil fertility and also puts adverse effect on human health. Inappropriate use of chemical fertilizer leads to the rapid decline in crop production in most parts of the world, and therefore there is a strong need for good cultivation practice. Biofertilizers and biopesticides have been used in recent years by farmers worldwide to preserve natural soil conditions. Biofertilizer, a replacement for chemical fertilizer, is a cost-effective and prevents environmental contamination to the atmosphere, and is surely a source of renewable energy. In contrast to chemical fertilizers, causes hazardous effects to plants and human Beings also causes damage to soil fertility. In reply to this, organic fertilizers will be the right solution without which gardening and growing healthy and natural food and crops could be possible. The use of biofertilizers is, therefore, inevitable to increase the earth's productivity. A low-input scheme is feasible to achieve farm sustainability through the use of biological and organic fertilizers. This study investigates the use of microbial inoculants (*E. Coli*) as biofertilizers to increase crop production.Let's make a conscious effort to use soil beneficial bacteria that are PGPB (Plant growth promoting bacteria) in enhancing the abiotic stresses like drought and temperature stress due to which the world is facing tremendous decrease in production in cereal crops like wheat.

Index Terms: **Bioinoculant, Biopesticides, PGPR, Microbial inoculants, Organic farming, Yield component.**

INTRODUCTION

The increase in global temperature has caused erratic weather patterns and is affecting human life, wildlife, vegetation and sea level. Currently, the increased global temperature has been caused mainly by the high emission of green house gases, urbanization, cutting of trees and imbalance of ecosystem. An analysis of annual and seasonal temperature trends in the country from 1901 and 2021, indicate that the country has been getting warmer continuously, consistently and rapidly. Abiotic stress like heat, cold, drought, flood, heavy metal etc is unfavourable condition for crop plant which affects its growth, survival and reproduction. Extreme conditions limit plant growth and development. An unfavourable environment comprising extreme high temperature, salinity and drought pose a complex set of stress conditions. Plants can sense and react to stresses in many ways that favour their sustenance [1]. They remember past exposure to abiotic stresses and even mechanisms to overcome them in such a way that responses to repeated stresses can be modified accordingly [2].Wheat (*Triticumaestivum*L.) is the second most important cereal crop providing food and nutritional security to the masses in India. Wheat production in India has progressively scaled newheights with phenomenal increase in area, production and productivity. In 2020, wheat production for Rajasthan was 10.92 million tonnes. Over the last 4 years, wheat production of Rajasthan grew substantially from 8.99 to 10.92 million tonnes rising at an increasing annual rate that reached a maximum of 8.33% in 2020. The wheat programme has been very vibrant during the last four decades, but still there is no scope for complacency and the programme needs to be more responsive to the new emerging challenges. The change in climate will have impact on agriculture and food production as these two are interrelated. Water stress and temperature stress adversely affects physiological status of plants including the photosynthetic capability. Prolonged water stress decreases leaf water potential and stomatal opening, reduces leaf size, suppresses root growth, reduces seed number, size, and viability, delays flowering and fruiting and limits plant growth and productivity [3]. These abiotic stress affects the plants biochemical nature by changing polypeptide, enzymes and metabolites.It increases the levels of sugar, soluble proteins, anti-oxidant enzymes, proline, chlorophyll, seed germination rate, quality is reduced at rapid rate [4]. Under severe conditions, loss in plant productivity is observed [5]. An increase in temperature is major cause of crop losses, which severely affects plant development and adversely influences crop production.

The various climatic calamities like temperature and drought are major key challenges in global agricultural production [6].Agriculture(wheat production) is facing considerable problems such as various abiotic stresses like heat and drought stress, which puts adverse effect on the production of cereal crops. The whole scenario of agriculture is at a confluence where one has to rethink and improve agricultural packages and processes in meeting the dreams of millions of people. Combating abiotic stress and sustaining crop production is major challenge the world is facing these days. The management of drought stress is necessary for securing sustainable agricultural production and sustenance of biodiversity.

In Rajasthan, the crops face a higher temperature range during their growing phase. The temperature sometimes reaches to 50^{o}C too.With growing intricacies of global warming these prevailing conditions are about to worsen only. It will not only deteriote the quality of crops but also reduces the quantity of the crops. Therefore, it is mandatory to identify the crop variety for drought or temperature stress property that sustains under higher temperature exposure.

Wheat varieties grown in RajasthanHD 4728(Pusa Malawi) -A durum wheat variety, released and notified for the timely sown irrigated in Kota and Udaipur divisions of Rajasthan. This is a semi-dwarf variety (90 cm) having high tillering capacity, 120 days maturity duration with the high degree of resistance to leaf and stem rusts. The variety that gives an average yield of 5.42 t/ha with the maximum genetic potential of 6.8t/ha, KVK introduced newly released thermo- insensitive wheat varieties Raj-4079 & Raj-4120 in the district through 390 FLDs in 108 ha. area. The variety Raj-4079 gave average yield of 38.86 q/ha which was 54.21 per cent higher over local check (variety Lok-1) due to its suitability to climatic condition of Banswara. Varieties Lok-1 and Raj-3077, Raj 4037, Raj 3765 and DBW – 90 were grown in large area, but after the introduction of Raj- 4079 in last two-three years, 60-65 % wheat area in the district is being covered under this variety. In addition to this, most of farmers of KVK adopted villages have also started to reduce their wheat seed rate to 150 kg/ha instead of 200-250 kg/ha.Such varieties could be further taken up for quality improvement purpose through different plant breeding methods. In Rajasthan different varieties of wheat crops which are grown are namely wheat (Lok-1, Raj 4037, Raj 3765 and DBW -90),Butmodern agriculture mostly relies on various inputs, such as pesticides, chemical fertilizers, assured irrigation, improved seeds, and herbicides. Their employment in agriculture increases the production, but their improper utilization has unfavourable impact on environment quality,soil productivity and of Couse the humans are facing various health hazards, which is a matter of concern.

a. Chemical fertilizers used in crop production and their effects on crops

There are various kinds of chemical fertilizers available. These are nitrogenous fertilizer, phosphorus fertilizer, and potassium fertilizer. These are used for increasing productivity of land, but it worsens the soil quality especially like urea, muriate of potash, single super phosphate (SSP). Continuous utilization of chemical fertilizers has a distinct impact on the soil's biochemical properties, which results in the shift of microbial populations. Alteration in nitrogen (N) content, SOC, moisture, pH, and the availability of nutrients to microbes has been observed as a result of incessant use of fertilizer in different crops such as wheat and other cereal crops like maize, rice and corn [7]. Plants require a number of soil nutrients like nitrogen, phosphorus and sulphur for their growth. But soil nutrient levels can decrease over time when crop plants are harvested, as nutrients are not returned to the soil, hence we need to add nutrients back to it and this demand is fulfilled by adding chemical fertilizers to the soil to promote soil fertility and increase plant growth. Today fertilizers have become essential to modern agriculture to feed the growing population. Uses of fertilizers, especially, the chemical fertilizers have brought in blessings on humanity, which helped contain hunger and death in different corners of the world. When we use chemical fertilizers; they do not help replenish soil nutrients and its fertility contrary to the popular belief; but, replenish only nitrogen, potassium and phosphorous. As we know phosphorous does not dissolve in water and its overuse may cause hardening of soil. Since salt content in chemical fertilizers are harmful to agriculture in the long run as salts are harmful for plants as well as soil.The plants will be more susceptible to pests and diseases as they lack good immune system and enough resistance against these forces. The chemical fertilizers can cause root burn or fertilizer burn, as theydo not allow enough water intake for the plants. There is also various risks to soil health [8] soil compaction, acidification, erosion, contamination, salinization, and decline in organic matter, which can affect P and N losses to water and air.These artificial fertilizer elements disturb the soil properties and also enhances the emission of nitrate. Crop growth is not sufficient after application of chemical fertilizers hence it results into decrease in crop production. Soil dwelling organisms, such as earthworms, which make the soil fertile, also are destroyed due to improper application. When N-fertilizer is applied in the field, only then bacteria already present can turn the nitrogen into nitrate. The use of fertilizers today is viewed as a technology needed for agriculture but the long-term application of chemical fertilizers has a negative impact on the physicochemical properties and soil biological properties.

b. Hazardous effects of chemical fertilizers on human health

Though chemical fertilizers increase crop production; their overuse is bringing hazards to human health and environment as well. It has already been proved how chemical fertilizers pose serious challenges to the balanced and sustainable growth. The nitrogen fertilizer is toxic to both animals and humans. These fertilizers are mixture of toxic chemicals which are absorbed into the plants, leading toxins to enter the food chain via vegetables and cereals and water creating health affects increase and spread rapidly through contaminated water may contain high levels of nitrates and nitrites, causing hemoglobin disorders. They may also contain Heavy metals such as Mercury, Lead, Cadmium and Uranium, which can cause disturbances in the kidneys, lungs and liver and cause even cancer. Over 29 popular fertilizers tested positive for 22 toxic heavy metals, including Silver, Nickel, Selenium, Thallium and Vanadium, all directly linked to human health hazards. If exposed to Ammonium Nitrate causes eye and skin irritation. Inhalation exposure can result in irritation of the nose, throat, and lungs, nausea, vomiting, flushing of the face and neck, headache, nervousness. Potassium Chloride interferes with nerve impulses, and affects heart functioning. It can cause all kinds of gastric and stomach pains,bloody diarrhoea, convulsions, headaches, mental impairments, redness or itching of the skin or eyes. Yet, human beings, via the gastrointestinal system, only uptake about 5% of the Cd through food. Hence, Cadmium poisoning could betake place and lead to bone and pulmonary damage.Chemical fertilizers are carcinogenic which is the major root cause of health issues in humansaccordingly;scientists and researchers are seen arguing in favour of organic fertilizers as the best solution to avoid such health hazards to humans and other threats to environment by overuse of chemical fertilizers. Hence the chemical fertilizers should be applied in due time and appropriate quantity,so to overcome these major health issues and threat to environment one efficient way which I came across is bythe use ofmultifaceted PGPB Plant growth promoting Bacteria (*E. coli*).

c. Functional Genomic approach in alleviating Drought Stress in Wheat crop

Development of heat and drought tolerant wheat genotypes is one of the major mandates of wheat improvement programmes. Different approaches being followed to achieve this therefore several experiments have been conducted to understand the mechanism by which wheat as a crop can adjust to heat, drought, salinity and other abiotic stress conditions. The very basic requirement is to evaluate the germplasm for existing variability for tolerance to heat and drought. In addition, there is a need for better insight into the environment such as Hadoti region of Rajasthan where these stresses are endemic (hot spots).The promising genetic stocks are utilized in crossing programme for combining better yielding and disease resistant traits with that of the tolerance to heat and drought. Work done on wheat crop for alleviating drought tolerance using functional genomics approach.

d. Transcriptomics

Deciphering drought tolerance using functional genomics approach in recent years, functional genomics approaches have been used to study the molecular basis of drought resistance at the transcriptomics, proteomics and metabolomics level. Serial analysis of gene expression (SAGE) and RNA sequencing (RNA-seq), genome wide transcript profiling has been widely used to identify drought responsive genes in wheat.

Next-generation transcriptome sequencing is also used for analysis of gene expression, the structure of genomic loci, and sequence variation present at expressed gene loci. The expression profile of transcription factors involved in abiotic stresshas beenstudied byresearchers in wheat. Activity of antioxidant enzymes: superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT) and the expression of their genes were studied in wheat genotypes under controlled severe drought [9]. The results indicated a unique pattern of activity and gene expression of antioxidant enzymes which suggested that each genotype showed different molecular and biochemical responses under the same drought conditions.

Proteomics: The key proteins/enzymes and metabolic pathways identified from drought tolerant wheat. Lines could be potentially targeted for designing drought tolerant varieties of wheat. A number ofproteomic studies have been done in wheat under drought stress as compared to stem proteome patterns of two divergent wheatlandraces (N49 and N14) under terminaldrought stress. The tolerant landrace (N49) was more efficient at remobilizing stem reserves than the sensitive landrace (N14). The maximum number of differentially-expressed proteins was noted at 20 days after anthesis in N49 when active remobilization of dry matter was recorded, thus suggesting potentialparticipation of these proteins in efficient stem reserve remobilization.

Metabolomics: Plants react to abiotic stresses by altering the composition and concentration of metabolites so that they can acclimatize to adverse environmental changes.

Metabolomics: Plants react to abiotic stresses by altering the composition and concentration of metabolic profiling hasaccelerated discovery of stress signal transductionmolecules and compoundsthat are integral part of plant response to abiotic stresses. To accelerate the trait-based analysis of complex biochemical process, metabolic profiling alongwith NGS techniques, transcriptomics and proteomics reveal the underlyingcellular biochemical events across diverse conditions. The metabolite profiling of given mapping population can be combinedwith thegenetic linkage makeupmaps to obtaingreater insights into the genetic map ofcomplex traits, thereby renderingmetabolomics particularlyrelevant tocrop breeding. Experiments have identified multiple metabolite QTLs in wheat under drought stress and pinpointed some genomic segments that controlled both agronomic traits and specific metabolites.

e. Identification and validation of stress-induced micro-RNA in wheat

Micro-RNAs (miRNAs) are a class of short endogenous non-coding small RNA molecules of about 18–22 nucleotides in length. Computational predictions have raised the number of miRNAs in wheat significantly using an EST based approach. Hence, a combinatorial approach which is amalgamation of bioinformatics software and Perl script was used to identify new miRNA to add to the growing database of wheat miRNA. Identification ofmiRNAs wasinitiated by miningthe EST (Expressed Sequence Tags) database available at National Centre for Biotechnology Information[10] investigated as many as 4677 mature miRNA sequences belonging to 50 miRNA families from different plant species were used to predict miRNA in wheat. Authors further identified five abiotic stress-responsive new miRNAs. Also, four previously identified miRNA, i.e., Ta-miR1122, miR1117, Ta-miR1134 and Ta-miR1133 were predicted in newly identified EST sequence and 14 potential target genes were subsequently predicted, most of which seems toencode ubiquitin carrier protein, serine/threonine protein kinase, 40S ribosomal protein, F-box/ketch-repeat protein, BTB/POZ domain-containing protein, transcription factors which are involved in growth, development, metabolism and stress response. Amongst the predicted miRNAs, validated expression of miR855 in wheat for salt tolerance. The result has increased the number ofmiRNAs in wheat, which should be useful for further investigation into the biological functions and evolution of miRNAs in wheat and other plant species. These findings indicate that diverse set of miRNAs could play an important role in mitigating drought stress responses in wheat.

f. Markers associated with drought stress tolerance in wheat

It may bestated that tolerant linesidentified wouldlead tohigher production andproductivity underabiotic stress situations that adversely affect the wheat crop. These lines may also be utilized in hybridization programme for developing next generation mapping (MAGIC and NAM) populations for fine QTLs scanning for stress tolerance. The trait specific germplasm, precision phenotyping and the selection criteria based upon indices will be rewarding for increasing grain yield in wheat under harsh environments.

Transcriptomics: With the advent of microarray, DNA chip technologies, subtraction libraries, cDNA-AFLP, Deciphering drought tolerance using functional genomics approach

g. Need to use Organic fertilizers

The organic fertilizers are not like chemical fertilizers; they are slow release which will allow time for microbial activity to break down the organic materials in the fertilizers. When we talk of microbial activity; we need to remember that natural microbes; which include beneficial insects, fungus, and bacteria found in the soil, are very much helpful for healthy soil and plant growth. Needless to say, use of chemical fertilizers will kill these soil friendly micro-organisms. In their larger threat to environment, animals and human health; chemical fertilizers will ultimately end up leaking into our water bodies; ponds, streams, ground water etc. and contaminate water supply as a result of which humans as well as animals may suffer numerous short term and long term hazardous chemical effects on their health and body. To combat this, organic fertilizers will be the right solution without which growing healthy crops and natural food could be possible. Let's keep our earth safe, for the present and the future. Let’s make a conscious effort to use soil beneficial bacteria that are PGPB (Plant growth promoting bacteria) in enhancing the abiotic stresses like drought and temperature stress.

PGPB can fundamentally support the plants in acquiring nutrients, resisting against diseases and tolerating abiotic stresses [11]. Microbial intrinsic metabolic and genetic capabilities make them suitable organisms to combat extreme conditions of the environment [12]. Their interactions with the plants evoke various kinds of local and systemic responses that improve metabolic capability of the plants to fight against abiotic stresses [13].

E.coli is the most studied micro-organism since its discovery in 1885. Based on studies done in the past few decades, the presence of environmental *Escherichia* is now well recognized. This environmental *E.coli* may be of animal-origin and have become adapted to their surrounding environments; or may retain the characteristics of their ancestral linage, which was environmental bacteria using soil and sediment as their primary habitat. More data are needed, especially genomic information of environmental *Escherichia* strains, to clarify the evolutionary history of environmental *E. coli*.

h. Abiotic stress tolerance in wheat crop using *E. Coli*.

Based on studies done in the past few decades, the presence of environmental *Escherichia* is now well recognized. This environmental *E. coli* may be of animal-origin and have become adapted to their surrounding environments; or may retain the characteristics of their ancestral linage, which was environmental bacteria using soil and sediment as their primary habitat.A number of challenge-testing studies have examined the fate of *E. coli* O157:H7 and S. enterica in the agricultural environment with the view of designing strategies for controlling vegetable contamination preharvest. It was examined by few researchers through different approaches that have been used to study the behaviour of *E. coli* O157:H7 and S. enterica in the manure, manure-amended soil, and in manure-amended soil-plant ecosystem during cultivation of crops.

The Another report confirmed that functional expression screening of LEA proteins *in vivo* in yeast or *E.coli* under abiotic stresses have been investigated successfully in various flowering plant species. Similarly, one *LEA4* gene from *Brassica napus* is able to enhance the cellular tolerance to temperature and salt stresses of *E. coli* cells [14]. More recently a SMP protein from tea has reported function as a chaperone enhanced tolerance to *E. coli* against stresses[15]. However, only a few LEA proteins increasing the tolerance to abiotic stresses had been studied and were primarilyfrom the major group *LEA3*, *LEA4* and *dehydrin*. The work done with model transgenic plants has demonstrated that cellular accumulation of mannitol can alleviate abiotic stress; they showed that ectopic expression of the *mtlD*gene for biosynthesis of mannitol in wheat improves tolerance to water stress and salinity. Wheat (*Triticumaestivum*L.cv Bobwhite) was transformed with the *mtLD* gene of *Escherichia coli.*The*beta* gene encoding choline dehydrogenase from *Escherichia coli.*The *beta* gene encoding choline dehydrogenase from *Escherichia coli was introduced* into common wheat (*Triticumaestivum* L.) by *Agrobacterium*-mediated transformation. Various levels of expression of the *betA* gene were confirmed by RT-PCR among the transgenic lines and different

levels of glycine betaine accumulation were detected in these lines. Several wheat transgenic lines with different *betA* expression levels in the T3 generation and wild-type (WT) were selected to test their performance under drought stress conditions [16]. It was concluded that the amount of injury to the wheat plants was negatively correlated with the level of accumulation of glycine betaine, and the glycine betaine acted as an important osmoprotectant in transgenic plants to improve root growth, and enhance the resistance of transgenic plants to drought stress.Cold shock proteins (CSPs) enhance acclimatization of bacteria to adverse environmental circumstances. Investigation of several stress-related parameters in *SeCspA* and *SeCspB* transgenic wheat lines indicated that these lines possessed stress tolerance characteristics, including lower malondialdehyde (MDA) content, lower water loss rates, lower relative Na^+ content, and higher chlorophyll content and proline content than the control wheat plants under drought and salt stresses. RNA-seq and qRT-PCR expression analysis showed that overexpression of *SeCsp* could enhance the expression of stress-responsive genes. The field experiments showed that the *SeCspA* transgenic wheat lines had great increases in the 1000-grain weight and grain yield compared to the control genotype under drought stress conditions. Significant differences in the stress indices revealed that the *SeCspA* transgenic wheat lines possessed significant and stable improvements in drought tolerance over the control plants. The results indicated that *SeCspA* conferred drought tolerance and improved physiological traits in wheat plants.
Escherichia coli clones from a pond water metagenomic library were studied to identify salt tolerance genes in uncultivable bacteria by growing at inhibitory NaCl concentrations of 750 mM. Genes from two clones encoding for proteins similar to a putative general stress protein (*GspM*) having GsiB domain with a putative enoyl-CoA hydratase (*EchM*) identified to have a role in salt tolerance. After purification, *EchM* was found to have crotonyl-CoA hydratase activity [17].

CONCLUSION

We have to re-examine our knowledge about the usage of *E.coli* as PGPB (Plant growth promoting bacteria) to combat the use of chemical fertilizers in wheat crop production. However, this seems contentious, as the capability of *E.coli* to evolve and survive in a in different environment .Growth efficiency and competitiveness under conditions of low available nutrient levels likely represent the most important physiological factors leading to the successful persistence of *E.coli* in nutrient-limited open environments [18]. Therefore, it is important to understand the mechanism(s) of adaptation to nutrient-limited environments, which affect the survival of *E.coli* in such conditions. Now days there are growing demands for biologically based agricultural practices. Recent surveys of both conventional and organic growers indicated an interest in using biofertilizer products suggesting that the market potential of biofertilizer products will increase in the coming years. *E.coli* potential strains can therefore be used as a PGPB agent because its application resulted in better growth even under stressed environment. This strain may be helpful in minimizing the impact of temperature stresses which is currently limiting crop production under low input conditions and give rise to a more sustainable agriculture.More data are needed, especially genomic information of environmental *Escherichia* strains, to clarify the role of *E. coli* in enhancing abiotic stress tolerance like drought stress in wheat crop in Halotiregion of Rajasthan. We can focus on development of transgenic plants using *E. coli*. It would be more cost effective and eco-friendly to develop easy to handle microbial inoculants to alleviate abiotic stress. This abiotic stress suppressor might appear to be a very effectivestrategy for Drought stress alleviation in other cereal crops as well. The bacterial strain and the consortium formulation require further field evaluation before being confirmed as a bioinoculants to combat various abiotic stresses like heat and drought stresses in agro ecosystem of southwestern Rajasthan. Research on basic and applied remains to be done to unfold some hidden potentials of *E.coli* which may not be known yet. More of hard work to be done to commercialised E. *coli* as Biofertilizer.
Despite of several potential PGPB is discovered by various researchers but they have not been commercialized efficiently. Some research needs to be carried out to understand how

microbes and plant interact and efforts still require to make*E. coli*an efficient technique in sustainable agriculture. There are many questions still unanswered, and needs further research toexplore the potential traits of *E. coli* as PGPB and its role in Abiotic stress alleviation.

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