# SAMPLE LANDSCAPE ANALYSIS ON

# GENETIC ENGINEERING FOR ABIOTIC STRESS TOLERANCE IN PLANTS WITH SPECIAL FOCUS ON PLANT STRESS PROTEINS

## CONTENTS

## Introduction

All living organisms must adapt to changes in the environment. Adaptation to environmental stress is essential for the survival of organisms since dramatic changes such as cold shock, heat shock, acid shock, pressure and osmotic stress are lethal for most organisms. Plants respond and adapt to continuous environmental fluctuations by appropriate physiological, developmental and biochemical changes to cope with these stress conditions. The stress in plants is an induced physiological situation when there is severe or constant change in the environment or when normal conditions are aggressive, altering the physiological and adaptive pattern of plants. As an example of the changes that induce abiotic stress in plants, we can mention the variations of temperature, moisture, aqueous saline, soil pH, radiation, and pollutants, such as heavy metals and mechanical damage. All of these environment modifications produce physiological reactions in their cells of genetic origin.

Abiotic stresses are serious threats to sustainable food production. Drought, extreme temperatures and high salinity are major limiting factors for plant growth and crop productivity. Abiotic stress conditions cause extensive losses to agricultural production worldwide, because they affect negatively plant development and productivity. Up to 45% of the world's agricultural lands are subject to continuous or frequent drought and 19.5% of irrigated agricultural lands are considered saline. Also, crops and other plants are routinely subjected to a combination of different abiotic stresses. In drought areas, for example, many crops encounter a combination of drought and other stresses, such as heat or salinity. Together, these environmental stresses reduce the average yields for major crop plants by 50% to 70%. It is estimated that increased salinization of aerable land will have devastating global effects, resulting in 30% land loss in the next 25 years and up to 50% by the year 2050 (Wang et al).

In their quest to feed the ever-increasing world population, agricultural scientists have to contend with these adverse environmental factors. If crops can be redesigned to better cope with abiotic stress, agricultural production can be increased dramatically. Advances in understanding crop abiotic stress resistance mechanisms and the advent of molecular genetics technology allow us to address these issues much more efficiently.

Plants have developed several strategies to overcome these environmental challenges either through adoption mechanisms which allow them to survive adverse conditions or specific growth habits to avoid stress conditions and also numbers of genes and their products respond to abiotic stress at transcriptional and translational level. Plants can sense abiotic stress and elicit appropriate response with altered metabolism growth and development (Cramer et al 2011 and Krasensky and Jonak 2012). At the molecular level abiotic stress tolerance can be achieved through gene transfer in plants such as by altering the accumulation of osmoprotectants, increase in production of chapreones, enhance superoxide radical scavenging mechanism, exclusion or compartmentation of ions by efficient transporter and symporter systems. At the cellular level, plants adopt a wide range of responses to cope with abiotic stress. The mechanism associated with sensing stress, transduction of stress signals into the cell is well-known, and it represents the initial reaction of plant cells to stress (Desikan et al., 2004).

Furthermore, plant acclimation to a particular abiotic stress condition requires a specific response that is linked to the precise environmental conditions that the plant encounters. Thus, molecular, biochemical and physiological processes set in motion by a specific stress condition might differ from those activated by a slightly different composition of environmental parameters. Transcriptome profiling studies of plants subjected to different abiotic stress conditions showed that each different stress condition tested generates a somewhat unique response, and little overlap in transcript expression could be found between the responses of plants to abiotic stress conditions such as heat, drought, cold, salt, high light or mechanical stress. Each abiotic stress condition requires a unique acclimation response, anchored to the specific needs of the plant, and that a combination of two or more different stresses might require a response that is also unique.

## Different Approaches to Abiotic Stress

Before the genomics era, classical or conventional breeding of plants with improved physiological characteristics was the only way to improve crop productivity. In the last 20 years, mapping of quantitative trait loci (QTL), genetic engineering, and their implementation in marker assistant (molecular) breeding have become increasingly important. Such genomics-based approaches rely on the identification of genes (gene discovery) that may be valuable candidates for crop improvement and were empowered by the advent of state-of-the-art molecular tools, such as DNA sequencing and expression profiling.

The improvement of crop abiotic stress tolerance by classical breeding is fraught with difficulties because of the multigenic nature of this trait. Further complications arise from the large variability in stress sensitivity at different periods during the life cycle of a given plant. Of the various general types of plant response to salinity and drought stress, avoidance mechanisms mainly result from morphological and physiological changes at the whole-plant level. These are less amenable to practical manipulations. By contrast, tolerance mechanisms are caused by cellular and molecular biochemical modifications that lend themselves to biotechnological manipulation.

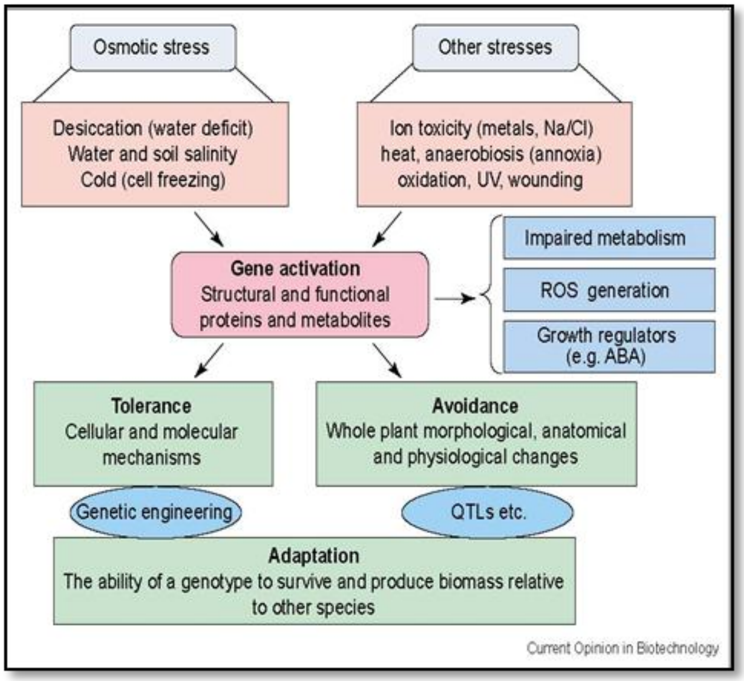


**Fig 1: Applied biotechnology: the interacting factors in molecular and conventional breeding for plant tolerance to abiotic stress**

All types of abiotic stress evoke cascades of physiological and molecular events and some of these can result in similar responses; for example, drought, high salinity and freezing can all be manifested at the cellular level as physiological dehydration. A full elucidation of abiotic stress tolerance mechanisms, and an intelligent breeding strategy for stress tolerance, requires clear and fact-based answers to a number of questions. Which genes and proteins are upregulated or downregulated by the different types of abiotic stresses? What are the functions of these stress-responsive genes and proteins? And, which can be used as genetic markers for the breeding and selection of stress-tolerant genotypes or otherwise successfully engineered in transgenic plants?

The development of genetically engineered plants by the introduction and/or overexpression of selected genes, such as the silencing of specific genes, seem to be a viable option to the breeding of resistant plants. Genetic engineering would be a faster way to insert beneficial genes than through conventional breeding too. Also, it would be the only option when genes of interest originate from cross barrier species, distant relatives, or from non-plant sources. Indeed, there are several genes whose correlative association with resistance has been tested in transgenic plants. Following these arguments, several transgenic approaches have been used to improve stress tolerance in plants.

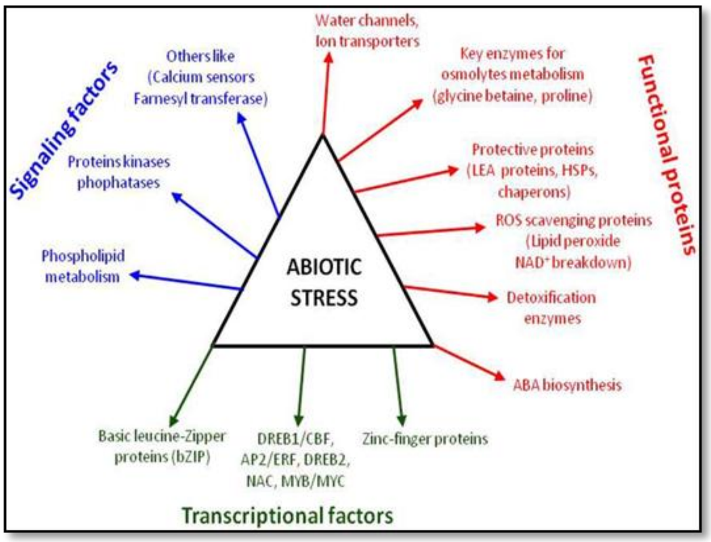


**Fig 2: Functions of drought stress-inducible genes in stress tolerance and their response**

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Gene products are classified into three groups. The first group includes proteins that probably function in stress tolerance (functional proteins), the second group contains protein factors involved in further regulation of signal transduction and gene expression that probably function in stress response (regulatory proteins), and the third group encloses proteins involves in transcription, gene regulation and expression most likely function in stress tolerance (transcriptional factors ).

## Plant Stress Proteins

### HEAT SHOCK PROTEINS

Temperatures above the normal optimum cause heat stress at different levels in all living organisms. Heat stress, often associated with salinity and drought stress, disturbs cellular homeostasis, and causes denaturation and dysfunction in many proteins, leading to severe retardation in growth, development and even death. Worldwide, extensive agricultural losses are attributed to heat, often in combination with drought or other stresses. The synthesis and accumulation of heat shock proteins (HSPs) are assumed to play a central role in the heat stress response and in tolerance to high temperatures in all plants, and other organisms.

The heat stress response is a highly conserved reaction caused by exposure of an organism tissue or cells to sudden high temperature stress, and it is characterized by rapid induction and transient expression of HSPs. These proteins primarily function as molecular chaperones to control the proper folding and conformation of both structural (*i.e.*, cell membrane) and functional (*i.e.*, enzyme) proteins, ensuring the correct function of many cellular proteins under conditions of elevated temperature. The primary protein structure for HSPs is well conserved in organisms ranging from bacteria and other prokaryotes to eukaryotes such as higher animals and plants. This conservation ensures a close involvement in the protection of the organism against heat shock and the maintenance of homeostasis.The induction agents of HSPs are high temperature stress, also by water stress, salt stress, low temperature stress and in some cases, by abscisic acid.

**OSMOTIC STRESS PROTEINS**

Osmotic Stress Proteins (OSP) includes water stress proteins and salt stress proteins. The agents which induce osmotic stress proteins are low water availability and salt stress. The characteristic features of OSP are varied molecular weights and cellular locations, are mostly the enzymes involved in diverse functions such as production of different osmolytes, protein degradation, signal transduction, gene regulation and transport.

**ANAEROBIC PROTEINS**

The anaerobic stress such as flooding or submergence induces anaerobic proteins (ANPs). These were discovered initially in maize and later shown to be universally present. Most of the ANPs have been shown to be the enzymes of the fermentative or the glycolytic pathway, most genes encoding ANPs contain anaerobic response elements in their promoters.

**COLD SHOCK PROTEINS**

Only one-third of the total land area on Earth is free of ice and 42% of land experiences temperatures below −20 °C. In such areas, plants require specialized mechanisms to survive exposure to low temperature. Cold stress can be classified as chilling (0–15 °C) and freezing (<0 °C) stresses. Generally, plants originating from temperate regions, such as spinach and Arabidopsis, exhibit a variable degree of chilling tolerance and can increase their freezing tolerance during exposure to chilling and non-freezing temperatures. This process is known as cold acclimation. On the other hand, plants of tropical and subtropical origins are sensitive to chilling stress and lack the cold acclimation mechanism. The molecular basis of cold acclimation and acquired freezing tolerance in plants, mainly Arabidopsis and winter cereals, has been extensively studied. To adapt to cold stress during cold acclimation, gene expression is reprogrammed and the metabolism is also modified. Cold response is a very complex trait involving many different metabolic pathways, gene regulations and cell compartments.

Low temperature affects several aspects of plant adaptation, e.g., freezing tolerance, plant growth, abiotic resistance and senescence. Among phytohormones, ABA, auxin, gibberellic acid (GA), salicylic acid (SA) and ethylene are related to the cold responses positively or negatively. Cold shock proteins are conserved proteins of varied sizes and functions, most cold regulated genes contain specific nucleotide sequence that stimulate transcription in response to low temperature.

## Patent Landscape

SCOPE AND COVERAGE

A. A background study was performed to understand the area of genetic engineering for abiotic stress tolerance in plants and the different plant stress proteins in various online resources and research journals. Based on the understanding, the following keywords were identified:

   Abiotic, stress, tolerance, plant, crop, protein, heat, shock, cold, osmotic, anaerobic, genetic or combination and other such relevant words.

B. A review of the International Patent Classification (IPC) was carried out to identify the patent classification code related to genetic engineering for abiotic stress tolerance in plants and plant stress proteins.

   The identified relevant IPC Codes were: **A01H1/00, C12N15/82, A01H5/00, C12N5/10, C12N15/00, C07K14/415, C07K14/245, C07K16/16, C12N5/04, C07H21/00, C07K14/00, C12N5/04, C12N15/63, C12N15/29, C12Q1/68, C12N 15/09**

C. The various patent and patent applications were extracted from paid database such as QUESTAL ORBIT and various free databases like Freepatentsonline, SUMOBRAIN, GOOGLE PATENTS and Patent Lens using the combination of the above identified keywords and IPC Code.

D. An approximately 2500 patent and patent applications in the area of genetic engineering for abiotic stress tolerance in plants in specific to plant stress proteins were extracted for the period 2003 to 2012 and were analyzed to present the patent landscape under two heads viz., Patenting Activities in the World and Patenting Activities in India

## Patenting Activities in the World

### 1. Patent Publishing Trend

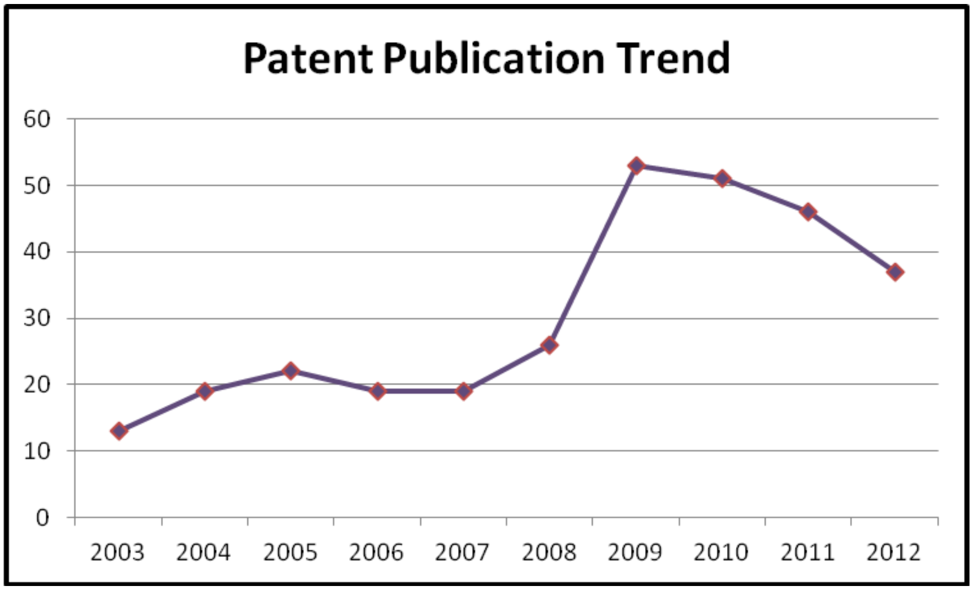


**Fig 3: Patent Publication Trend**

The analysis of the figure 3 shows that there is an increasing trend in the patent publication from 2003 to 2009 and decreases thereafter. This can be attributed to the impact of the global recession on funding the biotech industries.

### 2. Top Assignees

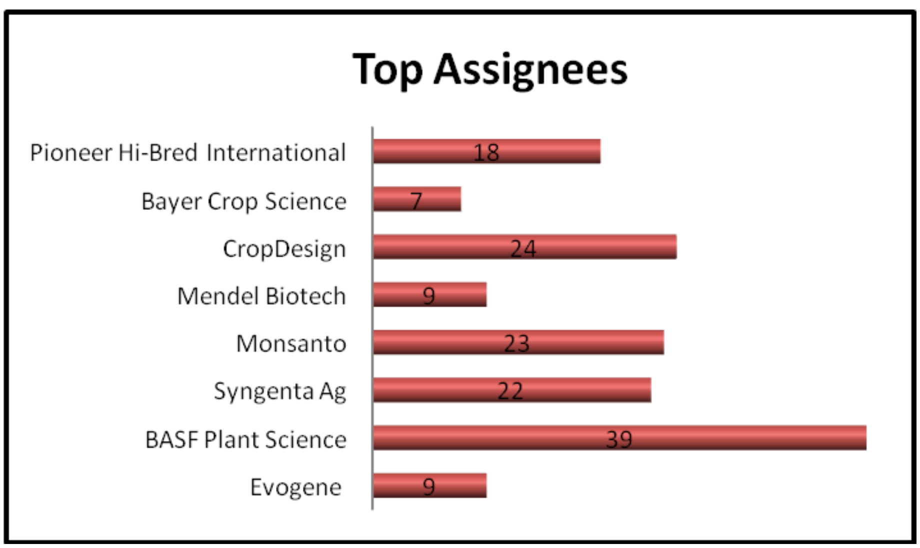


**Fig 4: Top Assignees in the field of Genetic Engineering of Plant Stress Proteins**

The above figure brings out the major players in agricultural genetic engineering. There is conglomeration of biotech companies in United States namely Monsanto, Pioneer Hi-Bred International and Mendel Biotech. BASF Plant Science and Bayer Crop Science are based in Germany while Syngenta Ag is based in Switzerland, CropDesign in Belgium and Evogene in Israel.

## 3. Top Patent Filing Countries

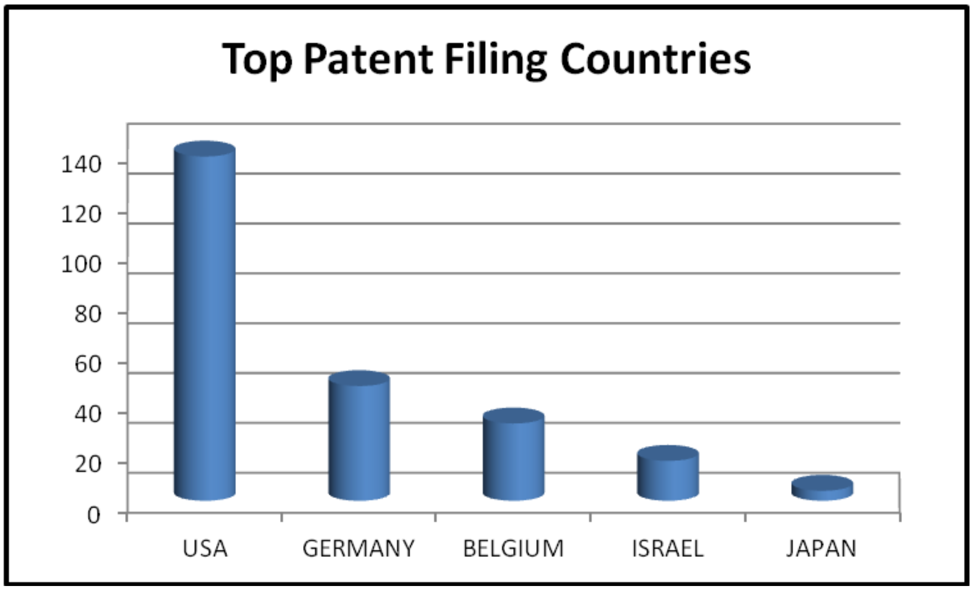


**Fig 5: Top Patent Filing Countries in Genetic Engineering of Plant Stress Proteins**

The analysis of figure 5 shows that USA tops with more filing of patents followed by Germany, Belgium and Israel. This is clear from the figure 3 where the top assignees have their basis in USA, Germany, Belgium and Israel.

## 4. Protein-Wise Distribution of Patents

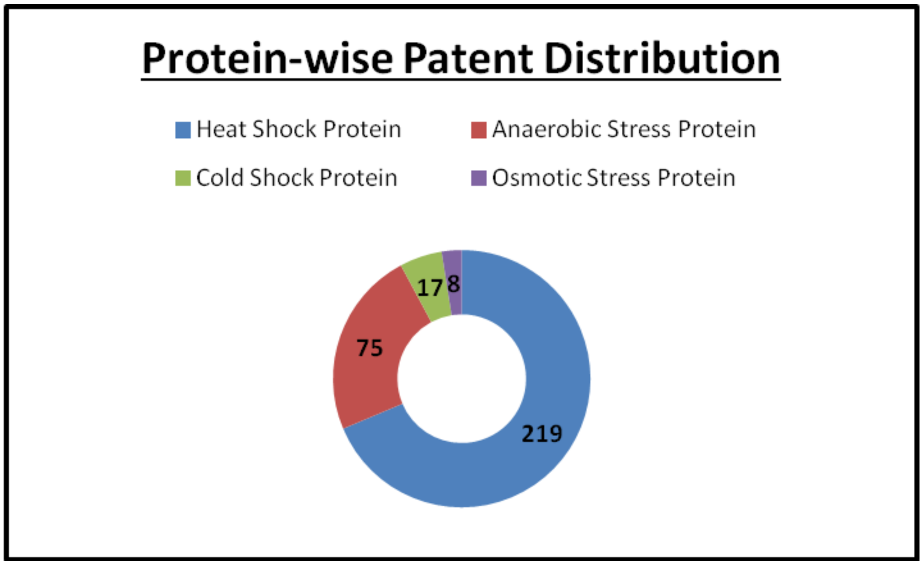


**Fig 6: Protein-wise Distribution of Patents**

The above figure elucidates the distribution of patents in plant stress proteins. There are more number of patents in the area of heat shock proteins, followed by anaerobic stress, cold shock and osmotic stress proteins. The main abiotic stress that affects the plant is drought which has made researchers to focus more on heat shock proteins.

## 5. Break Down of Patent Filings by IPC Code

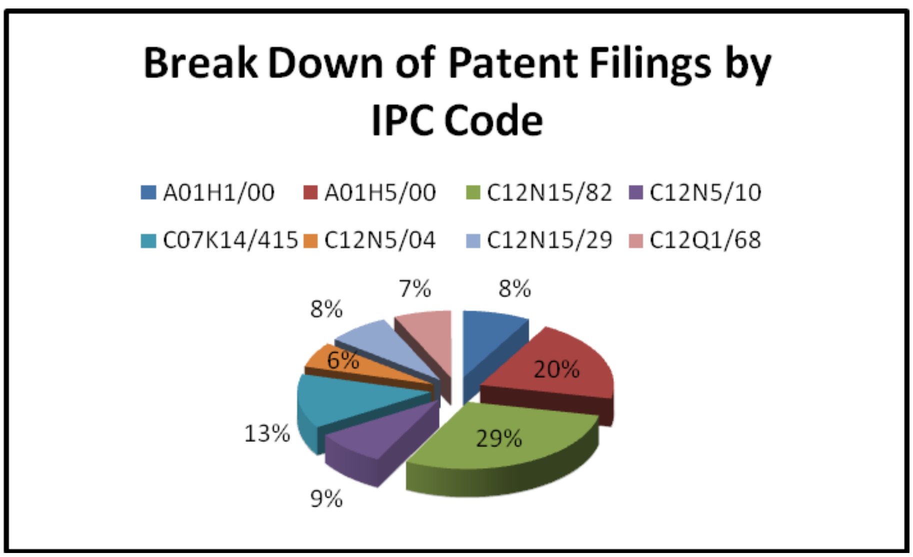

**Fig 7: Break down of Patent Filings by IPC Code**

The above figure shows the most relevant IPC codes in the area of genetic engineering for abiotic stress tolerance in plants in specific to plant stress proteins. More number of patents has been filed in C12N15/82 followed by A01H5/00 and C07K14/415.

**Table 1: Key Technology in Genetic Engineering**

| IPC Code | Definition |
|---|---|
| **A01H1/00** | Processes for modifying genotypes |
| **A01H5/00** | Flowering plants, i.e. angiosperms |
| **C12N15/82** | Mutation or genetic engineering; DNA or RNA concerning genetic engineering, vectors, e.g. plasmids, or their isolation, preparation or purification; Use of hosts therefor for plant cells |
| **C12N5/10** | Cells modified by introduction of foreign genetic material, e.g. virus-transformed cells |
| **C07K14/415** | Peptides having more than 20 amino acids; Gastrins; Somatostatins; Melanotropins; Derivatives thereof from plants |
| **C12N5/04** | Mutation or genetic engineering; DNA or RNA concerning genetic engineering, vectors, e.g. plasmids, or their isolation, preparation or purification; Use of hosts therefor Plant cells or tissues |
| **C12N15/29** | Mutation or genetic engineering; DNA or RNA concerning genetic engineering, vectors, e.g. plasmids, or their isolation, preparation or purification; Use of hosts therefor Genes encoding plant proteins, e.g. thaumatin |
| **C12Q1/68** | Measuring or testing processes involving enzymes or micro-organisms (measuring or testing apparatus with condition measuring or sensing means, e.g. colony counters,C12M 1/34); Compositions therefor; Processes of preparing such compositions involving nucleic acids |

## Patenting activities in India

Crop production encounters various biotic and abiotic stresses particularly in the arid and semiarid regions. The micro and macro crop environments pave way for the natural formation of different agro-climatic zones and the quantum of deleterious and adverse stress factors become realistic in limiting yield realization in crops. In this context, plant physiological approaches in crop production assume importance to unravel the abiotic stress mechanisms and to identify explicit tolerance traits for countering the detrimental stress onslaught during ontogeny.

India, being an agrarian country, the analysis of the patent in the field of abiotic stress assumes importance due to following factors. The total food grain production in 2011-12 was 257.44 MT while in 2010-11 it was 244.78 MT; projected demand by 2030 is 280 MT. Farm growth has dived to a dismal from 2.9% to 2.5% due to climate change - erratic rainfall and lower yield per unit. Agriculture and its allied activities accounted for about 13.9 percent of the GDP in the period 2011-12. Major crops such as wheat, pulses and oil seeds showed decline in output farm area with deficit rainfall increased by 40% from 28% during last 3 years and delayed irrigation projects have made matters worse.

There are a number of patents filed during the period 2003-12 in relation to proteins for abiotic tolerance of crops in India. The data extracted from Indian Patent database and ORBIT shows that around 182 patents were filed in this period for Heat Shock Protein in abiotic tolerance. Out of which 4 were filed by Indian researchers and rest were filed as PCT national phase applications. Similarly, for anaerobic proteins, total of 51 patents were filed as national phase applications. For Cold Shock protein, merely 15 applications were filed where only one has been filed by an Indian applicant and rest for National phase. Lastly for Osmotic stress protein category, total of 180 patents were filed where 13 were filed by Indian applicants.

**Table 2: Few Exemplary patent applications filed for Plant Stress Proteins**

| Application Number | Title | Original Patent Assignee |
|---|---|---|
| **1700/DEL/2011** | POLYNUCLEOTIDES ENCODING HEAT SHOCK TRANSCRIPTION FACTOR AND USES THEREOF | INTERDISCIPLINARY CENTRE FOR PLANT GENOMICS & DEPARTMENT OF PLANT MOLECULAR BIOLOGY UNIVERSITY OF DELHI SOUTH CAMPUS |
| **2437/DEL/2009** | METALLOTHIONIEN POLYNUCLEOTIDE FROM RICE CONFERRING ABIOTIC STRESS TOLERANCE IN PLANTS | PAREEK ASHWANI |
| **2460/MUM/2008** | IDENTIFICATION OF GENES RELATED TO ABIOTIC STRESS TOLERANCE IN JATROPHA CURCAS | RELIANCE LIFE SCIENCES PRIVATE |
| **0767/CHE/2007** | EMERGENCE OF THIONIN GENE FOLLOWING EXPOSURE TO HIGH SALINITY STRESS POSSESSING SIGNIFICANT AND PHARMA UTILITIES | AVESTHA GENGRAINE TECHNOLOGIES PVT |
| **US 20110258742** | Stress Tolerant Transgenic Crop Plants | Monsanto Technology Llc |
| **US 20070124839** | Active substances for increasing the stress defense in plants to abiotic stress, and methods of finding them | Bayer Cropscience Gmbh |
| **US 7786353** | Methods for enhancing drought tolerance in plants and compositions thereof | Monsanto Technology Llc |
| **US 20120030836** | Plants Having Enhanced Abiotic Stress Tolerance and/or Enhanced Yield-Related Traits and a Method for Making the Same | BASF PLANT SCIENCE GmBH |
| **US 7732667** | Nucleic acid molecules from rice controlling abiotic stress tolerance | Syngenta Participations Ag |
| **EP1925672** | Abiotic stress responsive polynucleotides and polypeptides | Syngeta Participations AG |
| WO2012145269 | Yield and stress tolerance in transgenic plants | Mendel Biotechnology, Inc. |
| **US20040128712** | Methods for modifying plant biomass and abiotic stress | Cai-Zhong Jiang |

Note: We can share a complete list of the patent documents on request

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