Transgenics: An emerging approach for cold tolerance to enhance vegetables production in high altitude areas

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Abstract

By the 2020, global population is expected to reach 8 billion. Conventional breeding methods alone cannot feed the extra hungry mouths. Despite the successes of the green revolution with substantial strides in food grains production, India is still classified by FAO as a low income, food deficit country, nearly 26 per cent of India’s population (268 million) are considered food insecure, consuming less than 80 per cent of minimum energy requirements and account for about a third of the world’s population that suffers from chronic hunger. Agricultural biotechnology has the potential to reduce crop losses from pest and diseases, to improve the nutrient efficiency of food and animal feeds; to extend post harvest losses with increased shelf life of fruits and vegetables; and to increase the stress tolerance of crop plants allowing them to tolerate various environmental extremes such as cold and drought. The biotechnology has the potential to enhance the productivity and also extend the cropping period especially in high altitude areas where vegetable cultivation is impossible in severe winter months. Modern Biotechnology has the tools to develop cold tolerant varieties, which enhance the productivity and profitability of farming. It empowers the hill farmers by boosting the productivity and also enhancing their nutritional security. The present article examine the use of cold tolerant genes in vegetables and also tools of biotechnology the DRDO agricultural laboratories employs towards transformation of hill agriculture through development of cold tolerant transgenics in vegetables.

Key words: Transgenic, cold tolerant, high altitude, abiotic stress

Introduction

Freezing temperature greatly limits the geographical distribution of cultivated plants and often causes severe losses in agriculture production and productivity. Conventional breeding methods have met with limited success in improving the freezing tolerance of important vegetables. Biotechnology offers new strategies that can be used to develop transgenics crop plants with improved tolerance to freezing stress. A number of genes have been isolated and characterized that are responsive to freezing stress.

The molecular tool makes it possible to select directly at the gene label without waiting for the phenotype to show up. Therefore it is important to use most appropriate tools that help in reaching the goals. The designed genotype should be better than the available ones and must reach the farmers. Transgenic technologies have opened up many exciting possibilities to improve products with added value with application in food, agriculture, animal husbandry, environment, medicine and industry. It also offers uncommon opportunities for improvement in genetic potential of plants and animals by introduction or removal of gene or genes that regulate a specific trait. The conventional breeding methods can be complimented by an array of bio-technological tools can be used to augment vegetable production by saving time and resources. The potential outcome be in the form of development of specific vegetable varieties that are more resistant to biotic and abiotic stresses, enhanced nutritional level of food items, enhanced shelf life of perishable farm produce, conversion of organic waste into bio-fuels.

As 100 million new people are added to the world’s population each year and world population is predicted to reach eight billion by the year 2010. The population increase in developing countries constitutes 97 percent of the global increase. Feeding 3 billion additional people will require dramatic increase in crop production, a formidable task by any standard.

There are limitations to conventional plant breeding technology either due to the limited gene pool or the restricted range of plant species between which genes can be transferred due to species barriers. Resource-poor farmers carry out 60 percent of global agriculture, but produce only 15-20 per cent of the world’s food. The crops face major challenges from abiotic and biotic stresses and limited access to external inputs like pesticides, fertilizers and irrigation. The newly acquired ability to transfer genes between organisms without sexual crossing provides breeders with new opportunities to improve the efficiency of production and increase the utility of agricultural crops. While the new tools, technologies, and products will come from rapid advances in molecular biology and genetic engineering. The rapid development of plant biotechnology, agriculture can be efficiently moved from a resource based to a science-based industry.

Present status of transgenic crops

The global area planted with transgenics crops has risen from 1.7 million hectares in 1996 to 90 million hectares in 2005 (James, 2005). Out of the total area of the world under transgenic crops, three countries
namely USA, Argentina and Canada, which cover more than 90 percent. The major crops occupying about more than 80 per cent area are herbicide-tolerant soyabean, Bt corn, herbicide tolerant canola, and herbicide tolerant cotton. The three varieties namely Mech 12, Mech 162 and Mech 184 of Bt cotton have been released in India for commercial cultivation. Several transgenic cultivars of major food crops, such as soyabean, maize, canola, potato and papaya have been commercially released incorporating genes for resistance to herbicides, insects, and viruses.

Transgenic crops under commercial production across the globe.

According to (Manjunath, 2005), 04 field crops, 03 vegetables, 01 fruit crop and 01 tobacco crops are presently produced commercially across the globe is depicted in table 1.

Table 1. Transgenic crops under commercial production at present

<table>
<thead>
<tr>
<th>Type of crop</th>
<th>Name of crop</th>
<th>Name of country</th>
</tr>
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<tbody>
<tr>
<td>1. Vegetables</td>
<td>Tomato &amp; Pepper</td>
<td>China</td>
</tr>
<tr>
<td></td>
<td>Squash</td>
<td>USA</td>
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<tr>
<td>2. Field crops</td>
<td>Soyabean</td>
<td>Canada, USA, Argentina, South Africa, Brazil, East Europe, Uruguay, Paraguay and Chile USA, Australia, Argentina, Mexico, China, South Africa, India and Colombia</td>
</tr>
<tr>
<td></td>
<td>Cotton</td>
<td>USA, Australia, Argentina, Mexico, China, South Africa, India and Colombia</td>
</tr>
<tr>
<td></td>
<td>Maize</td>
<td>Canada, USA, Western Europe, Argentina, South Africa, Uruguay, Philippines and Chile Canada and USA</td>
</tr>
<tr>
<td></td>
<td>Canola</td>
<td>USA, Argentina</td>
</tr>
<tr>
<td>3. Fruits</td>
<td>Papaya</td>
<td>Hawaii (USA)</td>
</tr>
<tr>
<td>4. Other crops</td>
<td>Tobacco</td>
<td>USA</td>
</tr>
</tbody>
</table>

Till date no transgenic crop variety tolerant to abiotic stress has so far been reported to be released for cultivation. However, 7 transgenic varieties exhibiting tolerance to a range of abiotic stresses underwent field testing in Bolivia (frost tolerant potato variety), China (cold tolerant tomato), Egypt (salt tolerant wheat variety), India (Moisture stress tolerant Brassica variety) and Thailand (salt tolerant and drought tolerant rice varieties). Most of the R&D activities on development of abiotic stress tolerant crops are being carried out in six countries of the Asian region namely Bangladesh, China, India, Indonesia, Pakistan and Thailand.

Major components for the development of transgenic plants

Despite significant advances over the past decade, development of efficient transformation methods can take many years. Effective regeneration and transformation systems are the prerequisite for successful genetic transformation (Sharma et al, 2005). Genetic transformation technology relies on the conceptual framework and the technical approaches of plant tissue culture and molecular biology to develop commercial process and products which comprises mainly the nine major components:

(i) The development of reliable tissue culture regeneration systems
(ii) Preparation of gene construct and transformation with suitable vectors
(iii) Efficient techniques of transformation for the introduction of genes into crop plants
(iv) Recovery and multiplication of transgenic plants.
(v) Molecular and genetic characterization transgenic plants for stable and efficient gene expression
(vi) Transfer of genes to elite cultivars by conventional breeding methods if required
(vii) Evaluation of transgenic plants for their effectiveness in alleviating the biotic or abiotic stresses and field performance
(viii) Bio-safety assessment including food and environmental safety
(ix) Commercialization of genetically engineered crop.

Important crops developed through genetic engineering

A number of transgenic plants developed so far through plant genetic engineering for different agronomic and qualitative traits are

(i) Herbicide tolerant transgenic plants have been engineered by using mutants EPSP (5-enolpyruvyl shikimate-3-phosphate) synthase enzyme e.g. soybean, canola, cotton and tobacco.
(ii) Transgenic for insect resistance have been developed introducing a gene from Bacillus thuringiensis coding a toxic protein (delta endotoxin) which inhibits insect growth. e.g. Bt cotton, Bt maize, Bt tobacco.
(iii) Transgenics for male sterility (Barnase and Barstar in Brassica spp. for hybrid seed production).
(iv) Stress resistance transgenics. e.g. chilling resistance in tobacco, a gene for glycerol-1-phosphate transferase from Arabidopsis.
(v) Transgenics for disease resistance such as fungal diseases, bacterial diseases, viral diseases.

(vi) Transgenics for food processing, Bruise resistance tomato (expresses antisense RNA against polygalacturonase which attacks pectin), delayed ripening

**Hill agriculture and prospects of cold tolerant genes**

Cold is an environmental factor that limits the geographical distribution and growing season of many plant species, and it often adversely affects crop quality and productivity (Thomashow, 1999). Most temperate plants can acquire tolerance to freezing temperature by a prior exposure to low nonfreezing temperature, a process known as cold acclimatization (Guy 1990, Hughes and Dunn, 1996, Browse and Xin, 2001). Plants of tropical and subtropical origins are sensitive to chilling temperature (0°C-100°C) and are incapable of cold acclimation. Many studies have suggested that cold regulated gene expression is critical in plants for both chilling tolerance a (Gong et al. and Hsieh et al. 2002) and cold acclimation (Knight et al. 1999: Thomashow 1999, Tamminen, 2001). Cold responsive genes encode a diverse array of proteins such as enzymes involved in respiration and metabolism of carbohydrates, lipids, phenylpropanoids and antioxidants: molecular chaperones, antifreeze proteins, and others with a presumed function in tolerance to dehydration caused by freezing (Guy 1990, Thomashow 1999 and Mohapatra et al. 1989).

The change in the gene expression occur in plant during cold acclimatization a developmental process that results in increased tolerance (Guy, 1990 and Steponkus et al. 1993). Since then, it has repeatedly been speculated that certain COR (cold regulated) genes might have role in freezing tolerance. To test this notion investigators have turned to isolating the characterizing genes that are expressed in response to low temperature. These efforts have led to the identification of a number of genes such as the COR 15a KIN1 LTI 78 fad 7 genes of Arabidopsis thaliana.

It is well known to farmers and scientists that low temperatures can kill plants. Low temperature stress is a major environmental factor that not only limits where crops can be grown but also reduces yields depending on the weather in a particular growing season. In addition to exceptionally stressful years that cause significant yield reductions, less extreme stress almost certainly causes smaller losses over large areas to produce comparable yield reductions every year. Even in cases when freezing stress does not result in yield loses., it often results in crop quality reduction. Each year, worldwide losses in crop production due to low temperature damage amount to approximately $2 billion. Some of the major losses include the 1995 early fall frosts in the US which caused losses of over $1 billion to corn and soyabean. The occasional freezes in Florida have shifted the citrus belt further south, and California sustained $650M of damage in 1998 to the citrus crop due to a winter freeze.

The inherent cold hardiness of the crop determines in which agricultural areas it can be grown. Crops that are more resistant to freezing stress would allow some geographical regions to grow more profitable and productive crops with less environmental risks. However despite continued efforts, traditional breeding has had only limited success in imparting crop plants with better freezing tolerance due to very little was known about the mechanisms that regulate chilling and freezing tolerance. With the advent of molecular genetics and biotechnology, it is now possible to genetically engineer plants to be more tolerant to many environmental adversities, including low temperature.

**Cold acclimatization and signal transduction cascade**

Plants vary greatly in their ability to withstand low temperature stress. As an adaptation strategy, most plants native to the temperature climates develop freezing tolerance after prior expose to cold, Non-freezing temperatures, a phenomenon called acclimatization. Many biochemical and physiological changes are known to occur during cold acclimation. With the onset of low temperature, putative temperature sensors at the cellular membrane generates stress signals which are transmitted and amplified through many steps that include Ca++ signaling and a stepwise kinase/phosphatase chain reaction termed the kinase cascade. The massage eventually reaches the nucleus and regulators of gene expression called transcription factors, which act as master switches to regulate the expression of groups of genes, resulting in the increase of proteins and other organic molecules that protect the cell from freezing damage. The plants response to cold acclimation is clearly complex and diverse, and the actual biochemical and physiological changes are still poorly understood at the molecular level.

**Technology development toward cold tolerance**

The cbf genes represent one of the most significant discoveries in the field of low temperature adaptation and signal transduction. All-important crops and few vegetables and tree species have contained this gene. The transgenic canola contain this gene are able to survive freezing temperature as much as 4-5°C lower than the non transgenic controls (Jaglo et al. 2001). Tomato plants have also been successfully engineered using the CBF genes to achieve chilling tolerance (Hsiech et al. 2002). Thus the CBF technology has a great potential for improving the cold and freezing tolerance in plants.

There is strong evidence that the decrease in fatty acids saturation that generally occurs in temperate plants upon exposure to low temperature contributes the cold tolerance of these plants. Ishizaki-Nishizawa et al. 1996 found that by introducing a broad-spectrum desaturase gene from a cyanobacterium into tobacco plants could increase the low temperature tolerance of the transgenic plants. Expression of a plant phosphatase (At PP2CA) in transgenic Arabidopsis thaliana can accelerate the development of cold acclimation and increase freezing tolerance. It has also shown that transgenic plants expressing a constitutively active kinase NPK1, is more tolerant to chilling and other abiotic stresses (Kovtun et al. 2000). Other transcription factors, including ABI3 and SCOF-1, have been used to successfully increases the cold tolerance of transgenic plants (Tamminen et al. 2001).

Transcription factor genes AbI3, abI3, cbf1, dreb1A, dreb1 and dreb2 from A. thaliana, osmolyte biosynthesis genes from Arthrobacter globiformis and afb (anti freeze protein) from A. thaliana are some other important genes which have effective role to cold tolerance in plants

**Genes used for transgenic development in tomato, capsicum and cucumber for high altitude region**

Advancement in plant biotechnology has led to the identification and isolation of a number /transcription factor(s) related to abiotic stress tolerance. Defence Research and Development Organisation has taken up the work on development of transgenics for cold tolerance through cloning and transformation of these genes into high value vegetable crops to cultivate them in higher altitude areas for the fresh requirement of armed forces and local populace. At present the work is carried out to develop transgenics with the following genes in tomato, cucumber, pea and brinjal and cloning of cold tolerant genes from seabuckthorn plant.

**Osmotin gene:** Among the various environmental factors, availability is the major factor influencing growth, development and productivity of crop plants. Accumulation of low molecular weight osmolytes such as praline, betaines and sugar alcohol is an important mechanism underlying adaptation to such stress factors. In addition, stress induced proteins whose functions, however, remain largely unknown and enzymes that toxify reactive oxygen species can also contribute to stress tolerance. Osmotin is a basic 24 KD protein that was originally identified in tobacco adapted to NaCl and desiccation. The stress induced synthesis and accumulation of the osmotin protein is correlated with osmotic adjustment in tobacco cells. The synthesis of osmotin is induced by ABA that accumulates in response to osmotic stress and subsequently plays a pivotal role in osmotic adjustment. It is known that expression of osmotin gene induces proline accumulation in unstressed and stressed plants and imparts tolerance to both salinity and drought stress. The gene has been transferred through *Agrobacterium* in tomato at DARL, Pitroagargh and transgenic plants are obtained and T3 plants are being tested in high altitude areas under controlled environments.

**Mannitol-1 phosphate Dehydrogenase (mtlID):** Mannitol-1 phosphate dehydrogenase (mtlID) is one of the gene encoding enzyme that synthesize osmoprotectants and enhance their expression in transgenic plants which leads to maintenance of osmotic potential during the stress period. The candidate gene has been isolated from *E. coli* K-12 strain and code for an enzyme called mannitol-1 phosphate. Mannitol-1 phosphate then converted into mannotol by non specific phosphates. Accumulation of mannotol in different parts of a plant leads to tolerance at cellular level by adjustment of the cytosolic osmotic potential when the concentration of electrolytes is lower in the cytosol than in the vacuole.

**OSISAP1 gene of rice and CBF1 gene of Arabidopsis:** The expression of these genes changes in the membrane lipid composition, accumulation of compatible osmolytes or cessation of plant growth. Cloning and expression of these genes has led to the development of strong tolerance to freezing through transgenic approaches.

**Dehydration responsive element binding factor (DREB/CRB3):** The gene is responsible for the expression of many cold tolerant genes during cold stress in plants

**Glyceraldehyde phosphate Acetyl Transferase (GPAT) gene:** The gene is responsible for the unsaturation of the fatty acids present in the plant cell wall, which give the cold tolerance to the plants during cold stress.

**Cold regulated (COR15A) gene:** This gene encodes for polypeptides that decreases the incidence of freeze induced lamellar to hexagonal II phase transitions, which increases freezing tolerance to the plants.

**Desaturase (desC) gene:** The expression of this gene increases the fatty acid unsaturation of the cell wall lipids giving strength to the cell wall of the plant during cold stress.

**Conclusion**

The high altitude regions have a very harsh climate and a short agriculture season. Only a few vegetable crops viz, radish, turnip, potato, cabbage and some leafy vegetables are grown traditionally, in these areas. The climatic and geographic differentiation segregates the region from rest of the world. Therefore, the agro-techniques for the vegetable production being
employed elsewhere is not suitable for these conditions and specific agro-techniques and vegetable varieties are required for these regions. Lots of works has already been done to identify the suitable varieties/hybrids and cultivation practices for these regions.

The transgenic technology is highly precise and beneficial for high altitude farming. The major limitation is the complexity of tolerance to abiotic stresses that is normally dependent on a number of physiological traits, each under multigenic control. The increased wealth of knowledge that is being acquired by means of genomic and other molecular biology studies and the cloned genes will certainly contribute to the development of tolerant genotypes. The successful integration of the cold tolerance genes into high value vegetable crops would increase productivity and production in high altitude regions. The transgenics having cold resistance gene may not only bring additional areas under cultivation but also help in optimizing productivity in high altitude and remote areas without any additional cost will enable or extend the cultivation of crops in abiotic stress, which are presently not under cultivation or are less productive.

References


