Production of high temperature tolerant transgenic plants through manipulation of membrane lipids

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In 1992, a major breakthrough was made when Murata *et al*.[1] produced transgenic tobacco plants with increased low temperature tolerance. Incidentally, this was the first report on production of a transgenic plant tolerant to any abiotic stress. This work involved over-expressing glycerol 3-phosphate acyltransferase gene from *Cucurbita maxima* and *Arabidopsis thaliana* into tobacco cells, resulting in increased fatty acid unsaturation. It was proven in this work that membrane lipids hold the key for improvement of photosynthesis under low temperature stress conditions and improvement of photosynthesis is a governing factor for stress tolerance against low temperature stress. Nearly ten years later, it is now shown that membrane lipids are important for improvement of photosynthesis against high temperature stress and improved photosynthesis means improved stress tolerance as well[2].

Supra-optimal temperatures cause a major stress (i.e. high temperature stress) on crops. Living organisms are adapted to grow, reproduce and complete their life cycle in a narrow range of temperature regime. Unlike homeothermic animals, plants are by and large incapable of maintaining body temperature optimal for their life activities. A slight increase in temperature, even transiently, may affect physiological and biochemical processes of plants to a great degree[3]. Adverse effects of high temperature stress have been noted during both vegetative and reproductive stages in various crop plants. Processes leading to floral development and quality of seeds are critically affected by high temperature stress. Though rice (perhaps the most important food crop in the world) is considered tolerant to high temperature stress, high temperature causes pollen and spikelet sterility in rice

in many parts of the world, including Punjab in India[4]. This effect of high temperature at the time of anthesis is so fatal that even 1°C rise in ambient temperature for just one hour can lead to a high level of spikelet sterility. In crops like wheat which are grown and consumed widely in India, high temperature stress causes a significant degree of loss in grain production. The problem of high temperature stress is expected to accentuate in future as global warming in years to come may have catastrophic consequences for the biosphere[5]. It is projected that the combined effects of increasing atmospheric concentrations of CO_2 and traces of other greenhouse gases (methane, di-nitrogen oxide and chlorofluorocarbons) might lead to an equivalent of doubling at the current level of 355 ppm of CO_2 as early as the 2040 AD; this means that the global mean equilibrium surface temperature will increase by 1.5 to 4.5°C, causing 20 to 140 cm rise of sea level from thermal expansion of water[5]. This scenario warrants an urgent need on the part of plant scientists for stabilizing crop production through greater resistance to environmental stresses.

Transgenic technology has emerged as a useful tool for improving genetics of crops for better survival, growth and yield[6]. During the past one decade, major success has been achieved in producing transgenic plants with increased tolerance to different abiotic stresses[7–17]. Generally speaking, it now appears that genetic engineering for abiotic stresses is within the realms of science of plant biotechnology.

Does this science (i.e. plant genetic engineering) have an answer to the global warming phenomenon, in terms of producing high temperature-tolerant crops? Principally, what makes a particular goal attainable or unattainable in genetic engineering experiments is governed by availability of the relevant gene which should lead to the desired trait when over-expressed in a transgenic host. Do we have genes identified which will lead to tolerance against high temperature stress? An effective approach for obtaining such genes is to bank on available physiological/biochemical information. High temperature-induced symptoms of impairment are noted on activities associated with seedling growth and vigour, root growth, nutrient uptake, water relations of cells, solute transport, photosynthesis, respiration, general metabolism, fertilization and maturation of fruits[3]. Altered levels of enzymes, membrane structure, photosynthetic activities and protein metabolism represent principal components of plant heat shock response[3]. The rate of photosynthesis in most species declines at about 35°C which is ascribed to protein denaturation, loss of membrane integrity, photoinhibition and ion imbalance. High temperature (a) induces photosynthetic ion imbalance, (b) affects chloroplast biogenesis and senescence, (c) causes disintegration of grana, (d) brings about disruption of the structure of membrane proteins, (e) influences protein–lipid interactions, (f) affects electron transport activities and (g) substantially decreases RuBP carboxylase enzyme activity[3]. Prominent heat shock-induced ultrastructural changes in plants have been reported for the nucleus, endoplasmic reticulum, mitochondria and plastids[18]. Considering the above complexity in the response of plants to high temperature stress, it has always been a difficult task to ask which genes would be important for genetic improvement of crops against high temperature stress.

However, this complexity in high temperature stress response has not been a limiting factor in production of high temperature-tolerant transgenic plants (Figure 1). In 1995, a group in Germany[19] achieved success in production of high temperature stress tolerant transgenic *Arabidopsis* plants by altering the level of expression of heat shock proteins (HSPs) through change in the expression levels of *Arabidopsis* heat shock transcription factor (AtHSF). In this work, it was shown that AtHSF of *Arabidopsis* is constitutively expressed but its activity for DNA binding, trimer formation and transcriptional activation of *Hsp* genes is repressed at normal temperature. They were able to derepress the HSF function by experimental means which led to constitutive expression of HSPs at normal temperature and increased basal thermotolerance. Malik *et al.*[20] have reported increase in thermotolerance in transgenic carrot cell lines and plants by constitutive expression of carrot *hsp17.7* gene (driven by CaMV 35S promoter). Another group of workers from Japan[21] raised high temperature-tolerant transgenic plants through increased osmolyte synthesis. Earlier workers have documented that *codA* gene

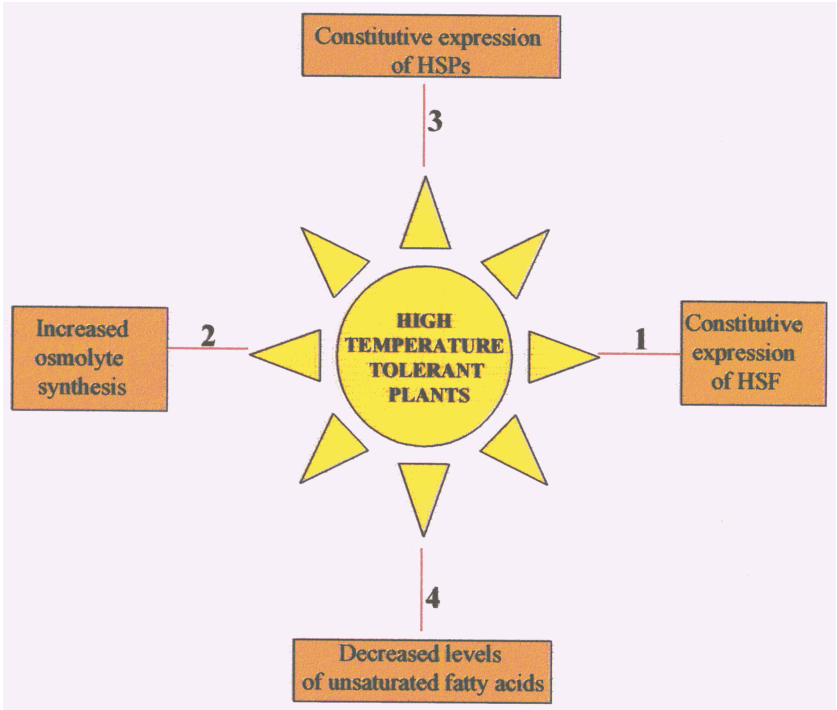

Figure 1. Strategies employed for producing high temperature-tolerant transgenic plants. Strategies 1 and 3 operate via increased synthesis of heat shock proteins. Strategy 2 leads to better water relations of the cell. Strategy 4 pertains to improved photosynthesis under stress conditions through requisite change in lipid biochemistry of the membranes.

obtained from soil bacterium *Arthrobacter globiformis* (encoding for choline oxidase enzyme) is responsible for choline to glycinebetaine conversion and high glycinebetaine level is important in providing protection against water stress, salt stress and low temperature stress[22]. In the study undertaken by Alia *et al.*[21], transgenic *Arabidopsis* plants over-expressing *codA* gene and over-producing glycinebetaine were found to exhibit high temperature tolerance as well.

As stated earlier, living cells adapt to lowering of extracellular temperature through alteration in membrane lipid composition by increased fatty acid unsaturation (i.e. incorporation of double bonds in fatty acyl chains) and chain shortening. Murata *et al.*[1] raised genetically altered chilling tolerant tobacco plants by introducing cDNA of chloroplast enzyme glycerol-3-phosphate acyltransferase (important for phosphatidylglycerol fatty acid desaturation) from squash (a summer plant) and *Arabidopsis* (a cold-loving weed) under the control of CaMV 35S promoter. Analysis of lipids of the transformed plants showed an increase in the number of *cis* unsaturated fatty acids and a corresponding decrease in chilling sensitivity (Figure 2). This work proved that increasing the unsaturation level of fatty acids in chloroplast membrane is critical for increased tolerance to low temperature. Recently, Murakami *et al.*[2] have shown that it is possible to genetically engineer high temperature tolerance in transgenic tobacco plants by reducing levels of unsaturated fatty acids (Figure 2). This work was based on the observations that the chloroplast membrane of higher plants contains an unusually high concentration of trienoic fatty acids and plants grown in colder temperatures have a higher content of trienoic fatty acids such as *a*-linolenic acid (18:3) and hexadecatrienoic acid (16:3). These workers accomplished the silencing of the gene encoding the chloroplast version of *w*3-fatty acid desaturase enzyme (Fad7, which synthesizes lipids containing three double bonds) in tobacco. Transgenic tobacco plants contained a lower level of trienoic fatty acid than wild type plants, and grew much better than control at higher temperature. Differences in growth rate were noted at 36°C, and transgenic plants survived for 2 h at 47°C, a treatment that killed their wild type counterparts. This work proves that thermotolerance is a function of the lipid profile of photosynthetic membranes.

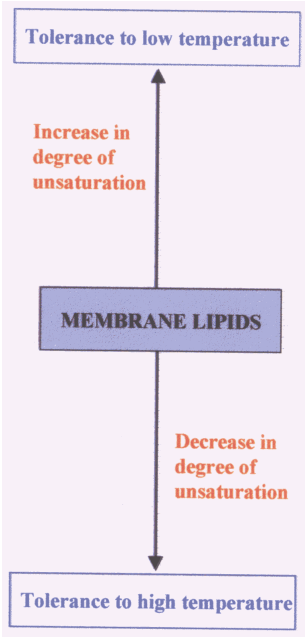

Figure 2. Schematic representation of the influence of saturation levels of membrane lipids on the temperature sensitivity of plants.

Future studies need to examine how the sensitivity of plants to lower temperature is affected with this change in unsaturated fatty acids. According to Murakami *et al.*[2], *w*3-fatty acid desaturase enzyme is expressed in nearly all species of plants and thus, may prove to be useful in high temperature tolerance in all plants. It should be useful to see if this gene works with important crops like wheat in future attempts. As availability of the relevant gene (but not the technique of introduction of the gene and its expression) is considered as the rate-limiting factor in plant biotechnology, this discovery adds a new member to the growing list of genes which have proven useful for tolerance to abiotic stresses. This work raises the hope that by pyramiding genes for membrane properties with increased osmolyte accumulation and increased HSP synthesis, it should be possible to raise plants to a higher level of high temperature tolerance in further attempts.

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