

## Towards season-free agriculture

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### Abstract

Applied molecular genetics, popularly known as biotechnology, has opened opportunities beyond imagination. It is likely to revolutionize the science of plant breeding in not too distant future. With the possibility of transferring genes across the biological world, it has become possible to create new plant genotypes carrying traits not only unique to closer or distant taxa, but even those from animal and microbial kingdoms. A thorough churning of cropping patterns should be possible by creating new varieties adaptable to unconventional environments. Two major environmental factors determine the acceptability of any crop or different varieties of a particular crop. These are temperature and photoperiod. Genotypes that are neutral (insensitive) to day length and simultaneously tolerant to high as well as low temperatures could be cultivated in any part of the year, especially in tropical and subtropical regions of the globe. Efforts will also be needed to make such varieties tolerant/resistant/immune to the various biotic (e.g. pests and diseases) and abiotic (drought, salinity etc.) factors which perpetually inflict crops, leading to huge economic losses. All the above properties are under genetic control. Genes controlling these traits, one way or the other, can be harvested from close and distant taxa and used in genetic transformation. Genes for opposite properties, e.g. simultaneous tolerance to high as well as low temperatures, can be pyramided in a single genotype, and their cultivation will not be season bound.. Consequences of such an eventuality will have tremendous impact on world agriculture, ultimately leading to solving the food problem of ballooning populations in the poorest countries.

**Key words:** Crops, unrestricted cultivation, gene mining, genetic transformation, unrelated donors of genes.

### Introduction

Agriculture, i.e. crop husbandry, is highly restricted by photoperiod and temperature. Crops are adapted to specific regions and seasons primarily because of their adaptability to short-day or long-day conditions. Day length is also associated with temperature conditions.

Parts of the year with longer days are also the period with higher temperatures (called summer), while the months with short day length are chilly (winter) and accompanied by frosts and snowfall. Through the history of agriculture, different crops were identified as adapted to the two seasons based on their primary habitat. Active crop cultivation is not possible during the snow-bound winter months in the temperate regions. Only a limited number of species can sustain life in dormant state during that period without entering into reproductive phase. All crop cultivation in the temperate regions is restricted to the summer months which also have longer days.

Crop cultivation is faced with numerous maladies. Pests and pathogens of crop species have coevolved with the plants and are always associated with the crops growing in open fields. In addition to insect-pest and disease control, nutrient and water supply are integral part of crop management. Millions of hectares are afflicted with other maladies like soil salinity which severely restricts the choice of crops and manifestation of their productivity potential. Fortunately, genes have been identified which in dominant or recessive condition impart a reasonable degree of resistance to the various biotic and abiotic ailments. Genes for resistance can be sourced from various related or unrelated donors and added to the genomes of cultivated plants. Such efforts are in progress on a massive scale all over the world. Indian efforts are also beginning to make impact in a few crops. There is no doubt that these efforts will spread to more crops and cover increasing number of economic traits.

### Drought tolerance

Of all the problems facing agriculture today, water deficiency is possibly most serious, and all predictions suggest that the whole biological world will face acute shortage of water in future. Since nearly 80% of the

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total amount of water under human consumption falls in the single area of artificial irrigation, one can imagine that agriculture will be exposed to maximum pressure of water scarcity. There are two ways to meet the contingency: through engineering works (construction of dams and canals, installation of tube-wells, diversion of surplus water from rivers over large distances etc.), and creating genotypes that can manage life with reasonable productivity under water stress. The total water requirement of such genotypes will be reduced by a considerable margin, leading to economy in water consumption and sparing water to other crops and regions. This is where genetics and biotechnology have a role to play.

Plants manage to survive under conditions of water scarcity by way of escape, avoidance, and tolerance or resistance of various degrees [1]. Drought tolerance is a multicomponent phenomenon and a series of genes are invariably involved in the total effect. Some of the major properties associated with drought tolerance, for which genes have been identified, are root development, thickness of the root tip, leaf rolling, osmotic adjustment, and metabolic products like dehydrin, osmotin, stomatal activity, nontoxic compatible solutes (fructane, trehalose, polyols, proline, glycine betaine, polyamines, abscissic acid etc.), water storage in (succulent) tissues, and economic use of water for metabolism, which is called water use efficiency. Many of these genes and gene systems are involved in joint pathways and, possibly, function in a coordinated manner under the control of regulatory systems. From a plant breeder's point of view, it is not feasible to tackle several components of drought tolerance simultaneously. A more forthright approach would be to develop a reliable screening procedure for drought response per se. The plant breeding procedure requires identification of individual plants in a segregating population. Individual segregants identified for drought tolerance may carry one or more components of drought tolerance. But the purpose is served as long as the selection process ensures that the plants identified maintain their ability to survive and reproduce under water scarcity in succeeding generations. Using this approach, even monogenic control of drought tolerance has been reported [2, 3]. The "stay green" trait ensures that the genotypes which do not lose leaves under induced drought for 15 days are able to survive on just 30% of the normal irrigation water, do not show signs of wilting, maintain high water content, and continue their photosynthetic activity throughout the dry period. They can grow on 70% less water, as claimed recently by

Rosa Rivero and Eduardo Blumward at the University of California, Davis Campus [news on line].

### Salt tolerance

Transgenics with high level of tolerance to alkalinity and salinity and varieties developed on their base have the potential of increasing productivity in the salt affected areas under crops. Large areas presently not accessible to crop plants due to high salinity can be brought under cultivation, thereby increasing total cropped area world over. There is a wide range of salt tolerance among the cultivated plants. Generally speaking, leguminous crops are more sensitive and cruciferous (safflower, brassicas), and other crops like melon, persimmon, tomato, tobacco etc. are more tolerant to soil or water salinity. Genes for different components of salinity tolerance can be cloned from them, hooked with a strong promoter, and the constructs used in genetic transformation. Many tree species, collectively called halophytes (salt loving) are expected to possess gene systems facilitating filtering out or metabolizing salts, or a combination of both. Several marine plant and animal species can be a rich source of genes for salt tolerance.

Genetically, salt tolerance can be enhanced by alternation of a single limiting factor in a complex chain of reactions through mutations in genes for specific molecules.

Salt tolerance, like many other biological properties, is a complex trait under multigenic control [4]. At least two sets of QTLs for salt tolerance are described: those acting at seedling stage [5, 6] and the others acting during vegetative phase [7, 8].

Several elements for salt tolerance have been identified. A few are listed below:

CDPK	Ca <sup>++</sup> -dependent protein kinase
CAX	Ca/H antiporter
EhCaBP	Calcium binding protein
Hal1	Yeast halotolerance gene
Hal2	Yeast halotolerance gene
Mn-SOD	Yeast mitochondrial superoxide dismutase
HKT1	High-affinity potassium transporter
HNX1	Proton sodium exchanger
RCI3	Rare cold inducible gene
RHL	Rice Hal2-like
SPD	Sorbitol-6-phosphate dehydrogenase

Many of the genetic and biochemical elements are common for salt and drought tolerance. A few genes and gene products have been identified:

Gene	Product	Enzyme
	BADH	Betaine aldehyde dehydrogenase
betB	CDH	Betaine dehydrogenase
	GST	Glutathione s-transferase
	GPX	Glutathione peroxidase
	HSP70	Heat shock protein
Hva1	LEA	Late embryogenesis abundant (LEA) protein
		Osmotin-like protein
		Proline dehydrogenase
	TRSP	Proline transporter

The LEA proteins, initially identified in plant seeds [10] were subsequently detected in bacteria, moss and nematodes. Expression of LEA genes is induced by abscisic acid (ABA) [11].

Resistance to some of the major stresses share metabolic pathways. As a result, genes simultaneously controlling tolerance to one or more abiotic stresses seem to be operating in higher plants [9]. As many as 114 genes are reported to be involved in simultaneous resistance to salinity and drought which also control the synthesis of abscisic acid. In another set, 20 genes determined resistance to cold and drought and are also involved in abscisic acid synthesis. Even if a few genes for drought tolerance in both sets are common, combining them with the other two major abiotic stresses of cold and salt tolerance should be within experimental reach. This is a very lucky situation as it shows a possibility of combining more than one resistance with much less effort than handling them in isolation and then making attempts to pyramid their genes.

### Biotic stresses

Biotic stresses affect crop growth and productivity in the form of diseases and insect-pests. Diseases are caused by a plethora of fungi, bacteria and viruses. Genotypes resistant to these parasitic organisms frequently occur in nature. Resistance is controlled by both dominant and recessive genes and manifests at

various stages in plant metabolism. Therefore, disease management is relatively more easily amenable through genetic means. Genes for resistance can be found in close or distant taxa and accumulated through conventional breeding procedures or transgenic route.

Breeding for insect resistance is far too tedious, mainly because of versatility of insect behaviour and migratory nature of the insects. The picture about insect tolerance is also faced with uncertainty because of true resistance, avoidance and escape mechanisms. Nevertheless, genotypes showing various degree of resistance to insects are found, especially if it involves physical features like hard skin, hairy or prickly surface and chemical composition (repulsive chemicals or antibiosis). Generally speaking, breeding for insect resistance is not as effective and productive as disease resistance. Biotechnology techniques can go a long way in creating new genetic architecture. Genes of plant or microbial origin can cause disruption in the digestive system of insects, leading to starvation and death. Amylase inhibitor enzymes inhibit digestion of carbohydrates and protease inhibitor genes block protein metabolism. The Bt gene from a bacterial source has been the most successful story in controlling lepidopterous (caterpillars) and coleopterous (beetles) insects. Such genes can be successfully employed in plants only if the final plant product is not be used as food for higher animals, including man, although some of the gene products (e.g. Bt proteins) may prove to be non-toxic for other biological systems even on consumption. Nevertheless, extreme care would be required when inhibitors of food digestion are to be incorporated in commercial varieties. Hopefully, situation will improve as the techniques of gene transfer are perfected.

### Crossing the seasonal barriers

The measures discussed above aim at protecting the crop in their areas of adaptation. These steps cannot alter the range of environments under which the crops are conventionally grown. Although a crop can be adapted to a wide range of whether conditions by creating new varieties, they still remain season bound. As a result, different crops can be cultivated only in different parts of a year. In the countries of tropics and subtropics, crop cultivation is possible round the year. But here again, the crops are categorized into summer and winter crops. The summer crops require relatively longer days and cannot withstand the chilling temperatures of winters in the subtropical band of the globe. In the tropical parts, e.g. south India, the same crops are cultivated almost

the year round because the temperatures in summer and winter vary in a narrow range and the difference in day length is also not so drastic as in the northern latitudes.

This pattern can be repeated and further expanded by manipulating genetically the photoperiodic response and temperature sensitivity (both low as well as high) of varieties in the two groups of crops which are now restricted to summer and winter cultivation. With appropriate combinations of genes mobilized from various sources it should be possible to evolve varieties that can be cultivated throughout the year even in the subtropics.

### Genes for day-length neutrality

The plants are, in general, categorized into three groups on the basis of their photoperiodic requirements: long day, short day and day-length neutral. The photoperiod decides the flowering time in accordance with the gene composition and the accumulated light hours during vegetative growth period. Many genes have been shown to play a primary role in regulating the circadian clock in arabisopsis. In pea, for example, specific genes are known to affect the photoperiod response [12]. Recessive mutations in some genes cause early flowering while similar mutations in other genes delay flowering in response to light conditions. They also include pseudo response regulator genes [13].

The genes controlling the overall response of genotypes to the total duration of light prior to flower initiation can be manipulated to the extent that varieties can be created which will adapt to varying photoperiod conditions and enter into reproductive phase within a reasonable period under different day-length conditions. The restrictive role of photoperiods in summer and winter will be minimized.

The genes determining response to vernalization (Vrn genes) are another example of genetic control of flowering time. These genes are responsible for converting crop species from winter type to spring type and vice versa depending on their dominant or recessive state in different combinations. Extensive work has been carried out on the Vrn genes in wheat and barley [14, 15] and promoter regions for some of them have been identified [16]. In wheat, for example, the dominant genes Vrn-1 and Vrn-2 determine spring growth habit, and their recessive alleles (vrn-1, vrn-2) make the plants winter type. Interestingly, this change to winter habit also creates a requirement for chilling temperatures in

the winter varieties. It is noteworthy that majority of crops do not possess Vrn genes. That is the reason why they all belong to the spring type. All the crops and their varieties cultivated in the tropics and subtropics are devoid of Vrn genes. It is worth exploring how the Vrn genes in dominant as well as recessive form will influence the physiology of non-Vrn crops when transferred to them through transgenesis.

### Tolerance to extreme temperatures

Plants grow best in a limited range of temperatures. The choice of season is severely restricted in the temperate zone as almost half of the year the soil is snow bound and not available for cultivation. On the other hand, temperature and photoperiod fluctuate in a much narrower range in the tropics and practically any crop can be sown any time in the year. This is particularly true for the annual crops. However, this does not apply to the tree species (for example, fruit crops) as they have specific need for chilling temperatures although cooler temperatures beyond a limit damage these crops also. The requirement in such cases, for the purpose of extending their adaptability to the tropical and subtropical regions, will be to introduce genes for acceptability of higher temperatures. This is a very challenging task.

The crucial area for increasing versatility of annual crops and increasing cropping intensity is the subtropical belt in which literally all crops are cultivated but are spaced out in time and consigned to two seasons, warm and cold. If plants can be changed into a module where extreme temperatures will not have inhibitory effect on crop growth, then such varieties can be planted in any part of the year. To achieve this, what needs to be done is enhancement of cold tolerance in the typical summer season crops and heat tolerance in the conventional winter crops. Tolerance to high temperatures is required even in summer crops to protect them from extreme heat. Xerophytes are excellent source of genes for this purpose. Genes for traits like heat and cold tolerance are available from many different sources. Their mobilization through genetic transformation needs to be achieved.

Broadly speaking, the objective is to create "temperature-neutral" plants just as photoperiod-neutral plants. An interesting point in this context is to examine whether the heat tolerance of summer crops and cold tolerance of winter crops will remain intact after genes for cold tolerance and heat tolerance, respectively, are added in their genomes.



Total crop production can be achieved by a combination of increasing productivity per unit time per unit area and higher cropping intensity, i.e. taking more crops from a single plot of land in a year. Plant breeding efforts over the history of crop improvement have resulted in manifold increase in the productivity potential of literally all food, fodder and fibre crops. Heterosis breeding is a time tested approach to enhance productivity and needs to be extended to as many crops as possible. Claims of genes for heterosis having been discovered are yet to be verified. But there are possibilities of increasing yield per unit area by suitably manipulating the genes controlling food synthesis, its transportation to sink, and increasing sink capacity. This seems to be an achievable task as several genes controlling these processes are already known and many more will be found in future.

Cropping intensity can be increased by combining properties like earliness, temperature tolerance (high as well as low), and day-length neutrality. As discussed above, this is not an insurmountable assignment only if irrigation water can be ensured. Even today, many countries of the tropics and subtropics, e.g. Egypt, have around 250% cropping intensity simply because all agriculture is practiced in irrigated areas. All other parts of the country are desert. More than two crops from the same field are occasionally harvested in south India. With concerted efforts and extensive use of biotechnology, a situation can be created that summer or winter crops are planted any time in the year in the tropical and subtropical regions as and when a field is vacated by the previous crop. Such genotypes, combined with earliness, will boost cropping intensity to great heights.

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