Genes and their promoters*

A national seminar on Gene Constructs was held recently in Bangalore. S. D. Shikhamany (Director, IIHR) in his address said that the objective of the seminar was to discuss the number of promoters available to increase the shelf life, improve quality, etc. and discuss the issue of IPRs regarding GM crops.

In his keynote address, Koundal (Director, National Research Centre on Plant Biotechnology (NRCPB), New Delhi), talked about genetic engineering and its tools that could be used to improve food crops. Precision breeding involving a target gene can lead to the elimination of junk DNA. NGOs are critical of GM crops, according to Koundal, but developing countries need GM crops to eliminate hunger. He was emphatic about countries where children suffer from malnutrition, not being left with any other option. Our scientists cannot work on borrowed genes because of IPR issues; we need our own promoters and genes. He suggested that we could probably work on target crops instead of *Arabidopsis*; experiments on a model plant system are fine for awards and theses writing. Koundal's concern was that a country like India cannot spend money on academic curiosity. He said that screening of bioresources is important; chick pea and pigeon pea transformation products are also important. The constructs should not develop resistance. There is scope for isolating genes for carotene content, iron, etc. and these crops can then be improved. Also, plant protein quality can be improved. Fungal disease resistance, e.g. to *Alternaria*, *Fusarium*, etc. could be developed in more crops like rice. Crops like cotton and sorghum can be made virus-resistant with Replicase or antisense technology. Indica rice that is eaten by the poor man, can be thus improved. Koundal also said that Indian agriculture can depend on irrigation only in part. We are dependent on rain and therefore we need to have abiotic stress resistance in plants – to waterlogging, salinity, etc. According to him, use of biotechnology to reduce metal toxicity and also in nitrogen fixation, paints a rather rosy picture of the techniques which are not practical. What is more important is that the food needs of the growing population have to be met. He stressed that it is everyone's responsibility to translate biotechnology into simple talk for the farmers. These ethical issues need to reach the people. An interdisciplinary approach where plant breeding, biotechnology, biochemistry, etc. all collaborate with each other will lead to better results.

M. Mahadevappa, a plant breeder, in his talk said that incorporation of genes has been going on since life began. Not realizing this is the problem. We need to educate people about the beneficial aspects. Educational authorities should try to incorporate this into primary and secondary education curricula. Stories or articles on GM crops will then reach the public. Scientists have to be updated on IPR issues. He said that this seminar will promote collaboration.

There were four sessions in the seminar, out of which the first session was held on the first day and the other three on the second day. Session IA was on 'Genes for biotic stress'. H. S. Savithri (Indian Institute of Science, Bangalore) spoke on viruses that infect different plants and how they can be controlled. One unconventional method is to develop transgenics when the correct virus cannot be identified. The other is by RNA-induced gene silencing. Transgenic virus-resistant plants show an increase in yield, e.g. in tomato by 40%. T. R. Sharma (NRCPB) spoke on cloning of a pathogen-inducible blast resistant gene that can be used for functional markers and transgenics. P. Ananda Kumar (NRCPB) spoke on resistance to insects and nematodes, while P. B. Kirti (University of Hyderabad) spoke on disease resistance.

Session B was on 'Genes for abiotic stress and other traits'. M. Udayakumar (UAS, Bangalore) spoke on genes and gene constructs for abiotic stress.

Drought is a major constraint in realizing the potential yield and the total loss is about fivefold higher than in biotic stress. Yield losses vary with diverse soils and also with different precipitation patterns. Drought traits with adaptive significance are associated with water mining, WUE and water conservation, traits associated with intrinsic tolerance traits like osmotic adjustment, tolerance to oxidative stress, protection to macromolecules, maintaining membrane integrity, hormones, ionic homeostasis and upstream regulatory mechanisms.

So far, nearly 2280 genes have been isolated in plants which are associated with drought. However, only 80 upregulatory genes and 112 functional genes have been validated in various plant systems like the *Arabidopsis*, *Nicotiana*, rice, wheat, etc. These genes are involved in ionic/osmotic homeostasis like proline, glycinebetaine, trehalose, fructans, pinntol, mannitol and some are involved in protection mechanisms like scavenging enzymes, lipid transfer proteins, BiP, chaperones, etc. The candidate genes for desiccation tolerance are transcription factors, functional genes and inducible promoters. Successful field trials for drought tolerance are going on HVA1 (LEA), a variety of wheat.

Based on phenotypic expression, both under laboratory and field conditions, a few candidate genes have been identified. These include functional and upstream regulatory elements like transcriptional factors that can be considered as potential candidate genes to improve drought tolerance. However, there is a need for cloning already identified and validated functional genes and transcriptional factors from our local plant species adapted to drought like horse gram, finger millet, pennisetum, etc. A number of important genes which regulate inherent traits like wax, root development and WUE are also of great importance.

Shivaprakash (MSSRF, Chennai) spoke on biofortification. In order to improve the nutrient and vitamin availability in our diet, increasing available content in foodgrains, vegetable and fruits is an important strategy and is called biofortification. Using Ferritin, an iron-binding protein, one can improve the available quantity of iron in the crop. This has been demonstrated in transgenic rice for Ferritin using endosperm-specific promoter.

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A. G. Shankar (UAS, Bangalore) spoke on improving nutrient availability. For improvement of nutrient uptake availability, there are two approaches. First, assess genetic variability for nutrient uptake and use developing markers which can be used in MAS. The other is the transgenic approach which needs better understanding of Zn and Fe homeostasis, identifying more or rather all genes involved in uptake like Fe, Zn transporters, cellular and tissue localization of these proteins, their expression and their interactions.

Usha VijayRaghavan (IISc, Bangalore), spoke on functional studies of transcriptional regulation. There are several regulatory elements that have been identified in plants for floral development like LEAFY, which controls conversion of vegetative meristem to inflorescence meristem. This has been studied in rice, where it has a role in panicle emergence and development. Studies in this direction will help in improving initiation of floral development, seed number and thereby the yield.

Session II was on novel promoters and the speakers were K. R. Koundal and K. C. Bansal. P. S. Rao, Chairman of the session, emphasized the need for novel promoters to reduce the input cost to farmers who are the end-users. Gene constructs with indigenous genes and promoters are highly desirable to reduce the input cost, e.g. transgenic seed cost.

Koundal spoke on isolation of seed-specific promoters from indigenous legumes and made the following points: (a) Pulses are a rich source of proteins, but average productivity is low. (b) Acceptance of the plant genes and promoter is high compared to genes and promoters from bacterial or animal origin. (c) Under different kinds of stress, it is important to maintain the assimilatory power of the plant to maintain productivity.

Bansal emphasized the use of tissue-specific/inducible promoters in place of the constitutive promoters. Genes cloned in the speaker's laboratory were osmotin, genes for delayed ripening and a lycopene enhancing gene. He opined that 5′-upstream regulatory elements are important in promoters, in the region between –1 and 2.0 kb. Bansal talked in detail about the tissue-specific expression by the promoters of *CAB1* gene which is mesophyll cell-specific, whereas the rbcS promoter is active in bundle sheath cells.

In case of delayed ripening, till now most of the genes have utilized the constitutive promoters resulting in non-ripening types. Gene expression at later stages of ripening is important for the delayed ripening process. Two promoters were found to be suitable candidates as the expression is noticed only at later stages of ripening, e.g. ACC synthase and Le EXP1. The 5′-upstream regulatory elements were studied in Le ACS4 and Le EXP1 promoters. These promoters may also be wound-induced and are useful in delayed ripening.

The next session was on gene constructs and transgene expression. Sunil Mukherjee (ICGEB, Delhi) spoke on post-transcriptional gene silencing and single stranded interference RNAs and the mechanisms involved in both these systems. He touched upon the possibility of resistance to viruses through these mechanisms. Non-translatable pathogen-derived viral sequences as a successful strategy in the case of TRSV and other viruses were also explained.

B. M. Prasanna (IARI, New Delhi) spoke on integrating transgenics in plant breeding. The importance of different factors influencing transgene expression such as copy number, gene construct, genetic background of the host, and position effect of the transgene were mentioned in his presentation. In addition, factors such as epistasis, somaclonal variation and the environmental effects were explained using examples of transgenic cotton expression, i.e. *Cry1Ac* expression. The importance of selection criteria of the genotypes to be used in transgenic work such as efficiency of transformation, elite agronomic background, good combining ability, and suitability to marginal environment was brought out. He highlighted the importance of position effect of the transgene with respect to heteorochromatin, telomere, centromere and strategies to overcome the position effects.

The strategy of private companies in generating a large number of primary events, although successful, is not necessarily cost-effective. The dosage effect of transgene with respect to golden rice was discussed. The importance of gametic competition, epigenetic variation and the importance of breeding consideration for developing the final product such as pure breeding lines, synthetic varieties and composites as well as transgene pyramiding were discussed.

Utpal Tatu (IISc, Bangalore) touched upon the significance of proteomics in finger-printing of plant materials, plant physiology and plant defence. The significance of heat shock-responsive proteins in plant defence was addressed by citing appropriate examples from *Drosophila* and malaria parasite systems. Plant scientists may benefit from research emerging from these animal models and evolve suitable strategies in plant systems. He invited collaborative efforts from scientists.

The last technical session was on IPR issues. The speakers Kalyan Chakravarty (IIM, Bangalore) and Khetarpal (National Bureau of Plant Germplasm Research (NBPGR) New Delhi) said that an IPR cell should be mandatory in every institute and IPR awareness should be enhanced. A patent facilitation cell should also be formulated in every institute so as to guide researchers on the modality of filing patents. It was advocated to involve the National Research Development Corporation, and Central Sericulture Research and Training Institute, Mysore, which help obtain patents. It would be helpful during commercialization of transgenic technology. All biotechnology projects should have an IPR clause. Elite genotypes responsive to plant tissue culture should be registered. Repository of all the genes/constructs should be made in the country. The repository may be maintained by NBPGR.

Recommendations made in the concluding plenary session emphasized that the focus of work in biotic stress should be on development of constructs with more than one gene for use in gene pyramiding/gene stacking. Functional validation of the gene/gene construct was strongly advocated. Focus should also be on the development of efficient, reliable and reproducible regeneration protocols for various crops. It was decided that genes/gene constructs/promoters will be isolated for various traits and the world literature would be compiled on the availability of genes, gene cassettes, promoters with respect to biotic and abiotic stress and their status in relation to Indian IPR issues.

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