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ACKNOWLEDGEMENTS. We gratefully acknowledge help from Prof. J. A. Creighton and Prof. A. E. Beezer of University of Kent, UK, in getting the SERS spectra of pyridine done in their laboratory. We wish to thank Dr T. Pal for helpful discussion. The work was supported by CSIR, India.

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Biosafety of transgenic crops: Precautions for case-by-case risk assessment

As contributors to commercial exploitation of rDNA technology in agriculture, transgenic crops were a hope a decade ago. Today, they are a reality[1]. The commercial cultivation of a respectable number and many more that are currently being field tested for biosafety and environmental clearance stands testimony to their relevance[2]. In spite of this and the very vigorous promotion of transgenics by proponents of the technology, concerns about potential negative impact of the transgenics on the health and nutrition of the consuming public and on the environment persist. The debate on adequacy of current procedures of biosafety testing and environmental risk assessment has become so intense as to create a vertical divide between the protagonists and the opponents[3,4]. The former argue that testing of the product should not be linked to the technology used for its production and be pragmatically based while the latter advocate that since genetically engineered plants are produced by a new technology, many consequences of which have not been fully tested, should not be equated with products of conventional breeding and the projected negative effects should be proved to be absent by experimentation.

Crops differ markedly in their requirement of various components of ambient environment for optimum growth and yield, modes of reproduction and agronomic husbandry. When variables like nature of transgene and its source; the characteristics of transgene product and the possibility to its modification by interaction with products of other genes and the environment; flow of the transgene into other crops, crop-related wild species and non-target organisms; the nutritional, health-related effects of the transgene product, etc. are superimposed on diversity of crops, a bewildering variety of complexities confronts us while determining biosafety and environmental risk assessment. No universally applicable guidelines are possible in such a situation for objective and acceptable risk assessment. For this reason, it has been accepted that the assessment should be made case by case[5].

According to the current procedures in countries where transgenics have graduated to commercial reality, the regulatory authorities typically require information on the following for granting permission for large open field tests and determining biosafety and environmental risk assessment for commercial release: Donor of genetic material, Recipient, Vector or vector agent used for gene transfer, Field trial plans (test site, experimental design, containment, etc.), Product of transgene (expression, toxicity, allergenicity, pleiotropic effects, product degradation, etc.), Environmental fate (gene escape, weediness, genetic diversity, effect on non-target organisms).

It is thus seen that concern is focused on the crop–transgene combination only. No account is taken of the possible contiguous cultivation of different cultivars of a crop containing the same transgene. This we believe is a serious lacuna, the relevance of which assumes even greater significance in a country like ours where farm size holdings are very small and difference among farmers for new technology absorption and practice is wide. For this reason the nature and details of case-by-case assessment in our situation will have to be different from that obtainable in the developed world. Let us illustrate this by a few examples.

First, a transgene mobilized into different crops that constitute components of a farming system. A gene for herbicide tolerance is moved into three or four crops that traditionally go into a rotation cycle. Self-sowns will inevitably contribute seed to the succeeding crop/s. Even if we do not go by the scientific definition of what is a weed, a sizable amount of rouge seeds can considerably diminish the commercial value of the crop produce. The second case where the same or closely homologous transgenes are moved into the same crop, the theoretical consequences can be even more serious. It is known that multiple copies of transgene or a combination of closely homologous transgenes can result in gene silencing[6]. It is also known that gene flow among genotypes of a crop cultivar can be sizable[7]. When different entrepreneurs and public sector institutions produce transgenics using a particular or similar gene for producing cultivars of the same crop, the fields will be mosaics of varieties containing the same gene. In such cases, following gene exchange among cultivars, the copy number of transgenes will increase in progeny generation. If such seeds are used to raise crops, gene

silencing will have serious consequences. When the gene in question controls a phenotype on which a farmer keeps a constant vigil, the adverse consequences of gene silencing can, in theory, be mitigated. An example of such a situation would be Bt-gene for insect tolerance. However, when the transgene has been employed to alter a characteristic not visible to the naked eye or for which no corrective measures are available against failure, the impact of gene silencing will go unnoticed or will have to be suffered. An example of such a situation would be a transgene, in sense or antisense orientation, that modifies chemical composition such as a changed fatty acid profile or elaboration of an allergin. In practical agriculture, gene silencing in both the situations leads to commercial consequences of significance. For situations like an insect pest epidemic, farmers cultivating a transgenic can be caught unawares because of conferred over-confidence that they are cultivating a product of high technology. The suffered losses could be considerable. The consequences of gene silencing of a quality conferring transgene can be even more serious.

The third example is of transgenics carrying viral coat-protein genes to resist pathogenic viruses. Heteroencapsidation of viruses is observed in nature when mixed viral infections occur on a host plant[8]. Similar transcapsidation of a virus with transgene-derived coat protein may help the movement of virus beyond its normal host range. Such movement is considered a dead-end event because the virus will not be able to spread to another plant. However, if the new host is a perennial or is clonally propagated, even limited movement becomes a serious concern. Furthermore, in the event that the new host is also a transgenic that produces a different coat protein, the virus will have opportunity of spread to new hosts. The consequences of extended host range of viruses, facilitated by transgenics, are difficult to quantify at the current level of knowledge.

In the light of foregoing discussion, it becomes clear that even in case-by-case monitoring of biosafety concerns and environmental safety assessment, countries with different agricultural practices will need to deliberate and decide on criteria on which to base assessment. Developing countries can profitably draw upon the experience of the developed world but would nevertheless need their own exercise to develop guidelines which combine scientific soundness with pragmatism. Only such guidelines will inspire public confidence and allow advantages of the new technology options provided by rDNA technology to be exploited. Allaying concerns through experimentation, scientific knowledge and valid arguments are necessary but fears that are either not verifiable or would take so long to verify that negates the cost-benefit equation should not hold back exploitation of products that arise from an innovative new technology.

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A new destructive disease of neem (*Azadirachta indica*) incited by *Phomopsis azadirachtae*

Neem (*Azadirachta indica* A. Juss.), commonly called 'Margosa' or 'Indian lilac', is an evergreen tree. It is one of the most versatile, multipurpose plant species of the tropics with immense potential to protect the environment while developing sustainable agriculture (Figure 1 *a*). Neem is well-known for its durable wood, insecticidal and various types of biomedical properties[1–4]. Presently India is the largest producer of neem seeds in the world[5] with Karnataka in the third place preceded by Uttar Pradesh and Tamil Nadu. This ecofriendly native tree of India is perhaps the most researched tree in the world.

Field survey conducted during the last four years has revealed that there is a destructive die-back disease on neem trees of all ages and sizes in many areas of Karnataka State, South India (Figure 1 *b*). It has caused almost always 100% loss of fruit production in severely infected individual trees. Presently, it is the major crippling disease of neem. Aseptic isolation from the diseased twigs throughout the year persistently yielded the same fungus. This fungus is a hitherto unknown species of *Phomopsis*[6]. Based on distinctive cultural and morphological characteristics as well as its limited host range, Sateesh *et al.* erected it as a new species, viz. *Phomopsis azadirachtae* Sateesh, Bhat & Devaki[7]. There has been no other report so far on the species of *Phomopsis* pathogenic to *A. indica* except a reference in the Monograph on neem. There it is referred that a die-back and twig blight are caused by unidentified species of *Phomopsis* (Khan, unpublished) in New Forest, Dehra Dun, North India[8].

P. azadirachtae was isolated from the diseased twigs once in every 15 days during 1995 and 1996 by inoculating surface-sterilized explants of *A. indica*