Fruit laxative and selection pressure

Uma Shaanker *et al.*[1] have raised an interesting possibility, namely that laxative property in fruit pulp may have evolved under selective pressure in connection with seed dispersal by birds.

The issue however may be more complex. While slow evacuation of seeds is likely to reduce their viability, rapid evacuation is not to the interest of the tree. As I have observed, in young bank mynah and jungle mynah, the evacuation rate is 18–44 min (ref. 2). This is just sufficient for transporting seeds from a fruiting fig tree like *F. bengalensis* to a significant distance, for, these birds spend a considerable time hanging around. The red eyed brown bulbul may defaecate after only 5 min[3] when it is perched on the same or another branch of the same tree. Another problem is that of destruction of seeds by birds, which keep gravels in their gizzard. Here, even quick evacuation will not help the tree, laxatives would only be a metabolic waste for the tree. Lack of laxatives and hard-coated seeds may also be selected for in an associative form. An evacuation delay of 360 min as in the case of imperial pigeon or of 530 min as in the jambu fruit dove[3], may help the tree in dispersing seeds to distant points. In case of the imperial pigeon, a large proportion of seeds are indeed crushed by the gravel in the crop but the small surviving[3] fraction is dispersed far away. It might be worthwhile to observe the evacuation patterns in various important bird dispersers like the hornbill, green pigeon and barbet, and compare the effect of laxative and non-laxative fruit pulp in their system. This can be done only in captivity and the present wildlife rules do not permit such experiments.

1. Uma Shaanker, R., Ravishankar, K. U. and Ganeshaiah, K. N., *Curr. Sci.*, 1997, **73**, 646–647.
2. Midya, S. and Brahmachary, R. L., *J. Trop. Ecol.*, 1991, 7, 537.
3. Lambert, F., *J. Trop. Ecol.*, 1989, **5**, 410; Lambert, F., *Ibid*, 1989, **131**, 512.

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NEWS

Sanctions hit the periodic table

We reproduce below, without comment, the following fax message received from the *Journal of Chemical Education* by IGCAR on July 17.

'We have received your purchase order ... dated May 31, 1998 for SP-17 Periodic Table Live CD for $ 120 USD.

Unfortunately, we have been informed by our [United States] State Department that at the present time we may not ship this material to India.

We will hold this on file and when the embargo is lifted we will process and ship your CD rom by the fastest way.'

RESEARCH NEWS

The terminator saga

K. P. Gopinathan

The 'terminator' concept and its impending consequences in agriculture have been hitting our daily newspaper headlines. The 'terminator' gene if expressed within a seed, will mark the death of the seed and the target can be cotton, millets, rice, wheat or corn. The expression of the 'terminator' itself can be modulated at will. The apprehension has been whether the engineered 'terminator' gene will eradicate particular species of crop plants by preventing the seeds to germinate. Worse still, what if the trait spreads to nontarget crops? These questions evidently are of great concern to our farmers who have been following the age-old practice of saving a portion of their harvest for sowing the next crop and these seeds do not germinate whereas the seed he procures from the industry behaves normally! I have tried to explain here the scientific principles of the terminator concept and how it can be technically achieved without taking sides for or against.

According to Hindu mythology Lord Vishnu, the Saviour, has taken ten incarnations (Dasavathar) and come to the earth periodically to save it from a perilous situation. In fact, the tenth avatar Kalki is still awaited at the end of 'Kali Yuga', the present epoch that we are living in. Kalki is the terminator and when this world is full of 'evils' (or corruptions!) Kalki is to appear riding horse back, with a sharp sword in his stretched hand and bringing the big deluge (Maha Pralayam) to submerge the whole earth to ensure that no one survives. The recent newspaper reports in the country that 'Kalki' has already appeared and people have spotted Him at various places notwithstanding, the 'terminator' function, I presume, still remains incomplete. The Kalki cult is growing strong at many places and He is ardently worshipped. But does it take much effort in our country to start a fad? Terminator or Creator, the people are known to worship.

In Hollywood and perhaps in most of the rest of the world, the conceptual Terminator is different. It is more like the six and a half feet tall, muscular, well-built Arnold Schwartzeneger clone possibly wielding a 12 gauge shotgun going around on a killing spree. Or did he terminate the terminator itself. The ending of the first incarnation marked the terminator sinking into marshes with a signal (or a threat!) that he is going to reappear and he did!

Today we hear about a different terminator in science in the context of agricultural biotechnology. This is about a 'terminator' gene which when expressed results in the death of the individual, in this case, a particular plant. 'Terminator' is an engineered gene or rather an engineered control of the expression of a gene that has been in existence.

The recent advances in genetic manipulations through recombinant DNA technology have made such operational controls feasible. So, the deal is when one permits or modulates the conditions appropriate for the expression of this gene 'terminator' within a seed, it ensures destruction of that particular seed and prevents it from germination. The original idea has been generated to 'eliminate' the inferior species of seeds, so that only the 'high quality' ones are propagated for agriculture purposes. The target plant/seed can be cotton, millet, rice, wheat or corn and the technology is applicable to any of these or more. The name 'terminator' itself for this killer gene was coined by RAFI (Rural Advancement Foundation International), a Canada-based organization which looks into the overall welfare and development, in areas like agricultural biodiversity and intellectual property. Evidently a matter of great concern because it straightaway interferes in the farmer's traditional right to save and utilize a part of his crop yield to sow the field for the next season. These seeds (harbouring the manipulatable 'terminator') fail to germinate but the seed that the farmer purchases germinates! In turn, the farmer has to depend on the commercial supplier for the next batch of seeds to be sown. Is this situation desirable? More than a billion farmers, especially in poorer and developing countries depend on farm-saved seeds. According to RAFI, this is a technology which comes in the way of developing locally adapted strains and can prove to be a threat to food security and agricultural biodiversity. Further, once such genetically manipulated seeds are sown in the fields, could this trait be spread to other species of plants through cross pollination or other means? This has been the centre of the controversy and the news item has hit many a headline in several dailies. Such apprehensions become considerably enlarged when the developers of the technology happen to be private industries and more so if they are multinationals. But are these seeds already in circulation or are they only being developed, is not completely clear.

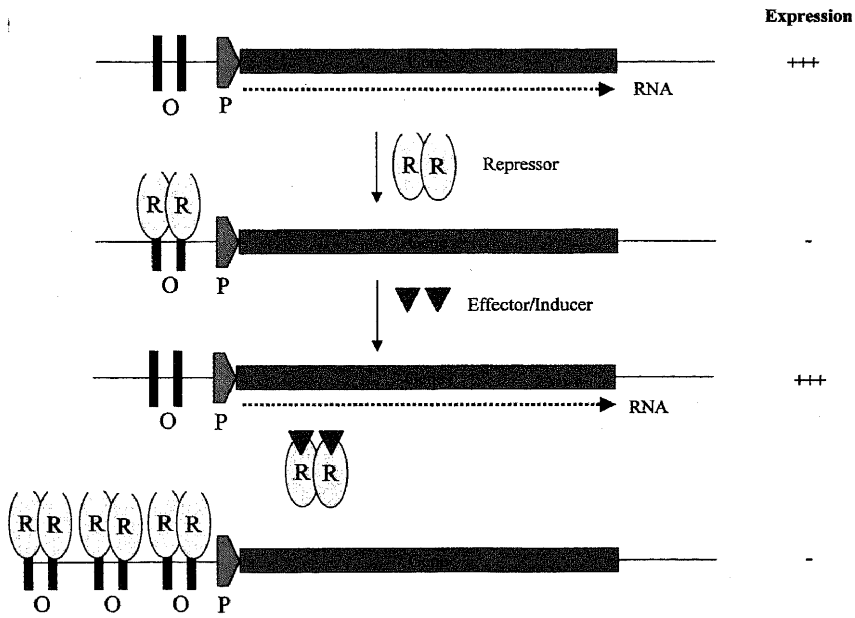

Figure 1. Modulation/control of gene expression – (Repression model). In the manipulation of terminator gene expression the principle of specific DNA–protein interactions of the classical Jacob–Monod model of gene regulation is used. P, the promoter site from which the decoding of the genetic information commences by binding of the transcription machinery. O, control site where a regulator molecule (either an activator or a repressor) binds. If activator is bound at O, the expression of gene is felicitated. If a repressor is bound at O, it creates a steric block for the transcription machinery and represses the gene expression. R, Repressor (aporepressor) is a protein molecule which binds with high affinity at O, the control site. When repressor is bound to O, the gene is not expressed. When the effector is bound to the repressor, the latter loses the capacity to bind to the O region and falls off. This opens up the promoter and the gene expression is turned on. By tandemly repeating the 'O' sites, the repression status gets strengthened. On addition of 'effector', the repression is released with the same ease and the expression of gene takes place. The 'O' locus used here comes from an inducible gene coding for tetracyclin resistance on a transposon. When the tetracyclin repressor is present, the 'terminator' gene driven under its control will be repressed. On addition of tetracyclin, the repressor binds to the antibiotic and falls off from the control site, O. As a consequence, the terminator gene gets expressed. By providing or withdrawing tetracyclin, one can modulate the expression of the terminator gene in this instance. Alternately, one can use other control elements such as 'stress response' (heat shock or metal ions) elements or other types of environmental stimulus to regulate the expression.

At least in our country, I understand that the Government has legally banned the import of 'terminator' seeds. But how effective or how complete this ban is, I do not know.

The science behind the *terminator*

Variously quoted as the 'Neutron bomb for seeds' or 'Trojan seeds', the immediate questions are what the 'terminator' is and how does it function or how does one modulate its functions by controlling the expression patterns. One makes use of the classical information of the basic principles of gene regulation by operator–repressor interactions (the well-known paradigm of control of gene expression proposed by the Nobel Laureates Jacob and Monod in the early 1960s), the current developments in transgenesis technology which permits the introduction of foreign genes into living organisms unrelated to the donor, as well as the application of our advanced molecular knowledge in differential gene expression and transposition of DNA sequences through the recombination process. The scientific principles behind the technology are depicted in Figures 1 and 2.

In fact, the 'terminator' can be any gene encoding for a protein or an enzyme, or any other toxic molecule which when expressed should result in the death of the organism. For instance, the uncontrolled presence of a suicidal enzyme like a protease which can degrade a vital protein essential for the survival, or a nuclease which can chew up the nucleic acids (DNA or RNA) may result in the death of the cell. The 'terminator' can be a mutant version of a constituent protein which when expressed may come in the way of the function of the normal counterpart, and prove to be detrimental to the life of the cell. Even overexpression of a normally 'harmless protein' can occasionally result in cell death. The results will be similar if the gene product is a toxin.

Genes encoding such products are indeed present in almost all organisms, animals or plants, as a part of their regular genetic make up. However, in normal life processes, the expression of these genes is well controlled and the cell ensures their 'on' or 'off' state of expression meticulously. The living organisms regulate the expression of the genes depending on its stage of development and what functions are required at any given point of time. Thus, in plants the functions involved in embryogenesis are turned on at its times of need which will be distinctly different from the needs for a process like seed germination. Similarly, the expression of a gene can be restricted to a particular tissue or organ while it is rendered silent at other parts. For instance, the genes specially involved in the flower development in a plant do not get expressed in the unconcerned regions. The recent advances in molecular biology have thrown a lot of light into our current understanding of the molecular basis for such differential expression patterns. The extrapolation of this basic scientific information has been instrumental in achieving the 'terminator technology', permitting one to modulate the expression of the killer gene functions, at will. The question therefore narrows down to, how does one control the expression of genes at will? Once again the principle is simple and exploits a whole bunch of basic scientific knowledge (see Figure 2).

The expression of the terminator gene results in the death of the organism but the expression can be modulated by bringing it under the control of a repressible element. One additional way to ensure that the gene is not expressed is to interrupt the encoded information with some stuffer DNA sequences. The nature of the stuffer is such that even if the manipulated gene escapes the cellular silencing mechanisms and gets decoded at low frequencies, the ensuing message would harbour 'nonsense' codons to ensure translational block.

The overall procedure can be summarized as follows: A transgenic plant is created which harbours three sets of genes. One is the 'terminator' (or the modified terminator harbouring the stuffer

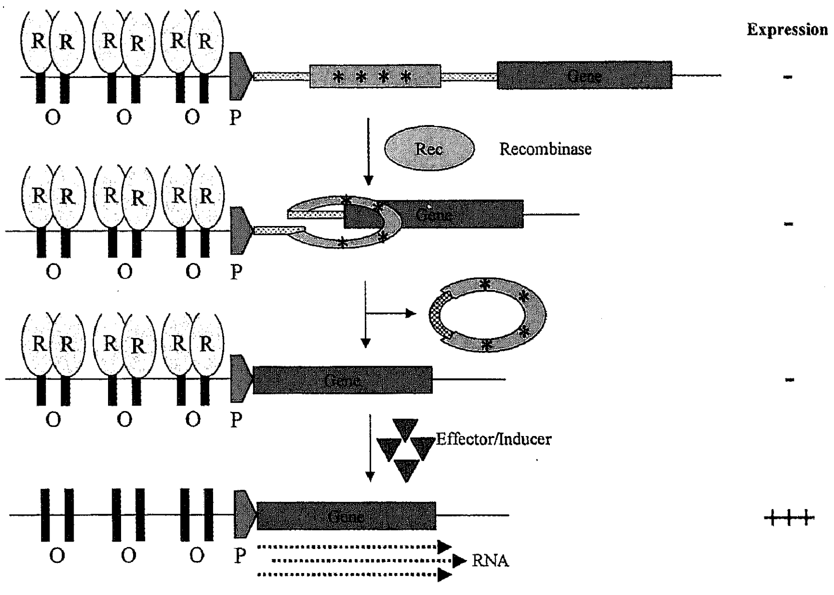

Figure 2. Modulating the expression of 'terminator' gene. A transgenic plant containing three 'transgenes' is created. One transgene is the 'terminator' itself under the control of a tissue-specific or development stage-specific promoter. The terminator coding sequences under the control of the repressor is also interrupted by a stuffer DNA with flanking regions recognized by specific recombinase. A transgene encoding a specific repressor protein under the control of a constitutively expressed cellular promoter is present as the second transgene leading to the presence of repressor at all times. A third transgene encoding the recombinase (capable of recognizing the flanking regions of the stuffer DNA inserted into the 'terminator'), is also present. The binding of the repressor to the control elements of terminator and the presence of stuffer DNA interrupting the coding region ensure that the gene is not expressed. However, once the effector (inducer) is added, it combines with the repressor and relieves the repression. The 'terminator' itself being controlled by a developmentally regulated promoter gets expressed only at a particular stage of development. The recombinase when present excises out the stuffer sequences from the 'terminator' which in turn results in the proper expression of the 'terminator'. On expression of the terminator the seed gets killed (for more details, see text).

sequence) under a defined control element (developmentally or tissue specifically regulated), and the second gene encoding for the repressor which interacts with the former control element. The repressor itself is driven under the control of any normal cellular gene so that it is constitutively expressed and consequently the terminator expression is completely repressed only when the effector is added the expression of the gene will be induced. The third transgene is a recombinase, which will specifically recognize the marked sequences (located at both ends of the stuffer DNA sequences) and promote recombination between them. Several sequence-specific recombinases are known (e.g. the *cre-lox* system from bacteriophage P1 or the *flp-frt* system from the yeast). In the *cre-lox* system, the phage encoded recombinase CRE recognizes the defined DNA sequence LOX and, as a result, the DNA fragment flanked by the LOX sequences get excised out as a circle, leaving behind the rest of the parental gene sequences intact. In fact this technology has been used in both animal and plant systems to specifically turn on a gene in a particular tissue or at a particular stage of development by modulating the expression of the recombinase, tissue specifically or development stage specifically by choosing the right sort of promoter. For instance, if the recombinase is engineered under the control of an 'embryogenesis' related gene (say, expressed only during late embryogenesis), the expression of recombinase will be confined to that period of time. Likewise, if the recombinase is under the control of a seed germination-specific gene, its expression will be confined to the germination process but not at the seed formation. The FLP–FRT system also works on similar principle as the CRE–LOX.

Thus, in a transgenic plant carrying all three transgenes, the expression of recombinase if confined to the seed germination period, the presence of an active recombinase at that time will lead to removal of the stuffer from the terminator gene and will result in its expression, when the effector is added. This in turn will end up in the failure of such seeds from germination. On the other hand, if the recombinase expression takes place during embryogenesis, the seed formation itself will be affected but once the seeds are formed it will have no effect on seed germination.

Although the above principles have been known for a while, how one puts them to use or misuse is the reason behind the controversy. The 'terminator' technology is feasible but whether one shall use it and with what consequences are the questions to be addressed. RAFI and other nongovernmental organizations have called for a global ban on terminator technology and the FAO Commission on Genetic Resources on Food and Agriculture was expected to condemn the technology. The long-term repercussions need to be carefully analysed before implementation of the technology.

ACKNOWLEDGEMENT. I thank Mr Gopal Raj the scientific correspondent of *The Hindu* for providing information downloaded from the Web sites. Information is available on the internet at www.rafi.ca and the issues containing details are available at US $ 10 a piece from RAFI. I also thank S. Sriram for help in the preparation of figures.

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More about the 'feathered' theropod dinosaurs from China

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No other extinct animal has sparked so many controversies about its life and extinction from earth as the dinosaur. Right from their evolution – whether or not descended from the reptiles, whether cold or warm blooded, and finally whether their extinction was gradual, induced by earthly causes, or sudden, triggered by extra-terrestrial agencies – in fact, every one of these has defied solution and still remains as enigmatic as ever before. It is no surprise, therefore, that their descendants, the birds, inherited, apart from their several skeletal and anatomical likenesses, some of the controversies too, particularly about the paternity with the dinosaurs. Now the detailed studies[1–4] on the 'feathered' dinosaurs, *Sinusauropteryx prima, Protoarchaeopteryx robusta* and *Caudipteryx zoui* discovered in Liaoning province of China since 1996, have brought out proofs to strengthen the dinosaur-bird descent and thereby adding fuel for the ongoing controversy among the avian palaeontologists about birds' ancestry.

Liaoning province in northeastern China (Figure 1) has been home to several recent discoveries of well-preserved fossils of insects, fishes, reptiles, mammals and particularly many bird-fossils. *Confuciusornis sanctus*, a pigeon-sized bird fossil was discovered here in 1994, in the Jurassic formations. This fossil, displaying a few bird-like features, till then believed to have evolved only during Cretaceous, startled many, as it dethroned *Archaeopteryx* as the earliest bird-fossil[5,6]. Soon, discoveries of other fossils of birds – such as *Liaoningornis* and *Chaoyangia* followed from the same site. These had toothed jaw, like a reptile, and resembled *Archaeopteryx, Hesperornis* and *Ichthyornis*, discovered in Europe and USA[7]. Very recently, a few more feathered specimens, *Protoarchaeopteryx robusta* and *Caudipteryx zoui*, apparently flightless birds, intermediate between *Sinusauropteryx* and *Archaeopteryx*, with clearly preserved feathers, have also been reported from here[2,3,8].

The dinosaur–bird link has been based essentially on several of the gradual modifications in the skeletal framework to enable flight and they were evolved over a period of time among the avian members (maniropteran theropods) of the *Dinosauria* that had divided into avian and non-avian lineages. The adaptations were essentially in the pelvis, hand (wrist and fingers), clavicle, and the tail; and the bones, overall, became hollow to lighten body-weight. Typical modifications were the enlarged claw on the second digit of the foot, development of disc-like bone in the wrist to enable flapping of wings, fusion of the clavicle to form the wish bone which has a vital