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Paradigm shifts in weed science and challenges they pose to India and weed scientists

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ABSTRACT

Inventions are the agents of change and have been the driving force behind the paradigm shifts that occurred throughout human history. There have been several paradigm shifts in the field of weed science too. The first one was the birth of modern weed science seven decades ago in 1944 when 2,4-D became commercially available. Since then, hundreds of newer organic herbicides have been developed and these became the mainstay of weed management in cropping and non-cropping systems around the world. The second paradigm shift was the evolution of herbicide resistance in 1968 when *Senicio vulgaris* was found resistant to 2,4-D. This was followed with hundreds of reports till today. The third paradigm shift in weed science occurred in 1994 when transgenic herbicide resistance crops, beginning with the approval of bromoxynil-resistant BXN cotton developed by Calgene and Rhône-Poulenc and glyphosate resistant soyabean developed by Monsanto, both in USA. These paradigm shifts has led challenges to India and Indian weed scientists too. This paper presents the various research challenges required to be worked, both directly and indirectly by the Indian scientists.

Key words: Challenges, GM crops, Paradigm shifts, Weed science

Inventions, the agents of change, have been the driving force behind the paradigm shifts that occurred throughout human history. Some of these inventions have a long-term effect, both direct and indirect, on the mankind. For example, the German Johannes Gutenberg's invention of printing press in 1445 changed the culture of people. Suddenly, numerous ancient scriptures, written hitherto on palm leaves, skin, bark, cloth, copper plates and the like, sculptured on rocks, engraved on stones, *etc*. became readily available. It also led to printing of voyage routes which aided many European sea explorers and adventurers in their quest for riches that changed the geography and history of the world forever. Similarly, the modern-age inventions of telephone, television, personal computer and the internet have impacted communication besides both personal and global business environments forever.

Paradigm shifts

There have been several paradigm shifts in the field of weed science too. The first one was the birth of modern weed science seven decades ago in 1944 when 2,4-D became commercially available. Its discovery was considered to be "amongst the greatest scientific discoveries of the 20th century" (Fryer 1980). Since then, hundreds of newer organic herbicides have been developed and these became the mainstay of weed management in cropping and non-cropping systems around the world. Other major landmark herbicide discoveries included triazines in 1958, paraquat in 1962, and glyphosate in 1974. These and scores of other herbicides have since saved billions of tons of global crop produce and thus enhanced agricultural productivity by that much.

The second shift was the evolution of herbicide resistance. This has its origin within a decade of continuous application of 2,4-D itself, but not discernable until 1968 when *Senicio vulgaris* was found resistant to it in a Washington nursery in USA where this auxin herbicide was being used since 1958 (Ryan 1970). This phenomenon of evolution of herbicide resistance that began as a trickle became a torrent beginning the mid-1980s consequent to commercialization of acetolactate synthase (ALS) and acetyl-CoA carboxylase (ACCase) herbicides. Currently, 237 species (138 dicots and 99 monocots) have evolved resistance to 22 of the 25 known herbicide sites of action and to 155 different herbicides. These species have spread across 82 crops in 65 countries (Heap 2014). Ninety of these weed species have multiple sites of action ranging from 2 to 11. *Lolium rigidum* has 11 sites of action, while two species *Echinochloa crusgali* var. *crusgalli* and *Poa annua* have nine (Heap 2014).

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Among weed families, Poaceae contributed the most number of resistance species of 75 followed by Asteraceae (37), Brassicaceae (21), Amaranthaceae (12), Cyperaceae (11), Scorphulaceae (9), and Chenopodiaceae (8), Alismataceae (6), Polygonaceae (6), and Caryophyllaceae (5) (Heap 2014). Of the various crops in which herbicide resistant weed species were found so far, wheat tops with 65 followed by maize (58), rice (50), and soya bean (46), rapeseed/ canola (20), and cotton (17) (Heap 2014).

The third paradigm shift in weed science has occurred in 1994 when transgenic herbicide resistance crops, beginning with the approval of bromoxynil-resistant BXN cotton (developed by Calgene and Rhône-Poulenc) and glyphosate resistant soyabean (Monsanto), both in USA. The same year also saw the Davis, California-based Calgene produced world's first transgenically engineered whole food tomato line '*Flavr Savr*', and the European Union approval of tobacco developed to be resistant to bromoxynil.

Transgenic plants, also known as "genetically modified" and "genetically engineered" plants, are plants which have a single or multiple genes transferred from a different species (Rao 2014). The inserted gene(s) may be derived from a completely unrelated kind of organism like bacteria or another plant of the same or a different species. The process of transgenic engineering involves several biotechnology techniques, collectively known as recombinant DNA (rDNA) technology. Its aim is to design plants which have specific characteristics by using artificial insertion technique of genes from other non-plant or plant species. The heritable transgene gene(s), prepared outside, is introduced either directly into the host or indirectly into the host cell or into the cell that is then fused or hybridized with the host as described under (Rao 2014).

a) Locating and identifying gene(s) of interest.

b) Isolating and extracting DNA.

c) Cloning the extracted DNA for mass production.

d) Designing and constructing the gene of interest for plant infiltration by adding a promoter and a selectable marker gene for expression of transgene in the plant.

e) Transformation, *i.e.*, genetic alteration of a cell resulting from the uptake, incorporation, and expression of exogenous material (DNA) from its surroundings and taken up through the cell membrane(s).

f) Testing to ascertain that the inserted gene has been stably incorporated by evaluating first in greenhouse or screen-house, followed by field testing.

g) Repeatedly back-crossing the transgenic crop plants that passed all tests with improved, elite varieties of the crop with elite breeding lines to obtain a high yielding transgenic line; and food and environmental safety assessment of the new transgenic crop variety for possible alteration of nutrient levels, allergenicity, known toxicants, new substances, antibiotic resistance markers, non-pathogenicity to animals and humans, toxicity to non-target organisms, etc.

The transgenic plant thus made now contains genetic material derived from another species. The primary benefit derived from transgenesis is an increase in the amount of genetic variability available for breeders to use beyond that is accessible by conventional breeding methods (Rao 2014).

Generally, there are two approaches in engineering for herbicide resistance (Rao 2014). One is to have the inserted (trans) gene modify a plant enzyme or other sensitive biochemical target of herbicide action to render it insensitive to the herbicide or induce overproduction of the unmodified target protein permitting normal metabolism to occur. The other approach is the introduction of an enzyme or enzyme system that degrades or detoxifies the compound in the plant before the herbicide reaches the site of action. Plants modified by both approaches may be obtained either by selection for resistance against a specific herbicide or by applying gene transfer techniques utilizing genes encoding herbicide resistance determinants.

Scores of transgenically modified herbicide-resistant crops have been developed during the last two decades. Genes used to transfer resistance traits belong to glyphosate, glufosinate, bromoxynil, sulfonyl-ureas, 2,4-D, dalapon, dicamba, atrazine, phenmedipham, paraquat, isoxafutole, mesotrione, *etc.*

Transfer of foreign genes from a wide spectrum of species, including viruses, bacteria, fungi, and animals into transgenic crops, has elicited perceived risks associated with this technology and opposition to their commercial deployment. Besides, concerns of consumers about the health safety and ethical justification of transgenic crops have led plant molecular biologists to employ alternative transformation methods. One of them is based on transfer of native genes (including regulatory elements) from plant sources, commonly known as 'intragenic' transformation. This method transforms crops with plant-derived transfer (P-) DNAs that consist of only native genetic elements, without affecting the overall structure of the plant's genome (Rao 2014). Intragenic method has been used successfully to develop crops resistant to imidazolinone and cyclohexanedione (sethoxydim) herbicides.

The next paradigm shift, the fourth, occurred in 2000. It involved development of ‘gene stacks’, ‘pyramided stacks’, ‘biotech stacks’, or simply ‘stacks’. They express two or more genes that code for proteins having different modes of action and different related traits in a single plant. The ‘stacked’ plant expresses resistance to more than one herbicide as in the case of dual-herbicide and triple-herbicide stacks. Stacking up genes resistant to herbicides with different modes (sites) of action broadens weed control efficiency and provides farmers more flexibility and options in weed management. For example, combining glyphosate-resistance gene *epsps* with the *pat* gene that confers resistance to glufosinate and/or with *dmo* gene conferring resistance to dicamba broadens the range of weeds to be controlled.

Biotech stacks are also engineered to have better chances of overcoming other myriad of problems in the field such as other biotic stresses (insects, diseases, viruses, fungi, nematodes, and parasites) and abiotic stresses (high/low temperature, cold, ozone, salinity, flooding, intense light, and nutrient imbalance, *etc*.) by stacking the concerned genes with those that confer resistance to herbicides so that farmers can increase crop productivity.

Insect pests became the second most important target for transgenic technology. Engineering of plants to express insect-resistance gene (*Bt* gene derived from the soil-dwelling bacterium *Bacillus thuringiensis*) offers the potential to overcome the shortcomings with continued heavy use of insecticides. These herbicide-cum-insect stacks confer resistance to herbicides and insect-pests so that farmers can increase crop productivity. Stacked-trait transgenics have now become an important feature of biotech crops.

The easiest and quickest way to stack up genes into a plant is to make crosses between parental plants that have different biotech traits, an approach known as hybrid stacking. Most of the commercially available biotech stacks are products of serial hybrid stacking which is widely adapted and accepted (ISAAA 2013a). Another method of gene stacking, known as molecular stacking, involves the introduction of gene constructs simultaneously or sequentially into the single locus of the target plant by standard delivery systems such *Agrobacterium*-mediated and biolistic methods (Halpin 2005, Que *et al.* 2010). In some stacks, molecular stacking has been done with conventional breeding approaches to put together the desirable traits (ISAAA 2013a).

The length of time in developing a transgenic plant depends upon the gene, crop species, available resources, and regulatory approval. It may take 6-15 years before a transgenic line is ready for commercial release (ISAAA 2012).

Ever since their first commercialization on 1.73 million ha in 1996 in the U.S., beginning with the glyphosate-resistant maize, farmers around the world have readily adopted transgenic crops such as soya bean, maize, cotton, rapeseed (canola), lucerne (alfalfa), and sugar beet. By 2013, the area under biotech crops reached 175.2 million ha, which is more than 100-fold growth (ISAAA 2013b). The nine major countries adopting transgenics include USA (70.1 million ha), Brazil (40.3), Argentina (24.4), Canada (11.6), India (11.0), China (4.2), Paraguay (3.6), South Africa (2.9), and Pakistan (2.8) in that order which together account for 97 percent of biotech crops (Table 8.1) (ISAAA 2013b). These nations excluding USA covered 100.8 million ha. This accounts for over 57 percent of the global biotech crop area. Over 81 percent of the global area under soya bean and cotton was accounted by transgenic varieties (GM Science Update 2014). These were followed by 35% for maize and 30% for rapeseed.

Currently, two transgenic traits dominate the global biotech crops: herbicide resistance accounting for 65%, insect resistance 15%, and a combination of the two (stacked) for 15%. This makes the herbicide-resistant transgenic crops for 80% of gross global biotech acreage.

The area under transgenic cultivation, which is doubling every five years, now accounts for some 12% of global arable land (GM Science Update 2014). At this rate, the gross area under these crops is poised to reach 400 million ha by 2030 when over 120 crops are expected to be transgenically engineered with desired traits and adopted worldwide (Rao 2014). This makes biotech crops the fastest adopted crop technology in recent history.

In the light of this possibility, the fifth paradigm shift is likely to appear in the next few years when herbicide resistance genes encoding enzymes will be stacked with those that help crop plants withstand other biotic stresses (other than insects) as well as abiotic stresses listed earlier. The more immediate will likely be the crops that carry herbicide resistance-cum-high yielding genes.

Future transgenic technology (second and third generations) is expected to be diversified to enhance both quantitative and qualitative traits of crops. Some of these include a) photosynthetic efficiency, b) nutrient (nitrogen, phosphorus, *etc*.) use efficiency, c) tolerance to salinity, drought, frost, flooding, *etc*., d) plant characteristics (panicle size, seed quantity per panicle, *etc.*), e) nutritional quality (â-carotene, iron, protein, *etc.*), f) antioxidants like flavonols and flavonoids in fruits, *etc*. Currently, active global research is in progress to engineer to insert these traits in plants.

Proponents of biotech crops consider that genetic engineering a panacea to attain the food and fiber needs of the burgeoning global population that is expected to reach 9.2 billion by 2050 from the current figure of 7.3 billion against the prospect of shrinking productive and cultivable land, depleting water/irrigation availability, escalating salinity in irrigated regions, shrinking labor force, enhancing demand for food and feed, increasing undernourishment, growing CO_2 emissions leading to global warming, *etc*.

Challenges

As mentioned earlier, transgenic crops have made a positive contribution to global crop production and food and fiber security and improved the economic status of farmers who adopted them. They provided farmers pecuniary or direct benefits in the form of net gain in farm income or profitability based on crop yields, market value of crop produce, production costs, costs of fuel and labor, *etc*. The most obvious pecuniary benefit is yield increase which is tangible and quantifiable. At the same time, biotech crops also accelerated changes in farming styles, affecting genetic diversity in agro-ecosystems of many countries that have adopted the biotechnology. For example, the adoption of herbicide-resistant transgenic crops has changed traditional weed management practices and the biodiversity of crop and weed species.

However, the commercial cultivation of transgenic crops with various desired, beneficial traits has aroused concerns about their biosafety, risks, and issues followed by debates worldwide by proponents and antagonists. These factors became crucial in the further development of transgenic biotechnology and their utility and wider application of transgenic products in global agriculture.

The risks are broadly grouped into agro-ecological concerns and food safety concerns. Agro-ecological concerns include a) outflow of transgenes from transgenic crops to landraces, wild/weedy relatives, non-transgenic crops, and unrelated organisms, thus leading to unintended consequences; b) evolution of transgenic crop-volunteer weeds; c) effect on soil ecosystem which accounts for 80% of soil-borne communities dominated by microbes (one of the least understood areas in the risk assessment of transgenic crops); d) soil microbe dynamics; and e) uptake and availability of soil nutrients (Rao 2014).

Food safety concerns include a) alteration of nutrient levels of foods and feeds derived from transgenic crops; b) allergenicity as a result of consumption of foods containing proteins and glycoproteins derived from biotech crops; c) horizontal transfer of transgenes from plants used directly as food or indirectly as feed to animals that are used as feed; and d) resistance to antibiotic gene used during transformation process leading to humans' loss of ability to treat illnesses with antibiotic drugs (Rao 2014).

There are several vital issues related to transgenic crops. These include the following (Rao 2014):

a) Production of terminator seeds derived from genetic use restriction technology (GURT) and trait-specific gene use restriction technology (T-GURT).

b) Intellectual property rights (IPR) of inventors granting exclusive ownership rights to their inventions and discoveries in a technical field.

c) Asynchronous approval of transgenic crops due largely to disparate regulatory procedures and standards in the countries that adopted biotech crops.

d) Biopiracy which, in fact, is the misappropriation and commercialization of genetic resources and traditional knowledge of rural and indigenous people of another country and making profit illegally from freely available natural biological materials that belong to it.

e) Coexistence of transgenic crops in the vicinity where non-transgenic, conventional, and organic crops leading to a socio-economic issue, but not necessarily a safety issue unless the foods derived from transgenic crops pose health risks.

f) Coexistence of transgenic and non-transgenic food products in food markets without proper segregation and traceability standards, thus curtailing the consumers' freedom of choice in buying the food they want.

Currently, development of transgenic crops is dominated by agro-biotech industry which considers it more as a profit-driven rather than need-driven process. Therefore, the thrust of the genetic engineering industry is not really to solve agricultural problems, but to create profitability (Altieri 1998). This is evident by the fact that over the last 30 years scores of multinational corporations have initiated transgenic research on a variety of crops around the world. Although several universities and public research institutions are also simultaneously involved in this field, their research agenda is being increasingly influenced by private sector in ways never seen in the past. The challenge these organizations now face is how to ensure that ecologically sound aspects of biotechnology are researched and developed while at the same time carefully monitoring and controlling the provision of applied non-proprietary knowledge to the private sector, farmers, and consumers and making such knowledge available in the public domain for the benefit of society (Altieri 1998).

Challenges to India

Regulation of transgenic crops is one of the most contentious roiling India whose existing regulatory rules have been heavily criticized for incompetence and non-transparency in the decision making process. The current regulatory procedures apparently tilt more in favour of agro-biotech industry rather than farmers and consumers. Besides, there is inadequate scope for, and consideration to, public debate. Many a time, decisions are taken arbitrarily regardless of farmer and consumer interests.

One clear-cut example is the way *Bt* brinjal, developed by Mahyco-Monsanto Biotech, was approved by the Genetic Engineering Approval Committee (GEAC) in October 2009 for commercial cultivation despite the serious concerns expressed by some scientists, farmers, and anti-GM products. Added to this was Monsanto's attempt in collaboration with Maharashtra Hybrid Seed Company to resort to 'biopiracy' of using native brinjal (*Solanum melon-gena*) varieties for the purpose of genetic modification in violation of the country's Biological Diversity Act, 2002 (Mercola 2012). It required the minister of environment to declare indefinite moratorium on the cultivation of *Bt* brinjal that contained the *Cry 1Ac* gene and interference of Supreme Court to decide on the issue of biopiracy.

In response to a public interest petition filed in 2005 for banning GM crops in India because of approval of field trials by GEAC without proper scientific evaluation of biosafety issues, the Supreme Court appointed on 10 May 2012 a five-member Technical Expert Committee (TEC). In its report submitted to the court on 7 October 2012, TEC recommended a 10-year moratorium on commercial release of all GM crops till all the systems are in place for independent research and regulation. It also recommended a moratorium on field trials of herbicide-resistant crops until an independent assessment has evaluated its impact and suitability.

In the long-term interest of farmers and consumers, the government should carefully consider all the facts (benefits, risks, issues, *etc*.) dispassionately, unbiasedly, and non-politically before opening the box. Before that, it should put in place strict regulatory mechanism at every level of administrative and research establishment in the country. Every new technology, though beneficial to mankind, has its own the attendant problems. Wise people foresee and resolve them before they become uncontrollable. The first responsibility of the government is its citizens which elected it, but not the ever profit-seeking industry.

At the moment, the European Union has the most stringent regulatory system than any country. India would do well to follow this system than the one followed by USA which has the same laws that govern health, safety, and efficacy, and environmental impacts of similar products derived by traditional methods. This North American nation which is in the forefront of genetic engineering technology treats foods or products derived from transgenic crops on par with those derived through conventional technology, regardless of the fact that the transgenic crops were the recipients of genes from non-plant sources.

Challenges to Indian weed scientists

Man with hoe has been a classical symbol of weed control for several millennia before the advent of organic herbicides. The early-era weed scientists were essentially botanists involved in taxonomy and biology of weeds. Later, they enlarged their sphere of research by testing these herbicides for crop selectivity and weed management in a wide range of cropping and non-cropping situations. This was further enlarged as herbicide resistant weeds began emerging. In the light of the rapidly expanding field of genetic engineering during the past two decades, the current crop of weed scientists face enormous challenges emanating from the rapid adoption of transgenic herbicide resistant (THR) crops. They are required to involve, both directly and indirectly, in the following areas.

a) Development of THR varieties (events, stacks) in different crops.

b) Interaction with various governments, research organizations, and biotech industry during various stages of genetic transformation.

c) Evaluation of THR varieties during pre-release stage.

d) Integration of THR crops with the existing weed management systems

e) Evolution of herbicide resistant weeds in THR crops.

f) Outflow of transgenes from transgenic crops to landraces, wild/weedy relatives and non-transgenic crops and find ways to contain them.

g) Evolution of transgenic crop-volunteer weeds.

h) Effect of cultivation of transgenic crops on soil ecosystem.

i) Play a decisive role in the THR-related regulatory and adoptive policies of the country and states.

In order for the weed scientists to meet these challenges successfully, they are required to equip themselves with adequate knowledge and expertise in the fast-expanding fields of molecular biology and genetic engineering. Future weed science curriculum in universities and other educational institutions needs to be broad-based to include these fields. This way, weed scientists can play a more active, greater, and vital role in the country's march towards a more open transgenic technology, particularly with regard to herbicide resistance.

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