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PLANT PHYSIOLOGICAL PARADIGM IN FOSTERING AGRO- AND BIO-TECHNOLOGY

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SUMMARY

The physiological principles and practices have been the basis for developing most of agro- and bio-technologies used in crop production and improvement. The agrotechnologies which are based on physiological paradigm are: depth of sowing/planting, seed rate, fertilizer requirement, irrigation scheduling, abiotic stress management, vegetative propagation, inter and multistoried cropping, pruning of shoot and roots, pre and post harvest management of flower, seed, fruit etc. Plant physiological paradigms have given the birth to weed science, environmental science and seed science/seed technology. The dwarf plant type concept for achieving higher yield, which in fact, brought green revolution was proposed by physiologists. Synchronization of flowering for crop breeding is solely dependent on physiological paradigm. The modern days biotechnology, but for, the plant physiological paradigms could not have attained the success. The rejuvenation of plants, development of disease free genotypes, germplasm conservation, enzyme engineering, fuel biotechnology, transgenic plants for yield, quality, prolonged storability and stress tolerance, chloroplast and mitochondrial transgenics, productions of metabolites, environmental biotechnology etc. have become feasible due to plant physiological paradigms. The challenges ahead and steps to combat the problems of productivity, breaking yield plateau and sustaining yield, quality and storability are discussed.

***Key words*: Agrotechnologies, biotechnologies, physiological paradigms, plant productivity challenges.**

INTRODUCTION

Plant physiology, besides being a basic science, is acclaimed to be an applied science. This science is essentially an application of biophysics and biochemistry to the understanding of plant. Studies in plant physiology depend strongly upon plant anatomy and cytology (study of cell) and also upon structural and functional chemistry. Today, the technology of applied physical sciences provides the instrumentation upon which research in plant physiology depends. At the same time, plant structural science such as anatomy/cytology becomes more meaningful because of plant physiology. The other sciences like plant pathology, agronomy, entomology, genetics and plant breeding, soil physics, soil chemistry, horticulture/sericulture, vegetable science, pomology/plantation crops etc. relish the outcome of plant physiology. Plant physiology, besides dealing with the fundamentals of plants life starting from seed to seed, i.e. seed germination, plant growth, flowering, seed formation and storage, plant productivity etc., also encompasses and gauge plants interaction with environmental conditions. Hence, most of the important agro technologies developed by different sciences are based on the physiological paradigms. Similarly the plant biotechnology and its approaches are based mainly on plant physiological principles and practices. A brief review on these aspects is presented here.

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A. AGRO TECHNOLOGY

Seed sowing/planting/transplanting

(i) The depth of sowing of seeds: In different crops, the depth of sowing of seeds is based on the elongation potential of plumule/hypocotyl, which is controlled by auxins. (Went and Thimann 1937, Bonner and Galston 1952). Sugarcane sets planted even at 20-25 cm depth perform better whereas, wheat, rice, groundnut etc. collapse because their hypocotyl/plumule do not elongate much and exhaust/fail to reach above ground surface. Consequently due to not reaching to light/inadequate exposure of hypocotyls to light, the entire crop fails right at germination stage. Physiologists suggested the depth of sowing/planting and that was developed as agro technology for crop cultivation by the agronomists, horticulturists etc.

(ii) Transplanting of seedlings/saplings: It is a most common practice followed in agricultural and horticultural crops cultivation. This is based on fast root initiation regulated by hormones specially the root cytokinins production. On partial root injuries, the augmented cytokinins are produced which help in fast establishment of seedling and significantly more number of synchronized tiller emergence (Torrey 1976). The Japanese transplanting technique of rice based on these physiological paradigm spread throughout the world. Raising of seedling/settling from tiny seeds of plants and thereafter their transplanting is a common agro technology based on aforesaid paradigm.

(iii) Spaced transplanting (STP) technique: This was developed by raising seedling/settling from single bud set in sugarcane, and is based on hormonal regulation. One month old settling are transplanted in the field. After establishment of settling, the synchronized and uniform tillering and thereby doubling of cane yield is obtained (Dwivedi and Srivastava 1993). Through this technique the vegetative seed (bud) to vegetative seed (bud) ratio is 1:40 as against 1:10 by conventional 3 budded set planting. Polybag technique of transplanting of sugarcane and other agricultural and horticultural crops is synonym to STP method of sugarcane crop.

(iv) Seed rate: In cereals, higher tillering is a common process, consequently even the small amount of seed would serve the purpose to cover the desired area. In wheat, Miller (1938) reported that the mother shoot bears more bold seeds as compared to tillers because of strong source, sink and root system. This concept came in practice in India, after the identification and production of dwarf gene wheat plants (Borlaug 1967). Wheat seed rate was suggested as 90-120 kg/ha as against 40-60 kg/ha seed used earlier in this crop. Higher numbers of seedling for transplanting were also recommended in rice. In sugarcane too, the mother shoot and sometimes primary one or two tillers were found to be superior to rest of the tillers in cane and sucrose yield (Dwivedi and Srivastava 1993). Hence, increase in seed rate (10-15 t/ha) and closure spacing was recommended, for attaining higher productivity. Pit method of planting of sugarcane developed by Yadav (1993) is based on this principle.

Based on physiological principles involved in crop expansion/tillering, light penetration in crop canopy, development of economic parts of crop and time required for their maturity, water and nutrient use efficiency and finally the optimal yield, the seed rate of different crops have been fixed which is already in use in crop cultivation since long.

(v) Time of seed sowing/planting: The recommended time of sowing of a crop, provides assured availability of suitable environmental conditions for completing the full span of crop life starting from seed germination to crop maturity/seed ripening. The crop life is consisted of various growth phases including seed germination, seedling establishment, crop expansion and tillering/branching which are accomplished due to proper functioning of various physiological and metabolic processes (Salisbury and Ross 1984). Hence, the fixation of time of sowing/planting of crop, which is very important agrotechnology is purely based on physiological paradigm.

Irrigation

(i) Critical growth stages for irrigation: The irrigation is given at different growth stages based on water requirement. In wheat, crown root initiation stage (CRI) (21-25 day after sowing) is most critical stage for irrigation. (Locke and Clark 1924, Kramer 1945, Bharadwaj *et al.* 1971). The physiologists worked out

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crown root stage, which gave impetus in developing other critical stages for irrigation in wheat crop. Based on the principles of maximum water use efficiency and maximum need of water at a particular growth stage, the critical growth stages for irrigation in different crops have been worked out.

(ii) Come up irrigation (CUI): In the state of Madhya Pradesh (India) sowing of wheat after rainfed soybean is a common practice. Irrigating wheat 60 days after sowing which is called as come up irrigation compared to crown root stage irrigation gives significantly higher yield. The superiority is based on reduction of unproductive tiller population and quick growth of new nodal roots to utilize soil moisture from heavy texture deep soil. (Verma *et al.* 2001)

Birth of weed science

Plant physiologists worked out the impact of different growth hormones/regulators including 2-4, D etc., which augment plant productivity at lower concentration and control/suppress weeds at higher concentrations (Went and Thimann 1937). The selectivity for killing broad leaves and narrow leaves weeds was worked out firstly under field conditions by Hamner and Turkey (1944). Thus the weed science developed and the agrotechnologies for weedicide application were formulated. In India, Bonsale (1950) and Arakeri (1951) were the pioneer agronomist who used herbicides to suppress weeds in sugarcane.

Besides competition with crop for growth resources, the allelo chemicals released by weeds suppress crop growth and to combat it, the need for application of herbicide in soil (pre emergence of weeds), post emergence of weed and at developed foliage stage of weed was realized. In addition to this the selective, non selective and systemic types of weedicide were identified which are widely used under plant protection technology since long (Whittaker 1975).

Plant type concept

Blackman (1905) gave the concept of dwarf plant to raise the productivity of crops. Borlaug (1967) developed two/three gene dwarf wheat to break yield plateau that brought green revolution in the entire world. Donald (1968) described plant type for high yield. Asana (1968) suggested plant type of wheat for drought tolerance (barani cultivation). A ear type of wheat was proposed whereby inverse relationship between grain number and grain weight could be minimized (Ghildiyal and Sharma Natu 2003). A plant type of *Brassica* for higher productivity was proposed by Bhargava and Tomar (1990).

Mineral nutrition

(i) Essential and beneficial inorganic nutrients: Plant physiologists have identified and demonstrated essential and beneficial inorganic elements for plant growth (Marschner 1995). Essential inorganic nutrients include N, P, K, Ca, Mg, S (macronutrients) and Zn, B, Mo, Fe, Cu, Co, chloride (micronutrients). Mineral elements which either stimulate growth but are not essential or which are essential for certain plant species under specific conditions, viz. sodium, silicon, selenium, aluminium and nickel usually called as beneficial elements.

(ii) Fertilizer requirement: Fertilizer requirement is worked out based on the need for optimal plant growth including apparent deficiency symptoms and hidden hunger (Marschner 1995). Visual deficiency symptoms of various inorganic nutrients have been demonstrated by plant physiologists (Agrawala and Mehrotra 1963 and Agrawala and Sharma 1979). The nutrients are applied to plants through soil or foliage. Hidden hunger is the state of plant suffering in which apparent deficiency symptoms of an element are not visible but plants respond significantly to the application of that nutrient. Dwivedi and Randhawa (1974) demonstrated hidden hunger of zinc in agricultural crop plants and recorded it by using carbonic anhydrase activity as rapid tissue test (Table 1).

(iii) Split application of fertilizers: Instead of one time application of fertilizers in the soil, based on the time of appearance of apparent and hidden hunger, the leaching losses of nutrients and maximum nutrient requiring crop-growth phase, the split application of fertilizers was advocated by physiologists. This was tested on large scale by the agronomists, horticulturists and it has now become an agrotechnology (Abrol 1999).

(iv) Foliar spray: Physiologists have also advocated foliar spray of inorganic nutrients under particular soil

Table 1. Biologically active zinc and carbonic anhydrase activity predicts the hidden hunger of zinc in crop plants (Dwivedi and Randhawa 1974)

Crop	CA activity (reaction mixture colour after 5 min.)		Zn in plant (ppm)		Physiologically active zinc in plant (ppb)		Response to applied Zn over control
	Zn_5 ppm	Zn_0 ppm	Zn_5 ppm	Zn_0 ppm	Zn_5 ppm	Zn_0 ppm	
Wheat	+++	—	40.8	41.2	269	64	25% rise in yield
Gram	+++	—	28.0	29.0	305	85	,,
Maize	+++	——	43.0	44.0	320	80	35% rise in yield
Mustard	+++	——	27.3	28.0	378	98	,,
CD at 5% level			NS			21	

(+) greenish yellow, (-) light blue/green/yellowish green; Zn_0 and Zn_5 ppm added Zinc in soil.

conditions, although, soil application of inorganic nutrients has definitely shown greater advantage than foliar spray. Foliar application of Fe (10-12 kg $FeSO_4$/ha) has been found to be much superior and economical than 50-100 kg $FeSO_4$/ha soil application on alluvial alkali soil in Punjab and Gujarat States. Similarly 2-3 spray of 0.5% $MnSO_4$ has been found to be 1.52 times superior to soil application of 40-50 kg/ha $MnSO_4$ in Punjab and Bihar (Takkar 1999). Foliar spray of copper sulphate + herbicides or herbicide alone has been found to be much superior and economical than pre-emergence soil application (Whittaker (1995).

(v) Fertigation/nutrigation: This agrotechnology is based on physiological paradigms in which easily soluble and readily available form of inorganic nutrients are applied in solution form at right site/organ to achieve maximum nutrient and water use efficiency in crops. In drip or sprinkler system of irrigation, the solution form of readily soluble and available fertilizers are administered with irrigation water and placed on leaves/roots to meet water and nutrient requirements of crop. This system is in operation in Israel and San Joaquin valley of California (USA) since long (Benes 2003). Brazil and Turkey are saving about ¾ of flood irrigation water and soil applied inorganic nutrient under field condition in vegetables, fruits and plantation crops, sugarbeet and cereal crops (Anonymous 2004, Kitron 2004). Brazilian farmers are getting maximum yield of sugarcane to a tune of 165-170 t/ha and thereby maximum profit following fertigation technology (Kitron 2004). Leaching of water and leaching and fixation of nutrients are least in this technique, which makes it more viable, economical, efficient and eco-friendly.

(vi) Fungicidal and insectidal use of mineral nutrients: Plant physiologists observed least attack of certain diseases and pests in the crops fed with certain nutrients. This resulted the use of few inorganic nutrients as an alternative for insecticides and pesticides in crop protection (Table 2).

Table 2. Insecticidal and fungicidal medium of few inorganic nutrients.

Element	Disease/pest	Ref.
Silicon	Rice blast	Volk *et al.* 1958
Ca	Twin stem (*Sclerobium sp.*)	Corden 1965
	Soft root disease (*Erwinia carotora*)	
	Fusarium Wilt	
$CuSO_4$ + Lime (Bordeaux mixture)	Bud rot- Coconut	Menon and Pandalai 1958
$CuSO_4$ + $CaSO_4$	Root infection take all (*Gaeumannomye graminis*)	Gardener and Flynn 1988
B	Red spider mite in oil palm	Marschner 1995
K	Puccinia disease (*Xanthomonas*)	

Drought (water deficit) management

To have a good crop under water deficit and rain fed conditions, the following agro techniques are followed worldwide. These agro techniques have been developed based on physiological paradigm.

(i) Soaking of seeds/sets/buds in lime water/ water: Soaking of seed in water starts germination process and hence the over night soaked seeds germinate quickly at low soil moisture level. The lime water (calcium hydroxide) augment colloidal functioning and helps in the uptake and retention of water in seeds and thereby ensure quick germination in the soils having inadequate moisture (Bonner and Galston 1952).

(ii) Drip/sprinkler irrigation: Water is made available directly to the feeding organs, i.e. roots and leaves. Thus with little water, plants meet their requirement and 50-80%, of water is saved as compared to flood irrigation. Flood irrigation of 30-35 nos. per year to sugarcane crop resulted in soil salinization (Dwivedi 1993). In Maharashtra state of India, drip/sprinkler irrigation is used in sugarcane that saved the soils from salinization.

(iii) Ridge and furrow system of sowing/planting and irrigation: Significant improvement in germination is attained by sowing seeds in furrow and ridge system. The irrigation is also done in this system which gives 30-40% water economy over flood irrigation (Yadav 1993). In certain crops like sugarcane, with the time, the furrow is converted into ridge and ridge into furrow. This gives physical strength to crops and reduces water requirement at later growth phases of crop (Dwivedi and Srivastava 1993).

(iv) Anti-transpirant spray: Anti-transpirants, e.g. Kaolinite, MH etc. are sprayed on foliage to reduce transpirational losses of water.

(v) Planting of drought tolerant crops: Species and varietal level differences in tolerance to water-stress have been observed. The genotypes with higher water potential and osmoticum and well developed water scavenging anatomical features, withstand water deficit. Such genotypes are scanty but a few have been identified in wheat barley, sugarcane, groundnut, sorghum, finger millet etc crops by plant physiologists. These are planted to combat drought conditions (Sinha 1987, Dwivedi 2000).

Salt stress, waterlogging and temperature stress management

To manage salinity, sodicity, temperature and waterlogging stress many agro technologies are available which are based on physiological paradigm. For example to withstand salt stress, the planting of a variety with high K/Na ratio in leaves (> 1.5) and root (> 2.0) (Nieman and Shannon 1976, Qadar 1987, Dwivedi 1993) and higher chemi-osmoticum in leaves (1600 μg/g dry matter) (Dwivedi and Qadar 2004) are suitable. On the other hand, for enduring waterlogging, varieties maintaining oxygen balance (i.e. redox-potential) in the shoot and root vis-à-vis higher arenchymatous tissues (i.e. porosity) are most suitable (Grable 1966, Dwivedi 2000). Chinoy (1947) showed that early wheats possess an advantage in yield over late ones by virtue of their capacity to escape high temperature. Soluble starch synthase of wheat grain was found to be extremely sensitive to high temperature. Terminal high temperature tolerance for grain growth in wheat has been shown to be related with the temperature tolerance of this enzyme (Prakash *et al.* 2003, 2004). The foliar spray of sodium meta silicate (2%) helps in enduring both low (<15°C) and high temp. (>39°C), since it provides a thin layer of sodium silicate on leaves which acts as bad conductor of heat (Dwivedi 2000). Consequently, the latent heat in leaves does not reach to the level of injury in the cytoplasm and the plant is saved.

For attaining higher yield and escaping from stress injuries the foliar spray of 0.5% zinc sulphate on crops growing on sodic soil, foliar spray of 2.5% urea and 1% $FeSO_4$ on crop growing under waterlogged/water saturated conditions, irrigating crops prior to onset of frost to escape plants from injuries are many known agrotechnologies which are based on plant physiological paradigm (Dwivedi 2000).

Birth of environmental science

Various imbalanced human activities on the earth including deforestation, industrialization and population explosion etc. have resulted in environmental degradation, global warming and depletion of ozone layer etc. These global climatic changes, if not checked would endanger human/living creature life, and thus have emerged as hot topics under environmental science. The changes in the structure and function of plants have proved as the best and rapid indicator for environmental disorder and also in the same or other forms as cure of disorders (Middleton 1961). Based on plant community structure, plant physiological functioning, processes and paradigm and various industrial activities, Faith (1959) proposed the methods of air pollution control. Thus practical environmental science came in to existence and mega share of plant physiological paradigm in combating the environmental disorders was recognized.

The green house gases, viz. CH_4, CO2, nitrous oxide, chlorofluorocarbon etc. cause environmental warming. Elevated atmospheric CO_2 does not have beneficial effect on all type of crops (Uprety 1999). Plants having larger sink capacity and which accumulate excess assimilates as starch in the leaves showed better performance under long term CO_2 enrichment (Sharma-Natu *et al.* 1997, Ghildiyal and Sharma-Natu 2000). Various measures have been suggested to control environmental disorders. To combat with rising CO_2 and methane level, planting of heavy feeder of CO_2 and degrader of methane has been suggested (Dasgupta 2003). Dwivedi (1994) recorded significant reduction in CO_2 and methane following sugarcane planting by the side of rice field. This finding explains the logic of following a known cropping practice, *i.e.* paddy and sugarcane plots side by side in India and many Asian countries, since time immemorial.

Vegetative propagation

This is most commonly used technology for growing horticultural crops and multiplying and improving their genetic make up. The entire success of such practices is based on growth hormone balance as advocated by physiologists. Grafting of scion bud on stock, fusion of branch of scion with stock and plant propagation through cutting of stem branch, rhizome are always practiced. Such processes are more successful in the plants where endogenous root hormones IAA, and IBA are abundant (Went and Thimann 1937). To increase the level of success the auxin, cytokinins and growth regulators are applied externally (Bonner and Galston 1952). The practice of rooting of cuttings for vegetative propogation using growth regulators in India is based on the pioneer work of Nanda *et al.* (1968). Commercial form like "rootex" etc. are widely available for initiating rooting of cuttings.

Prunning of shoot and roots

This has been most common practice in horticultural and plantation crops, whereby, the old twigs/branches or new growing buds and old and suberised roots are removed. Consequently the healing hormones, e.g. cytokinins, IAA, IBA, GA etc. are released (Went and Thimann 1937, Bonner and Galston 1952 and Salisbary and Ross 1984) and promote the growth of fresh branches/roots and multiply the branching of shoot/roots. Due to this, the fast uptake of water and nutrient takes place and more accumulation of photosynthates occur. Consequently, a high intensity of flowering and fruiting resulted in higher yields.

Inter-cropping and multistoried plantation cropping

Based on the physiological paradigm the concept of inter-cropping and multistoried cropping are in operation since time immemorial (Santhirasegaram 1966, Geus 1967, Dwivedi 1970). The availability and utilization of light by crops is the key basis for aforesaid cropping system. However, the other growth regulating resources also play some role, which are managed through agronomic inputs. The allelo-chemicals released by inter-crops become synergistic/antagonistic to the companion crop. Only that inter-crop is widely accepted where allelo-chemicals released by crop is neutral/synergistic to other crop and the problem of mutual shading does not arise besides that of imposing little competition for crop growth resources (Rice 1984, Bertin *et al.* 2003). Crop growth rate, maturity period of crop and utilization of space also determines companion cropping. In sugarcane, the inter-crops such as mustard, wheat, garlic, coriander have been found to be most successful and remunerative since these crops mature within 3-4 months when sugarcane crop growth rate is very slow and sufficient space exists for the availability of un-interrupted light, nutrient and water.

The multistoried cropping system, e.g. pineapple, cacao, pepper and arecanut alongwith coconut, is a widely accepted cultivation practice where the best utilization of inter-space between two lines of coconut occur (Child 1974). In this system, besides that of coconut-cacao inter-cropping (Ramdasan *et al.* 1976) little interference for light takes place and meager competition among plants for water, nutrients and other growth resources occurs. Many plants manage to grow under partial shades such as pepper and cardamom. In the evergreen spars forest, the commercial plantation of cardamom is a common practice. All these clearly reveal that physiological basis of crop growth determines the inter-cropping system of crop production (Evan 1975, Salisbury and Ross 1984).

Induction of flowering for breeding

The entire programme in the crops where flowering is not synchronized in different species/cultivars or where flowering is not assured, the plant physiologists make the plants to flower through physiological paradigm following photo-thermal manipulations (Garner and Allard 1920). In this regard, the automatic device developed by Sirohi and Hammer (1960) to control length of light and dark period gave great impetus in regulating flowering and targeted crop improvement. Photoinsensitivity has been a key trait for improvement and wider adaptability of many crop plants. Sirohi (1965) showed marked genotypic variability in photoinsensitivity in several crop plants.

Maturity/ripening and crop harvest

Maturity/ripening and crop harvest have been decided on the basis of physiological ripening. Maturity is defined as "the completion of the development of a fruit to a point at which it is physiologically mature enough to be separated from parent plants. Typically this is the point at which its seeds are viable". No other criteria except, the physiological maturity index, e.g. optimal sucrose accumulation in sugar crops, nicotine and fibers in tobacco leaves, caffiec acid in tea leaf and coffee pods, sugar and the ratio of total soluble solid/tritable acid and citric acid in orange (non climacteric fruit), soluble solids and optimal fruit size in apple (a climacteric and temperate fruit), starch in potato tuber, extractable oil in coconut and oil seed crops, decides the harvest of crop. Not harvesting the crop at physiological maturity results enormous loss in yield and deterioration in quality and shelf life especially, in fruit and vegetable crops. Storage of physiologically immature apple results bitter pit and scald in fruits (Grierson 1994). Utilization of physiologically mature tobacco leaves (developed fiber and optimal nicotine) produce biologically better product of tobacco (Carlson 1994). The harvesting of crop is therefore, based on physiological maturity and physiological paradigm.

Pre and post harvest management

Pre and post harvest management practices of vegetables, fruits and cut flowers with a view to enhance shelf life vis-à-vis to reduce the spoilage of produce, maintaining high quality, taste and colour for longer period and marketing the produce to far distant places/foreign countries are based on physiological principles. Considering the dynamics of respiration, permeability, ethylene production (Srivastava and Paul 2003), aeration, rancidity, firmness, hydration, dehydration, antioxidant defence system, interaction of specific gases and their diffusion, all the agro-flori-horticultural practices pertaining to pre and post harvest management technologies have been developed (Applbaum *et al.* 1977, Fishman *et al.* 1989). Giovannoni (2001) gave a vivid illustration on this aspect. Wrapping of fruits with paper and packing them in wooden boxes facilitate aeration and reduce temperature of box. Storage at low temperature improved shelf life of fruits due to its impeding effect on fruit deteriorating processes (Singh 2001). The use of ripening retardants , e.g. menadion 0.1% and ascorbic acid 2% in kinnow (*Citrus mobites* x *C. deliciosa*) significantly increase its shelf life over control (Nagar 1991).

Seed technology: seed production, storage, viability and vigour

Seed is an end product and beginning. It is the most compact software of plant which decode entire plant growth and development. Seed production, processing, certification, testing and multiplication processes were followed for getting seeds of homogenous vigour. Besides attention on genetic purity of seeds, the viability, germination and seedling vigour formed the quick and reliable parameter for healthy and homogenous seeds. Thus the chemical constituents of seeds and their physiological functioning were taken as important basis for seed quality (Kozlowski 1972).

Based on respiration rate, release of ethylene, loss of electrolytes, free radical induced oxidation-reduction and changes in seed coat permeability/diffusibility as the basic physiological paradigms, the storage conditions are changed. Besides this, the light affects viability/storability, dormancy and electrolytes release in many seeds including soybean. (Miller 1938, Bonner and Galston 1952). Hence, for storage and viability of some of the seeds, the polythene sheets of different gauge and colour (tirge) have been used with a view to manage/control moisture, temperature etc. so as to have prolonged seed viability. The existing all storage technologies, e.g. vacuum, very low temperature and irradiated seed storage in general (Basu and Sur 1988) and cryo, slow growth and DNA clones preservation in particular, for prolonged seed viability and good germination are based on physiological paradigm. The viability of stored seeds is very much affected by humidity, temperature and light (Basu and Sur 1988, Agrawal 1990). Pre-sowing hardening treatment in different crops (Chinoy *et al.* 1957, Sashidhar *et al.* 1977) and mid storage hydration and dehydration technology with or without chemical treatment of orthodox agricultural and horticultural seeds is widely practiced technology for raising seed storability, viability, germination and seedling vigour. These all are based on physiological principle (Basu and Sur 1988, Agrawal 1990). Prolonging seed dormancy is one of the ways to prolong seed viability and curtailing seed dormancy is another way to activate seed viability vis-à-vis quick use of freshly harvested dormant seed for germination. The seed industries, now a days, based on the physiological paradigm, treat freshly harvested dormant seeds of agricultural, horticultural, forestry etc. crops and make them to germinate. Freshly harvested dormant seed of sunflower germinated by the treatment of 25 ppm ethrel (Udaykumar and Krishna Shastry 1975).

B. BIOTECHNOLOGY

The term biotechnology is derived from the fusion of biology and technology. It is defined in various ways. However, as per Bullock (1987), the biotechnology is "controlled and deliberate application of simple biological agents living or dead, cell or cell components in technically useful operation either for productive manufacture or service operation".

Although the term biotechnology is of recent origin, the discipline itself is very old. Man began employing microorganism as early as 5000 BC for making wine, vinegar, curd, leavened bread etc. Some of these processes are so common and have become an integral part of usual kitchen technology of every home. Man has continued his quest for (i) improving natural capabilities of micro-organism (ii) making them capable of novel processes (iii) discovering micro organism with new capabilities (iv) modifying micro-organism and or other organism to create in them highly valuable, novel and naturally non-existent capability through recombinant DNA technology. In Agriculture, the application of this technology led to the development of transgenics for abiotic and biotic tolerance, environmental conservation and higher yield and better shelf life. Plant Physiology has played pivotal role in the development of biotechnology. It contributes to biotechnology, as illustrated in Fig. 1 (Singh 1998).

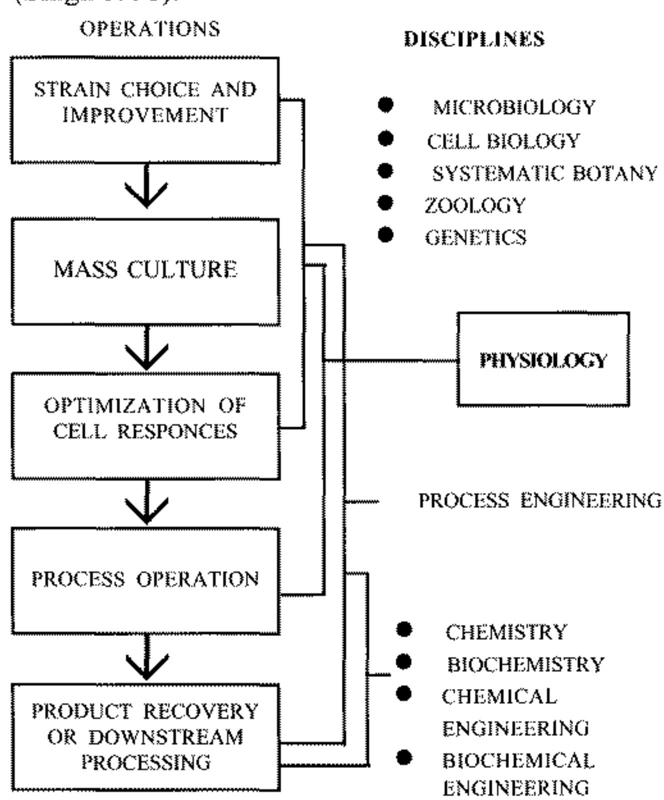

Fig. 1. A schematic representation of the various operations and the disciplines on which biotechnology is based in the production of a compound using microorganisms, animal cells or plant cells (i.e. a biotechnological operation)

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To have sound and systematic dent on productivity, stress tolerance, maturity/shelf life, health friendliness, biodiversity conservation and environmental friendliness through biotechnological approaches, genomic sequencing projects have been launched. India is a proud participant in the international rice genome sequencing to sequence 430 Mb of rice genome. For genomic sequencing studies six strides have been made, (i) structural genomics (ii) functional genomics (iii) comparative genomics (iv) proteomics (v) transcriptomics (vi) bioinformatics. Plant physiological paradigm gives the base for identifying functional genomics (by screening functions/characters), comparative genomics (by evaluating effectiveness for different traits) and transcriptomics (by finding out structural and process reproducibility) and thereby their modulation and transfer to desired set of plant cells for achieving the target vis-à-vis product or process. Few important product and processes of biotechnology, which have been developed on the basis of physiological paradigm, are mentioned below.

Rejuvenation of crop varieties and production of disease free plants

It is generally known that the outcome of biotechnology/plant biotechnology is "product" or "process". The development and evaluation of product of desired type say a transgenic or chemical accumulation/ synthesis have to pass through cell/tissue culture technology developed on the basis of physiological paradigm by the physiologists. The use of MS media formulated by the plant physiologists Murashige and Skoog (1962), and other media by White (1963) and Gamborg *et al.* (1968) are inevitable for the expression of products and processes developed through genetic manipulation. Further for their nurture and promotion to make them easily available to consumers, the uses of physiological paradigms are essential. The hormones (growth regulators) of different types are used in tissue culture media for promoting growth and activating/regenerating genes vitality. Gibberellic acid, since long, had been reported to modulate plant genome activity (Verner and Chandra 1964, Taiz and Stark 1977) and relaxation of DNA activity and thereby transcription and new vigor in plant (Chandra *et al.* 1988, Dwivedi 2001). The pioneer work on tissue culture in India started at Delhi University by P. Maheswari with chemical stimulation of egg *in vitro* to obtain haploid plant in 1957, followed by H.Y. Mohan Ram on physiology of sex expression. Sipra Guha and S.C. Maheswari discovered the anther culture technique for production of haploids (Maheswari 2003). Following the systematic cell/tissue regeneration processes, the rejuvenated lines and disease free lines are produced (Ravishankar and Venkataraman 1997).

Germplasm conservation

Germplasm provides raw material (=gene), which a breeder uses to develop commercial crop varieties. Conventionally, germplasm is conserved as seeds stored at ambient temperature. But many crops produce recalcitrant or short lived seeds and in case of clonal crop, the seeds are not the best material to conserve in view of their genetic heterogeneity and unknown worth. Root and tuber lose their viability quickly. Materials modified by genetic engineering are mostly unstable and need intact conservation for future. Methods of germplasm conservation include (i) freeze preservation (ii) slow growth culture (iii) desiccation of somatic embryos/ artificial seed and (iv) DNA clone (Table 3) are based on physiological principles. DNA segment clones in a suitable vector e.g. cosmid, plasmids or yeast artificial chromosome which could be preserved. These clones are produced and conserved, though a very costly affair but required for endangered species. The cryopreservation is based on suppressing physiological processes, viz. respiration, ethylene production, toxin accumulation, loss of electrolytes by employing super low temperature and liquid N refrigeration. The slow growth and DNA clone preservations are dependent on standard tissue culture technique and recombinant DNA technology respectively, which are well-known plant physiological paradigm based technology.

Chemical production

With the help of biotechnological approaches, the secondary metabolites in plants are being produced on large scale. These metabolites are of great importance as they act as antimicrobial, antibiotic, insecticide, molluscide, growth regulator (hormones) and valuable pharmacological and pharmaceutical agents/chemicals

Table 3. A comparison of the three approaches for *in vitro* germplasm conservation

Feature	Cryopreservation	Slow growth	DNA clones
Tissue/organ conserved	Shoot tips, zygotic or somatic embryos, cells, protoplasts	Slow growing shoots	DNA pieces as phage clones
Metabolic activity	Nil	Slow	Nil
Storage temperature	-196°C	4-9°C	4°C in lyophilized state
Storage in	Liquid nitrogen refrigerators	Ordinary refrigerators	Ordinary refrigerator/deep freeze
Attention needed during storage	Replenishing liquid nitrogen	Subculture every 6-36 months	Virtually nil
Risks	Loss in viability with time	Contamination	Virtually nil
Applicable to	All species amenable to tissue culture	All species amenable to tissue culture	Conservation of valuable genes
Sophistication	Sophistication	Less Sophistication	Highly Sophisticated
Cost involved	Costly equipments required	Standard tissue culture facilities needed	Very costly as it is based on recombinant DNA technology

and also used as flavour, fragrance, colour etc. Such chemicals are called as secondary metabolites (e.g. alkaloids, terpenoids, phenyl propanoids, quinines, steroids etc.), because in general, they do not involve in primary metabolic processes, e.g. photosynthesis, respiration, proteins, lipids etc. Using cell suspension technique developed by physiologists the productivity of such compounds has been raised by many folds (Table 4). For example eight run of bioreactor of 750 l with 600 l media using hairy root culture, cell suspension of *Lithospermum* would yield 9.6 kg shikonin, whereas, one ha *Lithospermum* root would yield about 9 kg after 4 years. Cell culture derived shikonin has been used in Japan since 1984 in the manufacture of cosmetics, lotion and soap.

Enzyme engineering

Structure and function of any enzyme molecule, for that matter of any protein molecule, are chiefly determined by its amino acid sequence, *i.e.* its primary structure. However, the change in enzyme property occurs usually when the amino acid sequence is altered in certain critical region of protein and not at any region. It is therefore,

Table 4. Selected examples of bio-chemicals produced in high concentration by plant cell suspensions.

Compound	Class of compound	Plant species	Yield (g/l)
Ajmaline	Alkaloid (indole alkaloid)	*Rauwolfia serpentine*	2
Anthraquinones	Quinones	Morinda citrifolia	2.5
Berberine*	Alkaloid	*Coptis japonica*	7
Coniferin	Phenylpropanoid	*Linum flavum*	2
Rosmarinic acid	Phenypropanoid	*Coleus blumei*	5.6
Raucaffricine	Alkaloid (indole alkaloid)	*R. serpentine*	1.6
Shikonin*	Naphthoquinones	*Lithospermum erythrorhizon*	4
Taxol*	Diterpene alkaloid	*Taxus* sp.	-
Ginseng*	-	Ginseng	19**

*Produced in Japan on commercial scale (Shikonin by Mitsui Petrochemical Ind. Ltd., Gingseng by Nito Denko Corp. Ltd.)
**Cell material (gingseng tissue, not cell suspension) on dry wt. basis, used as an additive for tonic drinks, wines, soups, herbal liquor etc.

essential to know critical regions for the various functions of an enzyme and to be able to predict the effect of specific amino acid change in these areas on the various function. The summary of steps involved in enzyme engineering, such as isolation and cloning of concerned gene, desired change in base sequence by site directed mutagenesis, changes in amino acid sequence, data base information on three dimensional structure and properties of enzyme etc. is presented in Fig. 2.

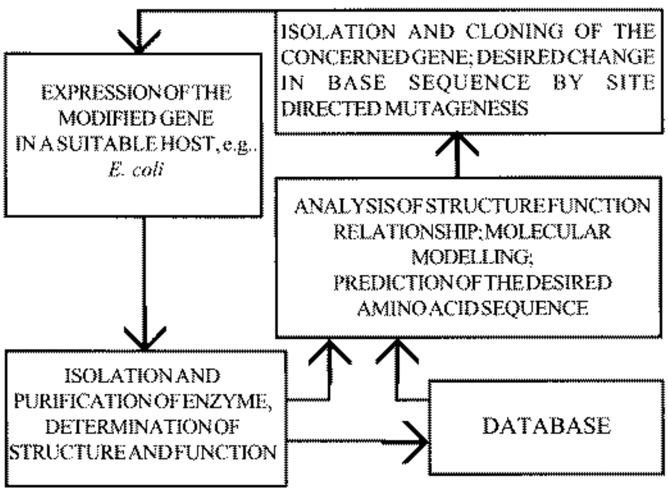

Fig. 2. A summary of the various steps involved in enzyme engineering

Fuel biotechnology

The ultimate source of energy for fossil fuels and biofuels is the solar energy, which is locked through photosynthesis in biomass from which both the aforesaid sources have been produced. Biologically produced fuel vis-à-vis biofuels are biohydrogen, biomethane (CH_4&CO_2mix), bioethanol, biobutanol and biodiesel which are used as substitute of declining fossil fuels (Petrol) to maintain clean environment. Energy from different sources and their requirement vary in developed and developing countries (Table 5). Developed countries use biomass energy to fulfill hardly 2% of energy requirement while developing countries still use it to full fill 42% of their total energy need.

Biofuel is being produced employing biotechnological approaches by developed countries, because (i) most of biofuels are derived from biomass (photosynthetic products), which is renewable, low cost and locally available. (ii) in general biofuel lead to low CO_2 production in comparison to fossil fuel. (iii) they do not contribute to environment pollution due to gas like SO_2 etc. (iv) the substrate is often waste including municipal waste (mainly organic biomass). This is low cost substrate and help in cleaning the environment.

Plant physiologist worked out the efficiency of solar energy harvest by crop and the release of energy through respiration for maintenance and growth. The process of glycolysis in plant physiology meaning the breakdown of sugar to ethyl alcohol was introduced in 1909 by German Scientists (Lipman 1975, Cori 1983). However, most cell produce pyruvic acid, when normally aerated, instead of alcohol. The anaerobic respiration by plants/microbes results fast degration of starch, lipid etc. and production of ethanol, a useful source of energy, is known since long. Anaerobic respiration does not continue in plant for long time since O_2 depletion and accumulation of ethanol and other toxic substances etc. become rate-limiting factor. Hence, bacteria were used to degrade substances in faster rate and produce large amount of fuel through biotechnological approaches. These processes were demonstrated by physiologists long back in 1782 (Salisbury and Ross, 1984).

Table 5. Pattern of energy consumption in developed and developing countries.

Feature	Developing countries	Developed countries
Energy consumption/person/yr (GJ, *rough estimate)	5-10	>5000
Total energy consumption (by all countries, GJ)	$92x10^9$	$208x10^9$
Energy from non-renewable sources (coal, oil, nuclear)**	57%	96%
Energy from biomass	42%	2%

*GJ=Giga Joules = 10^9 Joule (4.2 Joules = 1 calorie).
**The remainder energy from hydroelectric projects.

It has been noted that energy crop having ratio of more that 5 between energy produced and energy used for production/cultivation, are most suitable sources (Table 6). Dwivedi (1994) recorded this ratio as 17.2 in sugarcane. This is the most important crop for bio-fuel production at national and international level (Anonymous 2004a). The bacterial (anaerobic or aerobic) degradation is fast to yield higher biofuel. Therefore, considering physiological principles, the biotechnological approaches are followed to produce bio-fuel employing anaerobic bacteria.

Table 6. A list of some energy crops and the predominant mode of their utilization as biofuel under normal conditions and anaerobic bacterial attack.

Nature of biomass	Plant species*	Estimated annual prod. (tons)	Predominant mode of energy use
Wood (lignocellulose)	*Butea monosperma, Casurina equisetifolia, Eucalyptus globules, Leucaena leucocephala, Melia azadirachata, Tamarix dioica*	$1.3x10^{10}$	Firewood (50% of harvest)
Starch	Cereals, millets, root and tuber crops, e.g. potato	$1.9x10^{9}$	Bioethanol
Sugar	Sugarcane, sugarbeet	$1.2x10^{8}$	Bioethnol, e.g. in Brazil
Hydrocarbons	*Euphorbia lathyris, Asclepia speciosa, Copaifera multijuga, algae*	-	Biodiesel
Wastes**	Crop residues, animal/human refuge, sewage etc.	-	Biogas
Hydrogen⁺	Algae like *Chlamydomonas*, anaerobic bacteria like *Clostridium*	-	Stored as metal hydride, used as H_2 (pollution free)

*The listed plant species are fast growing and promising for biomass (wood) production to be used as energy source.

**This item does not represent an energy crop but is based on biomass of diverse origins.

⁺These species yield an inorganic gas (H_2), which is used to generate energy. Since they use solar energy to generate H_2, they are also regarded as energy crops.

Production of metabolites

Studies and evaluation of organic and inorganic chemical accumulation, their synthesis and effect on physiological process and plant growth are the inevitable task of plant physiologists. By accelerating or blocking or altering enzyme activity the metabolites production has been augmented/suppressed (Conn *et al.* 1987). This has resulted in higher accumulation and depletion of chemical or production of new chemicals. Based on this physiological paradigm, the production of metabolites such as (i) acetone-butanol (ii) alcohol (iii) antibiotics (iv) enzymes (v) organic acids, lactic acid-40000 t./yr. and citric acid 300,000 t./yr. (vi) amino acids- glutamine 40,000 t./yr. and lysine- 300,000 t./yr. (vii) vitamins (viii) ligno-proteins and many other organic metabolites have been accelerated using bacteria as strong source of enzyme for industry level production. Plant physiologists knew and have been working on such aspects since 1950, now focused as biotechnological attainments (Dunn 1974).

Gene transformation vis-à-vis transgenic plants production techniques

In the process of transferring genes to a plant, a specifically constructed gene assembly usually from unrelated organism is called as transgene and the resultant plant, containing this gene is called as transgenic plant. Most of the transformation techniques, viz. agrobacterium mediated gene transfer, agro-infection and direct gene transfer as discussed below for transgene in plant vis-à-vis production of transgenic plants, are based on physiological paradigm/practices.

(a) Agro-bacterium mediated gene transfer: This technique involves two steps, which are based on physiological paradigm, viz. (i) culturing and co-culturing with tissue explant and (ii) plant transformation through imbibition process as discovered by physiologist as early as 1827 by Robert Brown (Salisbury and Ross 1984).

(b) Direct gene transfer: In this technique the genes are transferred without involving biological agents. Direct gene transformation methods, based on physiological paradigms are discussed here.

(i) Chemical method : Certain chemicals e.g. PEG (polyethylene glycol), polyvinyl alcohol and calcium phosphate are used to enhance the uptake of DNA by plant protoplast. Such technology has been evolved from the work of plant physiologists who demonstrated the regulation of the uptake of nutrient, water, organic acid and organic complexes using aforesaid chemicals (Thomas *et al.* 1956, Baker 1978, Palmgren 2001).

(ii) Electroporation method: DNA is introduced in to cell by exposing them for very brief period to high voltage electric pulse which is thought to induce transient in the plasma lemma. Long back since, the era of Janse (1897) and Maeda (1954) this type of technique in which endo and ecto mycorrhizae form symbiotic system with plant roots and regulate nutrient uptake and thereby inorganic nutrient acquisition in plants is known by physiologists (Meyer 1974 and Clarkson 1980). Besides this, protein channels and pores in plasma lemma and differential voltage (energy) existence and its manipulation thereon, inside and outside of plasma lemma and in vacuole have been found as the basis of nutrient entry in to cell by the plant physiologists. (Thomas *et al.* 1956, Oertli 1968, LeRudulier *et al.* 1984, Barkla and Pantoja 1996, Maeschima 2001).

(iii) Microinjection method: DNA solution is injected directly inside the cell using capillary glass micropipette with the help of micromanipulator. The plant physiologists have used micropipette in introducing inorganic salt and herbicide in plants as early as in 1937 (Went and Thimann) and subsequently in 1952 (Bonner and Galston) for examining their effect on plant growth and development. Utility of such techniques in electrolytes and different ions in plasma lemma and tonoplast has been emphasized by Palmgren (2001) and Maeschima (2001).

(iv) Macroinjection method: Injection of plasmid DNA in to lumen of developing inflorescence using hypodermis syringe is called macro injection. Hypodermis syringe technique for introducing inorganic nutrients in tissues was developed by physiologists long back (Miller 1938, Salisbury and Ross 1984).

(v) Direct DNA uptake by mature zygotic embryo: This technique is based on simple physiological principle of imbibition of seed, known since 1827 (Salisbury and Ross 1984).

Production of transgenics for abiotic stress, shelf life and higher productivity

Through biotechnological approaches, especially with the aid of certain molecular markers, some successes have been achieved to develop transgenic plants for attaining higher productivity, salt tolerance, water deficit tolerance, higher shelf life etc. (Table 7). The parameters suggested by physiologists, viz. increasing the activity of photosynthetic enzymes and reducing photorespiration for higher productivity, different osmoticum and ABA regulated stress tolerance, K accumulation and Na/K ratio for salt and water stress tolerance (Boon-Long 1941, Bernestein and Hayward 1958), ethylene production, electrolyte losses and Ca regulated membrane permeability for altering shelf life (Burg 1962, Crane 1964) have formed the basis for selecting gene for transformation and thereby the production of transgenic plants (Hasegawa *et al.* 2000, Giovannoni 2001, Grover *et al.* 2003).

C. CHALLENGES AHEAD

The above review reveals that plant physiological studies have provided the basis for development of agro- and bio-technology for increasing productivity under varying environment. However, much is needed to be done with respect to (i) breaking of yield plateau (ii) improving quality of crops (iii) raising shelf life of plant produce (iv) developing abiotic stress tolerant plants (v) developing biotic stress tolerant plants (vi) investigations on seed standards and raising of seed storage and viability

Table 7. Selected examples of transgenics where genes/proteins for osmoticum, quality and productivity under abiotic stress environment have been used.

Gene (Protein used)	Source	Cellular role	Trans-host/(Promoter used)	Characteristics
Oscdpk7 (Ca dependent protein kinase)	*O.sativa*	Protein kinase	*O.sativa* *(CaMV35S)	Salinity/drought stress tolerance but not cold
Sod. (superoxide dismutase)	*A.thaliana*	Dismutation of toxic reactive oxygen intermediate	*N.tobaccum* *(CaMV35S with duplicated enhancer and a terminator)	Transforments showed 20% higher photosynthesis during chilling compared to control plant
Atmhxl (Na/H antiporter)	*A.thaliana*	Vacuolar Na/H antiporter	*L.esculatum* *(CaMV35S)	Tolerant to 200mM NaCl with no accumulation of Na in fruit resulting good fruit quality.

Abbreviations in parenthesis represent Protein/Promoter/Osmolytes used. (Source-Grover *et al.* 2003)

(vii) environmental conservation: checking the rise of environmental (abiotic and biotic) impediments and imbalances in the constituents of edasphere and atmosphere and (viii) crop sustainability and food security.

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