Azolla- a boon for making agriculture and allied sectors rearing resilient

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Abstract

India boasts of having the largest agriculture and allied sector across the globe. To supply the requirement for agricultural activities and for quality by-products, people's need exists to upgrade them by employing new strategies. Because, as world's population is constantly increasing, the land mass is constantly decreasing. Hence, to produce efficiently in restricted land mass is the need of the hour. Though India has highest agricultural production, it lags behind many other developed countries when it comes to average production because we don't cater to balanced nutrition and there is less availability of fertile soil and inadequate availability of fodder during draught. There arises a need to find some sustainable and substitute sources so that we can overcome the hindrances in these sectors and achieve a good production. Azolla is no doubt a one stop and easy solution for this problem. It contains all the nutrients, minerals and amino acids (essential) with no adverse effects. And it can be easily grown as well in short period of time. It acts as ideal manure during cultivation and ideal feed supplement for livestock as well. Apart from this, azolla extracts possess a therapeutic potential that helps in prevention of acute and chronic inflammation.

Keywords: Azolla, Agriculture, Animal feed, green gold mine.

Introduction

Azolla is one among the seven species of ferns, floating freely over surface of water and retains the aerial nitrogen by symbiosis with the cyanobacterium namely *Anabaena azollae*. Usually the cyanobacteria symbioses in the leaf pockets of Azolla and get a favourable environment, thus converting aerial nitrogen into a form, which is usable during cultivation as well as supplements in the livestock feed. Hence it plays a crucial role in production and cultivation, since it acts as a green manure for cereal cultivation and used as fodder for "cattle and poultry". In Asia, Azolla grows naturally in ponds and water bodies, that lead to its recognition eventually as manure. It is found in tropical and temperate climatic conditions, in water lodging areas of agricultural lands or when effluents accumulate at one place, where it forms a thick mat during favourable seasons. Other aquatic plants Riccia, Lemna, Salvinia, Pistia, Spirodela, and Ricciocarpus also grow along with it floating freely over water surfaces. This fern is one among the "fastest growing aquatic macrophytes" of the globe, that gets doubled within two to five days (Taghi-Ganji *et al*., 2005; Zimmerman, 1985)

Modern sustainable agricultural demand has been supported by various organic manures to reduced chemical-fertilizer, where Azolla plays major role as a potent 'source of nitrogen'. Also in livestock sector, it is supporting the fodder deficit by acting as a potent source of protein. The cost of production in agriculture and allied sectors can be reduced by using Azolla and also optimum productivity can be achieved. Azolla has also various medicinal uses in both livestock and human health. Looking towards these benefits, the review was done to know about production methodology and different factors affecting productivity and its multiple uses.

History and Taxonomic classification

Though origin of Azolla cannot exactly be traced back, first report of existence and its utility were recorded in Vietnam during eleventh century. Azolla comprised of two words from Greek dictionary, i.e. "Azo" meant drying and "Alloy" meant for killing. Botanically its recognition was created by Lamarck, as the genus "Azolla" in the year 1893, which has again 2 subgenus, namely Rhizosperma and Euazolla, based upon characters of sporocarp (Sood *et al*., 2007). The subgenus "Azolla" has five species and Rhizosperma has three species (Raja *et al*., 2012) as described in the figure 1.

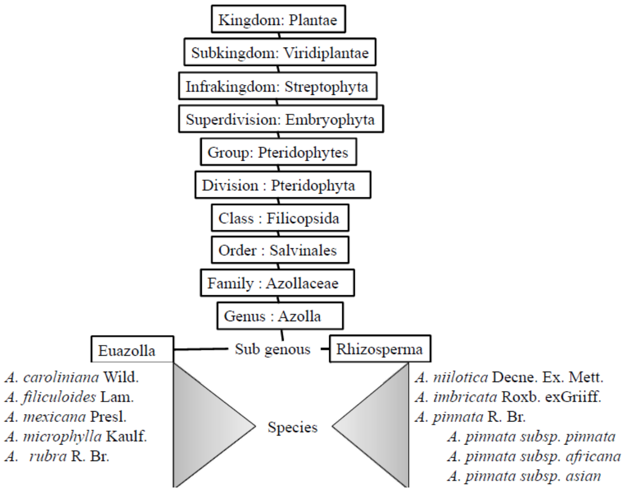

Fig 1. Taxonomic classification of Azolla

Production methodology

Dry matter production of azolla under natural condition is 0.5 t $ha^{-1}day^{-1}$. But the optimum production is 4 t ha^{-1} day^{-1} (agritech.tnau.ac.in). Azolla spp. grows very fast and gets doubled within 5 days when applied in rice field under favourable growing condition. Azolla can be grown in pits lined with polythene sheet in backyard or courtyard with adequate sunlight.

Azolla can also be grown in nursery bed of rice field. Only 10% of nursery area is generally taken for azolla production. Small plots of 2 m × 4 m with bunds of 30 cm height all round the beds are prepared. Beds are also lined with "polythene sheet" to prevent leakage. Water is being flown into the beds and application of azolla is done @ 0.1 to 0.5 kg m^{-2}. After 20 days, azolla is harvested from the beds with keeping the initial amount of azolla for regrowth of this fern. The quantity of azolla harvested is enough for applying in the main field of paddy cultivation. Azolla should be harvested on regular basis. Overcrowding can hamper the quality and productivity. The area used of azolla production can also be used any time for rice cultivation after harvesting of azolla.

Factors affecting the productivity and growth

1. **Water:** Azolla, an aquatic fern cannot grow without water. Stagnant water is required instead free flowing water for its growth and productivity. Depth of the water bodies is also important. Very shallow depth (< 5 cm) of water can inhibit the growth and multiplication of azolla. Generally, 5-10 cm depth under water bodies is maintained for azolla cultivation.
2. **Air Temperature:** 20-30°C is favourable temperature for azolla production. Temperature > 30ºC and very low temperature (< -4ºC) can inhibit the growth of azolla.
3. **Soil pH:** The pH must be within 5.0 to 7.0. At pH 5.0, high light intensity increases the growth of azolla but at pH 6 and 7 low light intensity is suitable.
4. **Nutrients:** 4 kg/ha P_2O_5 substantiate the productivity of azolla in nursery fields of rice while 6-8 kg/ha P_2O_5 is required for rice cultivation. Single super phosphate is generally preferred for P application. Azolla turns reddish when nutrient content in water is less coupled with high light

intensity. The colour also changes from green to red or brown under shady condition during hot summer and cool winter months.

5. **Light intensity:** Light intensity mainly regulates the photosynthetic activity of azolla which ultimately affects its growth and the amount N fixation by azolla. Azolla of thick mat size provides around 40 kg/ha N. The light intensity < 10 Klux can reduce N fixation in Azolla (Costa *et al.*, 2009).
6. **Relative humidity (R.H.):** Lower relative humidity (< 60% R.H.) makes the azolla dry whereas too high relative humidity (> 80% R.H.) is also not good for its growth. 65-75% R.H. is necessary to maintain the optimum production of azolla.
7. **Wind and wave velocity:** In shallow water bodies, high wind velocity has both indirect and direct harmful effect on azolla production. It causes mechanical injury on plant body as direct effect and increases siltation of water bodies as indirect effect. High wave velocity makes the azolla fragmented and killed in many circumstances.
8. **Soil salinity:** Under saline condition elevated nitrate concentration makes the azolla spp. salt tolerant. The salinity tolerance of *A. pinnata* has been found to be higher than that of *A. filiculoides*. Salt concentrations above 10mM NaCl and 40 mM NaCl inhibit the growth of *A. filiculoides*, and *A. pinnata*, respectively.

Nutrient composition

Azolla contains on an average 2.50% N, 0.60% P and 1.70% K. The other minerals are Ca (1.10%), Na (0.90%), Mg (0.50%) etc. It also contains crude protein (around 20.5% of dry matter), crude fibre (around 15.0% of DM), lignin (around 11.5% of DM) and starch (around 4.0% of DM) (FAO, 2015).

Multiple usages of Azolla

A. As an agricultural input

The most commonly reported use of Azolla is as a bio-fertilizer in rice production system. It produces around 300 t green bio-hectare year^{-1} in India which is similar to 800 kg N. Azolla generally fixes 3 times higher atmospheric N than the legumes. It fixes atmospheric N @ 50-60 kg ha^{-1} in soil when applied @10-12 t ha^{-1} as basal in paddy cultivation which ultimately decreases 30-35 kg N ha^{-1} application through chemical fertilizers (Roy *et al.*, 2016). Broadcasting of azolla @ 0.5 t ha^{-1} is done in the main field after 5-7 days of rice transplanting.

B. As animal feed supplement

In agriculture, animal food product plays an important role by meeting the demand of society through supply of meat, milk and egg. The nutritional value is well described leading to its increasing demand. For meeting quality food production, both health and nutrition play a significant role. Livestock farming is also a lucrative business since it is considered as an all weather farming and thus meeting livelihood of many poor small and marginal farmers in India. But the fodder shortage due to unavailability of land and climatic resilience, has necessitated the supply of alterative food sources like Azolla. Azolla, the water fern has been used in animal feed as supplement due to its high nutritive value. Livestock like sheep, goat, cow, buffalo and pig has a good impact on productivity upon feeding Azolla. It also fulfils the animal feed shortage of proteinaceous fodder/ feed due to unfavourable climate. Pigs were mainly fed with *Azolla filiculoides*, partially replacing the protein feed needed for growth and fattening. *Azolla pinnata* is considered as a key source for protein supplementing cow, buffalo, sheep, goat and poultry. Since, protein is being produced "four to five" times and biomass is being produced "four to ten" times by it than that of Hybrid Napier and Lucerne (Lumpkin, 1984).

C. Medicinal use

Indian Ayurveda has been one of the ancient treatment guides across the world by using different plant materials or any part like flower, root, stem, leaves, bark etc. The global health has started depending on herbal medicine for prevention and cure purposes to avoid the adverse effect, toxicity and resistance

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of synthetic drugs. Ayurvedic remedies are nontoxic and contain active pharmacological ingredients. In ancient literature medicinal properties of plants have been used against various health ailments (Himal *et al*., 2008). Medicinal plants are categorised based on presence of bioactive metabolites (secondary) in it, which are helpful in new drug discovery. At preliminary stage, the phytochemical screening isolated the pure active ingredients and further characterized their activity against different microbes (Ganiyat *et al*., 2010). Many authors have worked on antimicrobial effect of the fern on various infective conditions like dental caries. Decay of tooth due to infection is a common anomaly of public health and majority of infections are caused by *Streptococcus mutants*. *Azolla pinnata* has been recorded to pose significant antimicrobial effect (Selvi *et al*., 2017) and antioxidant effect due to flavonoids, phenolic compounds and tannin (Mithraja *et al*., 2011). Also, there are studies in pipeline that are demonstrating use of *Azolla pinnata* as an alternative bio-insecticide. The chemical components from *Azolla pinnata* extracts has shown promising result for developing emulsions against vectors causing dengue. Undoubtedly, azolla can rightly be termed as "green gold mine" for its versatile functions, in the sector of production and health.

Conclusion

To cultivate azolla low input and less time is required. The process involved is so easy that any layman devoid of any expertise can cultivate azolla. As chemical composition reflects high amino acids, minerals and protein it is useful as both substitute and conventional feed for livestock and potential manure during cultivation increasing the soil fertility. It has also versatile uses in human health too and many more properties yet to be discovered.

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Advances in Horticultural Crops

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Abstract

Horticulture is the branch of agriculture and it is the science and art of the development of sustainable crop production, marketing and use of high-value, intensively cultivated food. Horticultural crops arediverse, including:Fruit Science, Vegetable Science, Floriculture (with Landscape plants) and Annual and perennial species. Horticultural crops are major part of food, nutritional, and economic securities in India. The increasing demand of crop production, functional mechanisms of microbial communities are need to explore through advance approaches such as modern molecular approach, Canopy management, Development of GM crops, modern approaches of rootstock breeding, development of tolerant and resistant variety of horticultural plants against different insect, diseases and weather conditions. Good agricultural practices are also very important in horticulture crops production, Horticulture also provides the quality of life, and the beauty, sustainability and rehabilitation of our environment and the human condition.

Keywords: Modern molecular approaches, Resistance varieties, Rootstock breeding, Sustainable crop production,

Introduction

India is the second largest producer of horticultural crop production. Maharashtra state having highest area and production but productivity in Madhya Pradesh in fruits crop. Karnataka state having highest area and production but Kerla state having first in productivity in plantation crops. West Bengal state having highest area and production but Tamil Nadu state having first in productivity in vegetable crops. Rajasthan, Gujrat and Andhra Pradesh having highest area, production and productivity respectively. Horticultural crops are diverse agro-ecological conditions in the Indian agricultural economy. The **horticultural crops having** very higher yield per hectare in comparison to the other field crops. Horticulture is the important branch of agriculture in which horticultural crops are day by day leading in area and production as long as demand such as, mostly fruits and vegetables crops. Horticultural crops are an important source of carbohydrates, proteins, organic acids, vitamins and minerals for human nutrition due to pandemic critical problems, human need to with quality and healthy food for boosting energy in body and tackle this pandemic problem, so we have to introduce recent advance methods/approaches of horticultural crops production like, sustainable crop production, organic crop production, integrated plant protection, genetically modified approach etc.

Advances in Horticultural Crops:

1. Recent in Horticultural Crop Production:

In recent years of horticultural crop production many of approaches and technologies are developed which make advance to horticultural crop production with high quality and potential value. Major reasons are available of unique advantages of plastic materials such as-

- Greater visibility of produces and better light transmissibility

- Superior flexibility and higher resistance against chemicals
- Best in smoothness and help to increase of shelf-life of the produces
- Favorable weight ratio, moisture barrier and gas permeability
- Better property of thermal insulation

Some important advanced in horticultural crops are given below: -

A. Nutraceuticals and Phytochemicals for health boost up:

In today's era, overpopulation is the major problem in the world due to more crisisin food production but also of a number of diseases that are arising significantly but this problem may be destroyed itself by nature. Nutraceuticals are the nutrients which are use as a diet supplements that exhibit a physiological benefit, provide protection against chronic diseases, enhancing health, making medicine by delivering biologically active components of food in a non-food matrix. Fruits and vegetables are major source of nutrients and energy for daily activities of human body of which consumption requirement is day by day increasing. Fruits, vegetables along with spices and nuts provides antioxidants, carbohydrates, vitamins and polyphenols are important to growth and development of human body. Fruits such as apple, berries are a rich source of antioxidants while other such as Banana, Guava are used as laxative and are astringent (Rai et al., 2009; Imam and Akter, 2011).Basically, all-natural products are derivative of plants which contains antioxidants. Bioactive compounds of tropical fruits (Apple, Banana, Mango), vegetables (Tomato, Potato, Broccoli) spices and nuts having many health benefits to cure from many diseases to human body and some vegetable such as cabbage, broccoli and tomato having carcinogenic property which helping in maintaining the blood cholesterol, digestive system etc.

B. Role of Plasto-culture in Horticulture:

Application of plastic in horticulture is one of the most important sources to managing horticultural practices such as nursery management, surface covering, water management, innovative packaging, creation of controlled environment, food grain storage etc. mostly plastic culture is used in water management because of it plays important role in judicious usage of water and it is estimated that application of plastic in micro-irrigation can save the water 50-70% and help in increasing productivity by 30 to 100 per cent with significant saving of water. Although less than 200-gauge film is very dangerous to animal health because of consumption of it.

➢ **Role in Nursery Management:**

Many equipment's are developed for nursery management such as different trays, coco-pits, poly bags, pot-trays, plastic plugs, hanging baskets etc.

➢ **Role in Water management:**

Such as films (ponds film, lining of canal film, reservoirs), Sub-surface drainage equipment, PVC and HDPE pipes used as a water conveyor, Drippers and Sprinklers etc.

➢ **Role in controlled environment:**

Mostly controlled environment structures such as Green house, Shade net houses, Plant Protection nets, Low tunnels etc. are prepared from plastic and these plastic films may differently thickness and size according to structures purposes.

➢ **Role in Surface Covering:**

- **Soil Solarization:**

This is a hydrothermal process of disinfecting from pests to soil by solar power and this method first time described by Katan in 1976. Synthetic mulches play a major role in soil solarization process.

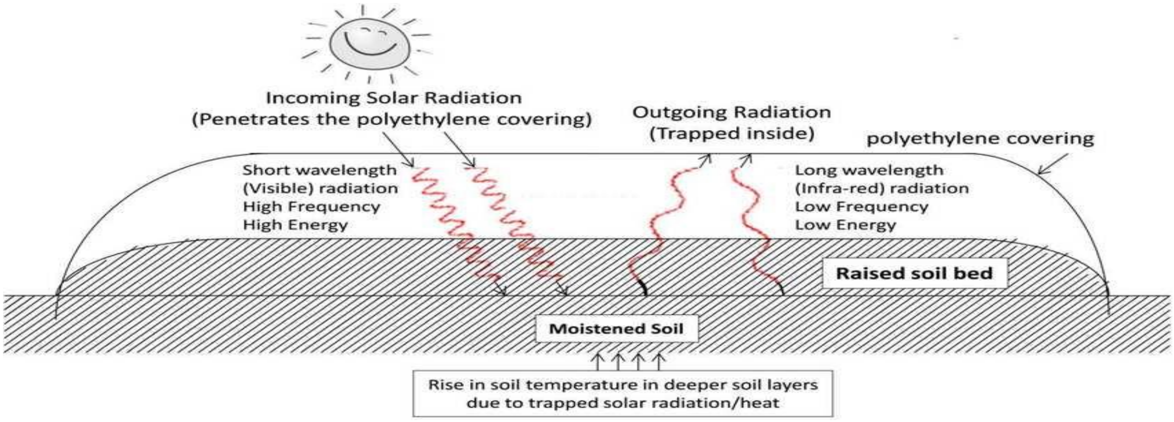

Fig. 1.Rise in soil temperature in deeper soil layer due to trapped solar radiation

Fig. 2.Solar solarization practice in Ginger crop.

- **Plastic Mulching:**

Plastic Mulching is a such type of processing in which soil surface near to plant is covered with application of plastic sheet and this madebenefits like, completely weed reduction, conserve soil moisture, works as a barrier to soil pathogen, maintaining warm temperature in night to quickly seed germination, reduction in plant nutrients, prevention in direct evaporation of moisture,

➢ **Role in Packaging:**

Many innovative packaging equipment are developed such as Modified Atmospheric packaging (MAP), Controlled Atmospheric Packaging (CAP), Plastic crates, Boxes, Leno bags etc.

Future Prospects:

As per estimates by 2025 about a third of India would be under absolute water scarce condition because water availability for irrigation is expected to come down from ~82% in 1997 to ~72% by 20251. In India, more than 330 million people living in 254 districts under the spell of drought. Plastics such as PVC, LDPE, LLDPE, HDPE, PP, PTFEE etc find good applications in the sector in India. Last two decades, the ground water table fallen of high agricultural productivity, at rate on one meter per annum. Water distribution remains a concern as three-fifth of the water is lost in conveyance and about half of through seepage. These and other factors contribute to a wide gap in crop productivity in India which stands at ~40- 60% of world's average while raising serious questions on sustainability of agriculture and eventually on food security. Due to heavy pre and postharvest losses which contribute to low availability of fruits and vegetables. There are substantial benefits of employing the Plasticulture techniques to improve the productivity, saving the water consumption and minimizing the post-harvest wastages. As we are aware, there is a huge unrealized potential of further growth of plastic industry as indicated by the present very low per capita consumption level in the country.

C. Advance Protected Cultivation in Horticulture:

Protected cultivation is mostly used in Fruits, Vegetable, Flower production and medicinal crops in horticulture. But why we use protected cultivation in some most of horticultural crops? Major reason is that, India having very diverse agro-climatic conditions in which all types of crops may cultivated but under open field condition quality and appropriate is lesser than protected conditions because of temperature fluctuation (high/low), rainfall (higher/lesser) and frost occur during growing and developing stage of plants. Due to these environmental calamities reduction in quality and yield along with increase in disease and pest infestation so protected cultivation is the best option to cultivation of horticultural crops. Protective structure gives better response than open field condition with respect to minimum number of days taken to sprouting, highest graft success, graft survival, number of leaves and length of sprouted shoot and other growth parameters and congenial condition inside the structure prevent desiccation of the scion and help for better survival of grafts. (Chander *et. al*., 2016). In fruit crops production, protected cultivation-maintained health of plants along with yield and quality of fruit due to favorable climatic condition under protected condition.

Why Need to Protected Cultivation?

- Under protected cultivation having a best climatic condition in which we can make a new environment and can grow off season fruit production.
- We can improve the quality and yield of fruits, vegetables and flower through create superior environment under greenhouse.
- We can do hardening and acclimatization of tissue culture plants but under open field condition it’s not possible.
- We can proper utilize the field resources like, water resource, field equipment, nutrients and labor.
- It protects crops from frost, rainfall, hails, birds, insect’s pest and diseases.
- It reduced the extra expenditure from weedicide, insecticide etc.
- Crops produced from protected structures helps to meet the market demand.

Major Horticultural Crops for Protected Cultivation:

There are some major Crops are cultivated under protected cultivation such as given in **table 1**.

1.	Major Fruits Crops	Strawberries, Grapes, Citrus, Banana, Mango etc.
2.	Major Vegetable Crops	Tomato, Spinach, Cabbage, Beans, Eggplant, Chili, Okra. Melon, Onion, Turnip, Cucumber, Peppers, Squash etc.
3.	Ornamental Crops	Roses, Carnation, Gerbera, Lilies, Orchids, Chrysanthemum, Anthodium etc.
4.	Other	Nursery Plants, Tobacco Etc.

Principles of protected cultivation:

- The transparent covering material act as a radiation filter.

- Greenhouse temperature coupled with available sunlight helps successful production crops.
- Greenhouse temperature raising due to trapping of solar heat.
- The heat retention capacity enhanced due to lack of air exchange between ambient and greenhouse condition.

Major Protect Structure for Horticultural Crops Cultivation:

a) Polyhouse:

Polyhouse are major structure of protected structure which are made up of transparent, flexible, cheap, and tight covering material and crop can grow in any season of the year under polyhouse structure because humidity and temperature controlled inside polyhouse. These are two types-

- **Natural ventilated polyhouse:** Natural ventilated polyhouse having only good ventilation and fogger system so in this system doesn't having any control system to damage from weather condition, insect pest and diseases attack.
- **Environmental controlled polyhouse:** This type of system is fully environmental controlled which helps to maintained the growing season as well as permits off season crops production of Horticulture by way of controlling humidity, light, CO_2 level and temperature under polyhouse.

b) Green house: A greenhouse is a framed structure covered with a transparent material in which crops can be grown under the conditions and which is large enough to work within it to carry out cultural operations. Average dimensions of structure for fruit crops production is 5-6 m high, covering 1.25 ha. Typical greenhouse structure is made of galvanized posts, 6 m high in the centre and 5.5 m high at the edges.

c) Net house: Net house prepared from high density polyethylene and flamed structure covered with shade net which is inset proof having 40-60 mesh free from viruses. This is helpful to control weather conditions resulting in best output and quality.

d) Glass house: Glasshouse are prepared from glass so need to proper maintenance and having higher air infiltration, better ventilation, and provide the most congenial conditions to growth and development of plants.

e) Lath house: This is made up with the shading object and it protects to tender seedlings and cuttings. Lath house also reduce the loss of moisture from leaf as well as soil surface.

f) Plastic tunnel: Plastic tunnels are small green house like structure which are covering the plants along with the row and are18" high with 30" wide at the base. Basically, these tunnels are available in different sizes according to customer requirement. These are two types- 1. High tunnel 2. Low tunnel

g) Mist chamber: This is very important structure of protect structures because this chamber provides the mist to rooting of cutting of difficult to roots plants during day time which increase the relative humidity surrounding the leaf surface. This structure also under self-controlled and human controlled.

h) Cold frame: Cold frame basically used in home garden and vegetable farming and they create the microclimate that provide different degree of air and soil temperature and shelter from wind. These structures mostly used in seedling growing that are transplanted under open field condition. A cold frame functions as s miniature greenhouse which increase growing season.

D. Application of Natural Farming in Horticultural Crop Production:

Natural farming approach was postulated by Masanobu Fukuoka in Japan andits refers to a way of cultivation of crops in which do-nothing and it's related to organic farming, natural biodiversity or sustainable crop production and it's the novel concept of farming. In this system the laws of nature are applied to horticultural practices. Conventional farming also related with natural farming but due to over application of chemical fertilizers, insecticides and pesticides that not only destroy the soil micro flora and fauna but also increase in the cost of production. Natural farming is decreasing the cost of production, enhancing soil fertility and nurtures the soil naturally. Soil is a dynamic entity and cannot be replaced quickly. Soil commands a major effect on plant growth besides it acts as a facilitator in organic matter decomposition, nutrient mobilization and decomposition of xenobiotics (Doran *et al.*, 2000). On a whole, soil quality is the most important factor in quality and sustainable crop production. Due to many adverse effects of modern farming system in horticultural crop production have necessitated the need to conserve and improve soil health fertility and also should be develop new innovative technologies of farming system like, organic farming, natural farming, zero tillage, and controlled traffic farming because these farming systems can enhance soil micro flora and fauna, increase soil organic matter, reduce soil compaction and improve soil aeration.

Essential soil microbial population greatly impact on farming systems and these are the important living organism which influence the health of soil and play a vital role in enhancing soil fertility like decomposition of organic materials, soil carbon sequestration, protection against various pathogens and degradation of xenobiotics.

Zero budget natural farming: This is the such type chemical free farming system in which same rules are applied as like organic farming and sustainable crop production which has been popularized in many states of India, mostly in southern India like Karnataka where it was firstly evolved. ZBNF gained prominence when Finance Minister Nirmala Sitharaman mention this topic in her budget in 2019 and it was very important farming system in which farmer can double his income. There are various components of ZBNF

- Jivamrita or Jeevamrutha
- Bijamrita or Beejamrutha
- Whapasa/Moisture
- Acchadan-Mulching etc.

Benefits of Natural Farming System:

The using of natural farming system there are many benefits-

- Improving the soil health like, reduce soil erosion by using cover cropping and grrn manuring, soil structure, soil texture, soil fertility, soil flora and fauna, increase beneficial soil micro-organism and enhance organic material.
- Reduce harmful chemical content in plant and human food chain like fertilizers, insecticides and pesticides etc.
- Improving food quality, production and productivity.
- Applying natural farming system, sustainability can be increased.
- Reduce the cost of crop production.
- Use of all farm resources and equipment.
- Ground cover is maintained by maintaining the weeds, herbaceous legumes.
- Poultry component forms vital part in the orchards and in rice fields, ducks and carp coexist.

Conclusion

India is the second largest producer of horticultural crops and it is major source of nutraceutical and functional food and this shows that horticulture can play a major role in the growth of functional food market of India. We need to produce quality food by using different natural approach or techniques like, organic farming, or zero budget natural farming (natural farming), sustainable crop production, protected cultivation etc.Organic or natural farming is the way forward in this direction that can restore the soil fertility by managing the inherent natural vigor of the parent soil without compromising on the production. These methods should be conveyed and should be apply to farmers and aware from disadvantages of chemical fertilizers. Protected cultivation one more very important aspect of quality crop production but it may be very expensive to small farmers to install the protected cultivation.

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Implication of Biotechnology Services in Insect Pest Management

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Abstract

Insect pest causes severe damage to the food production and known to spoil around more than 60 per cent of agriculture production either in field or during storage. The application of Biotechnology in the field of insect pest management proved to be efficient and target specific. The biotechnology uses different tools and techniques like genetic engineering, molecular biology and plant biotechnology. In the chapter, we have discussed extensively practiced methods in insect pest control comprising of alternative genes as biological weapons, transgenic crops, gene pyramiding and Host plant resistant. The alien genes in the transgenic plant helped in developing resistant varieties against particular pest e.g. Bt cotton against bollworm complex. Some researchers have found application of genetic engineering in improving the bio agent's potentials including microbial and macrobial natural enemies of insect pests. Therefore, application of these biotechnology services can bring new dimension in reducing the pest infestation without causing much impairment to the environment.

Key words: Genetic improvement, IPM, insect pest and transgenic crops.

Introduction

Biotechnology helps in providing new measures which are eco-friendly and efficient in reducing the insect pest load in agro-ecosystem. Insect pest causes wide range of damage to the crops in form of herbivore including defoliators, borers, sap sucker and miners. Insect pest also act as vectors for multitude of micro-organisms and viruses; hence reducing the yield indirectly. Out of total insect species recorded so far around 67,000 species of insects are known to have detrimental effect on the plantations in various forms and intensities. They share the biggest part in total losses occurred to the food production by causing 20-30 per cent loss globally. The severity of pest attack and their management depends on various factors *viz.*, agronomic practices, crop density, cropping pattern etc. Thus, a rational strategy should to develop which is broader in nature and can be used in various set-ups.

Since the green revolution more emphasis has been given to the chemical to bring the immediate and cost effective results in reducing the insect pest infestation,in spite of the fact that it adversely affect the natural ecosystem. The unselective and blanket use of these synthetic chemicals brought down the biodiversity in nature. The broad application of insecticide had negatively impact the human health and non-targeted organisms including beneficial insects, natural enemies and plants feeders. So, for this vary reasons a shift in the approach towards insect pest management is indispensable.

The biotechnology services in reducing the insect pest load can be explain as controlled and premeditated manipulation of biological system to accomplish efficient insect pest control (Gupta and Jindal, 2014). Organism's inherent multitude of capabilities and by exploiting apposite trait and specific living entity, efficient management of these harmful insects will come handy. In conventional techniques more emphasis was given on single gene base resistant being highly effective and compatible with other breeding procedures. Conversely, these resistant can be easily nullify by genotype development in insect pests. The new and recent application of Recombinant technologies has effectively augmented the traditional approaches used by incorporating genes from varied sources resulting in improved microbial species, plant varieties and bio agents.

In recent times, use of transgenic approaches is commonly follow in all the dimensions of agriculture, which aiming at significantly higher productivity with lower level of chemical application. The first transgenic plant was developed in case of tabacco having cowpea trypsin inhibitor gene against

Heliothis (Hilder *et al.*, 1987). However, this gene failed to provide insect protection in transgenic rice (Xu *et al*., 1996) and potato (Bell *et al*., 2001). The incorporation of Cry gene of *Bacillus* bacteria in various crops against lepidopteran pests like cotton, maize etc. The application of such gene technologies have surely made the environment more suitable and decreased the concentration of harmful deposits. Nevertheless, the journey to rely on these techniques is a long way and as a fundamental role it requires new and sustainable approaches in modern day farming.

Molecular techniques for insect pest management

1. Wide hybridization: The techniques concerns the gene transfer from one species to another species by breeding. This is a conventional approach in which resistant genes are being transferred from various sources.

Example- The transfer of WBPH gene from *O. officinalis* to *O. sativa* against white backed plant hopper

2. Somaclonal variability: The technique uses the variations derived in vitro by various agents and exploiting these variations to develop resistant varirties.

Example- The sorghum variety developed by somaclonal variation against *Spodoptera litura*,

Sugarcane variety developed by electroporation which is resistant against borers.

3. Transgenic plants: These plants are developed by incorporating the one or more additional genes into the plant genome by genetic engineering. These additional genes are responsible for the resistance against insect pests. There are multitude of gene which have been used to develop transgenic plants such as-

a. Bt endotoxin from *Bacillus thuringiensis*

b. Protease inhibitors

c. Amylase inhibitors

d. Lectins

e. Enzymes

a. *Bt* endotoxin gene: *Bacillus thuringiensis*, is soil borne and gram positive bacteria which produces a delta endotoxin. The toxin is crystalline in nature and works as a stomach poison. It is used against lepidopteran insects, as there gut pH is alkaline in nature. The gene which mediate the production of toxin is isolated from the bacteria and cloned into plants such as cotton, maize etc.

Plants	Used against
Cotton	Bollworm complex
Rice	Leaf folder and stem borer
Maize	European corn borer
Tobacco and Tomato	Cut worms
Potato	Colarado beetle

b. Protease inhibitors: Protease is an enzyme responsible for the protein digestion in insect gut. Those substance which hinder the enzymatic process of protease are termed as protease inhibitors (PI). The gene responsible for PI have been isolated from various plants and cloned to different plants, to produce transgenic plants.

Example: Transgenic plants of apple, tobacco, rice developed from incorporation of Cowpea trypsin inhibitor (*CpTI*) isolated from cowpea are resistant against *Heliothis virescens.*

c. Amylase inhibitor gene: The enzyme amylase helps in the digestion of carbohydrates and Amylase inhibitors (AI) hinders the process. The cloning of AI gene is done to produce transgenic plants, which can hinder the digestion process in insects.

Example: Transgenic tobacco and tomato having AI gene against lepidopteran pests

d. Lectins gene: The lectins are protein in nature and prevents nutrient uptake from mid gut. When insect ingest lectins, it bind to carbohydrates (chitin) in peritrophic membrane of gut and cause the adverse effect on insect.

Example- Pea lectin gene used in tobacco against *Heliothis spp.*

e. Enzyme genes: There are several gene which can be cloned to developed transgenic plants and help in reducing the insect pest infestation.

Example- chitinase enzyme and cholesterol oxidase gene shows insecticidal properties

Pyramiding genes

The process of stacking the multitude of genes in single genotype to combine the various traits through rDNA technology and conventional breeding and sometimes termed as second generation of genetically engineered (GE) plants.

1. Transgenic tobacco developed by cloning *CpTi* and pea lectin gene together

2. Transgenic potato having lectin and chitinase gene

Potential of Biotecnology in IPM

1. There is slow resistant development in case of transgenic plants.
2. The gene expression in all the plant parts resulting in broader effectiveness.
3. There is no use of additional insecticide applications.
4. It can reduce the tedious and time consuming monitoring.
5. These techniques are economic and efficient in overall application.
6. There is no environmental injuries and use is completely safe.
7. There is no pollution caused by any mean in the ecosystem.
8. It is safer to non-targeted organisms.
9. These approaches are better than synthetic chemicals.
10. There are no residual problems in environment.

Constraints of Biotecnology in IPM

1. The development of new transgenic is time consuming process.
2. There are limited tools for inter specific gene transfer.
3. The biotechnology application for new transgenic is costly.
4. In some cases, adverse effects of consuming GM crops has been previously reported.
5. The farmers are not so ready for useyet.

Conclusion

As mentioned earlier at various platforms and literature through studies conducted that insect pests are one of the major constraint in agriculture productions and they causes significant losses. Farmers use number of hazardous chemicals in great quantity compared to bio-pesticides which are safer in use. These causes detrimental effect on natural enemies (parasitoids, predators and pathogens), non-targeted organisms (humans, beneficial insects and herbivores) and environmental pollution. The indiscriminate use of synthetic chemicals led to degradation of biodiversity and adversely affect the ecosystem.For that reason, a shift towards new approached like transgenic crops is needed.

However, search for new genes having different action could develop supplemented or additional resistance in transgenic crops. Thus future prospective of biotechnological services for crop protection against insect pests can bring new dimensions in agriculture.

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Advancement in Crop Improvement

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Abstract:

Crop improvement is a necessary and urgent need of today in plant breeding sector. Due to tremendous population growth and sudden depletion of fossil fuels, there is more and more demand for plant based new products.Some modern developmental technologies will contribute to a great extent of crop improvement. Genetic engineering techniques with mutagenic properties possessing physical, chemical and biological factors helps in studying the particular genes and identification of molecular mechanisms in improving the crops.

Keywords: Genetic engineering, Gene transfer, Hybridization, Mutagenesis.

Introduction:

Crop improvement, is actually, the engineering of plants for the beneficial of humanity and society, but it's as old as agriculture. Before thousand years ago, the old age people create a sudden transit from hunting and foraging to cultivate the necessary plants and crops. There is strong evidence that the agricultural sectors are quietly degra ding, exacerbated due to the loss of biodiversity and of increasing unknown uncertainties of climatic change.Due to this switch on, there is more development of new and modern process, for the improvement of plants, in which human beings depend for food, feed and fiber. Crop improvement is a necessary and urgent need of today in plant breeding sector. Due to tremendous population growth and sudden depletion of fossil fuels, there is more and more demand for plant based new products.

The 20th century insights a tremendous increase in crop yields due to crop improvement. There are so many reasons for the continuous improvements in crop yield. Mainly, there is hike development due to modern plant breeding technologies and introduction of agronomical interventions. Because of these improvements, people were strengthened with safer food that lowers malnutrition (Ali *et al*., 2015).

There is a great challenging effort in this century, faced by the human populationfor the intense need of food security. It was estimated that, in 2050, there will be increased population ofabout10 billion. So, compulsory, there should be increase in food production of about 70–100% (FAOSTAT, 2016). But, there are so many demerits to overcome crop development; they are change in climatic condition, lack of large lands, both biotic and abiotic stresses, low agricultural facilities etc.

Some modern developmental technologies will contribute to a great extent of crop improvement. Genetic engineering techniques with mutagenic properties possessing physical, chemical and biological factors helps in studying the particular genes and identification of molecular mechanisms in improving the crops. (Ma et al., 2016). Mostly, the conventional breeding is most widely used in crop improvement. But, this approach is labor intensive and also ensures many long years to progress from the starting stages of screening the phenotypes and genotypes to the first crosses and particularly into commercial varieties.There is mainly two techniques applicable for the improvement of crops - selection and breeding.

The selection technique will make use of genetic variation inherent in plants. In earlier times, the farmers select the plants with potential traits, with largest fruit to harvest. The selection of plants and seeds will be applicable for establishing the next year's crop. The natural selection, enhance the survival of species. By selecting the suitable and choicest plants, the farmers influence for cross-pollination. Through selection and isolation, the gene pool was modified for each and every crop.

In breeding technique, the farmers will select two plants and further crossed them, in order to produce offspring with desired traits of both parents. The early plant breeders do not have idea on genetic transmission of traits and unable to predict about the particular cross. But, the valuable traits arise must be maintained in the specified population.

Crop improvement by Genetic Engineering:

Genetic engineering methodologies involve transfer of genes between unrelated species of plants that signifies a prominent role in crop improvement. Due to transfer of different genes, various important traits like disease resistance towards pests, insects, nematodes, parasites, drought and stress resistance, and bacterial, viral and fungal disease resistance has been transferred to crop varieties. Recombinant DNA methods are widely accepted to transform the genetic information from one variety to other. Genetic engineering has pave way to transfer the genes among dissimilar genera or species (Ali *et al.*, 2011a; Ali *et al.*, 2011b). Genetic engineering is an exceptional way of extending genetic base in breeding, when compared to that of conventional breeding (Ahmad *et al*., 2015a).Also, it avoids the problem of linkage drag, which is most commonly associated with the conventional breeding. This method is acceptable as it is very effective and less time consuming.

Nowadays, genetic engineering techniques were widely used to improve agriculture with advancement in crop improvement. Mostly, the transgene integration into the specific host gene becomes sometimes specific. Research has been carried out in many plants to improve the agricultural sectors and feed the humanity of the whole world. In maize, research work includes the induction of nitrogen usage, tolerance of stress and drought, long storage of corns, germplasm storage, insect and pest resistant strategies. The continuous research work implemented good varieties, more yield, commercial applications and further sustainability. Soyabean has been considered for its improvement with insect, pest and nematode resistance. Also, there is more improvement in germplasm storage that makes future enrichment to the society. Many small grains were considered for research to enhance the nutrition, good yield and many important agronomical traits(Ahmad *et al.*, 2015b).

In *Fusarium*sps., the specific genes that possess antifungal and antitoxin properties were induced to increase the resistance characters. The Ribosome-Inactivating Protein (RIP) gene *b-32* from maize was integrated into the wheat-inhibited *F. culmorum*, clearly shows more percentage of reduction in Fusarium Head Blight (FHB) in the transgenic plants. Similarly, the wheat PR protein having ribonuclease activity but inhibits *F. culmorum* was also done. The virulent nature of fungus was reduced due to the integration of genes that inactivate toxins into cereals.

Till today, there is development of many genetic engineered crops and have been commercialized with improved production efficiency, increased market focus, and enhanced environmental conservation. Some example of crops are long post-harvest storage tomatoes, insect resistant cotton, insect resistant maize, virus resistant potato, herbicide resistant soybean etc. (Ahmad *et al*., 2015b).

Hybridization Techniques In Gene transfer:

Intraspecific Gene Transfer

During 19th century, the plant breeding strategies began with many new discoveries, in which the plant traits are inherited. Plant breeding was carried out by selecting the plants with gene of interest and manipulating or modulating them with the process of cross fertilization. Desired and improved variety of plant is formed, during the back-cross with wild variety (Khan *et al.*, 2015).

Interspecific Gene Transfer

During 20th century, plant breeders start using inter-specific hybridization technique for gene transfer from a non-cultivated plant species to convertible crop species. *Avina sativa* (oat) *and Beta* *vulgaris* (sugar beet) has been transformed with interspecific gene transfer with respect to high yield of 25-30%, and stimulating sugar beet nematode resistance in the plant.

Non-Sexual Gene Transfer

The cells, tissues and organs of plant can be cultured in vitro; hence transfer of genes between plants is possible by non-sexual methods. Non-sexual gene transfer methods possess the ability to produce fully differentiated plants from non-sexual organs and tissues. As a regeneration material, stems, leaves and undifferentiated clumps of cells in culture can be used as starting material for regeneration. Single somatic cells can also be used in some experimental studies (Butt *et al.*, 2015).

Cell and Protoplast Fusion

In 1960s, cell and protoplast fusion become popular to prepare single plant cells without their cell walls. Fusion could be induced by electroporation, chemicals and liposomes. Callus induction is the initiative step of this method. In some plants, whole plant can be regenerated from callus tissue. Sexually incompatible species possess choromsomes combined by the use of cell fusion method. This method has some limitations for commercial agricultural uses.

Direct Gene transfer–Manipulation of DNA

Different genes from plant, animal, bacteria and virus were selected and injected into the crops. Tissue specificity, duration and expression of genecan be done by the alteration of DNA (Qamar *et al.*, 2015).

***Agrobacterium-Mediated* Gene Transfer**

Agrobacterium tumefaciensis is the pathogenic bacterium that can transfer some part of its genetic material into other plants through transformation. The T-DNA genes encoded in Ti plasmid causes "crown gall" disease. This bacterium is genetically modified for transferring the gene of interest into plants. The *Agrobacterium* approach is widely accepted because of minimum cost and equipment (Aaliya *et al.*, 2016). Efficient vectors are designed with virulent genes for increasing the level of expression of virulence genes.

This is a best method for treating insect and disease resistance varieties. This is adopted for non-sexual gene transfer, in which crops act as candidates for agriculture. By recombinant DNA technique many plant and bacterial genes that encodes enzymes has been engineered to produce a safe and tolerant herbicide. Usually, the bacterial genes are engineered to suppress the enzyme activity towards herbicide and then transferring into the plant. The genes from *Bacillus thuringienesis* has been engineered and transfer to plants for acting as a insecticide (Shahid *et al.*, 2016).

Biolistic Transformation

This method uses the direct delivery of micro projectiles of tungsten or gold coated with DNA and targeting into the target cells with acceleration force. Acceleration was given by electric charge, carbon-di-oxide, gun powder and gases and further DNA can be directly passed into specific cells and tissue.

Microinjection

The microinjection technique is a powerful direct physical approach, for introducing substances under microscope into the defined cells without damage. Microinjection was accepted for the plant crops, because the plant can be produced through transformed cells.

Mutagenesis

Mutagenesis can be referred as the development of specific alterations by breeding program for the improvement of crop. Mutational breeding helps in raising plant varieties with desired traits that are applicable towards food crops and horticulture. More than 2,000 plant varieties with mutation induction have been cultivated commercially (Rizwan and Akhtar, 2015). Mutations are the source of changes in the genome either permanent or temporary. Spontaneous mutation occurs with 10^{-6} frequencies (Wessler, 2006). Induced mutation occurs due to UV radiation, X rays α- particles and β particles. Induced mutations show specific importance towards conservation and preservation parameters and can increase the genetic diversity of crops (Watson, 1992). According to the FAO / IAEA Mutant Varieties Database, there are 1,357crop species considered as mutant cultivars, 490 as mutant ornamental varieties and decorative plants (1,284 entries) with seed propagation, vegetative propagation crops with nearly 73 varieties. Among that, 869 varieties of cereals, 333 varieties of rice, 261 varieties of barley, 147 varieties of bread wheat, 49 varieties of maize, 25 varieties of durum wheat and many other varieties.

RNA Interference

RNA interference is mostly used to increase the crop yield, biotic and abiotic stress resistance and also enriched the nutrients in fruits.

RNAi imparts silencing of gene at the post transcription modification level (Kamthan *et al.*, 2015). RNA interference are mostly endogenous microRNA and exogenous like small interfering RNA (SiRNA). It occurs due to breakdown of dsRNA by the ribonuclease enzyme DICER or DICER like enzymes (DCL). RNA induced silencing complex (RISC) is activated by the incorporation of single stranded RNAs. RISC has protein, that imparts ribo nuclease activity, which ensures degradation of mRNA and RNA binding regions. In *Arabidopsis thaliana*, RISC has argonaut protein, for performing the process of slicing (Thorpe, 2007). The modulated and activated RISC- RNA will specifically binds to the specific sequence by base pairing and hence degradation occurs..

Biotic Resistance

Transgenic potato resistant against Spindle Tuber Viroid (PSTVd) infection was produced with dsRNA against PSTVd sequences. RNAi has more effectiveness against DNA viruses. Resistance was developed in rice with specific protein gene and CP gene from *Rice Stripe Virus.* Crown gall disease was managed by using RNAi against tumor formation gene in *Arabidopsis thaliana*. In resistance pattern against fungal diseases fatty acid genes were targeted. The suppression of gene *OsSSI2* in rice result into resistance to blast fungus *Magnaporthe grisea* and leaf blight bacterium *Xanthomonas oryzae* (Jiang *et al*., 2009).

Abiotic Stress

Activated C-kinase 1 receptor gene was targeted in transgenic rice plants to enhance drought tolerance. In miRNAs, miR393 provoke the expression for stress tolerance. Osa-miR319a as a transgene, when over expressed in rice plant, enhance the tolerance against drought and salt stresses (Mohamed *et al.*, 2015).

Importance of Crop Improvement:

High yield: Increases the crop productivity inorder to ensure more yielding varieties.

Good Quality: Increase and improve the quality of many crops like grains, pulses, oil crops etc.

High Resistance: High resistance was adopted in biotic and abiotic factors. Biotic factors includes insect resistance, parasite resistance, disease tolerance, etc. and abiotic factors like stress resistance, drought tolerance, salinity, etc.

Maturity Level: Adopts to reduce and enhance uniform maturity level. The short maturity period ensure the farmers in multiple rounds of cultivation with low level of expenditure in a short period. Uniform maturity of crops helps in easy harvesting with more profit.

Adaptation: Initiate in developing tolerance and making crops more adaptable to extreme conditions like high salinity soil.

Desirable Agronomic Qualities: Helps in developing desired agronomic characters like dwarfness in cereals that may reduce the consumption of nutrients but does not affect the crop yield.

Conclusion

Recently improved genetic engineering techniques ensure a great boon to the society by producing new traits of crops. These modified crops are more useful for the farmers and the consumers. The production of improved food, feed and new varieties of crops and plants paves a new way for the users in the world market. Research should be encouraged among the young minds to modify many new crops with accountable benefits to the society.

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