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Agroforestry and Crop Production

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Abstract

Agroforestry frameworks in type of established hedgerows have a drawn out custom as land-use frameworks. Planting of trees and bushes into farming frameworks have efficient advantages as they give lumber and fuel wood and different items. It is all around perceived, that these shelterbelts upgrade biodiversity and affect the environment working. In those contemporary agroforestry frameworks, choice of tree species and their administration are all the more monetarily arranged towards a streamlining biomass creation, use and reap, particularly in short-revolution frameworks for Bio-energy. Hedgerows on microclimate and crop creation in an agroforestry framework. Numerous smallholders produce almost 90% of the world's palm oil, have embraced intercropping in youthful oil palm estates. Oil palm yield from intercropping or domesticated animals mix, palm oil and cocoa creation from agronomic and financial viewpoints. Monocultures of ware crop akin to oil palm, espresso and cocoa aren't habitually referenced in this unique circumstance, regardless of agroforestry's capacity to work on such harvests' maintainability and at times, yields. Shifting direction starting mono-cropping to different multi-crop ranches have a couple of bottleneck, yet analysts be attempting headed for assist ranchers in the midst of agroforestry at the same time as the mix of timbered perennials resembling vegetation and bushes amid yearly harvests is notorious and setting positive nut tree market.

Keywords: Agroforestry system, Alley cropping, Rubber, Nut, Oil Palm.

Introduction

Indian horticulture faces assorted difficulties and limitations because of developing segment pressure, expanding food, feed and grub needs, normal asset debasement and environmental change (Dhyani *et.al.* 2013) along these lines an administration framework should be contrived that is fit for delivering food from minor agrarian land and is likewise equipped for keeping up with and working on nature of creating climate (Dobriyal, 2014). Agroforestry is anything but another idea in spite of the fact that it has been drilled in India for millennia (Puri and Nair, 2004) on agrarian terrains for various purposes like for food, grain, natural product, kindling, restorative trees, bio-manure, Non-lumber woodland items (NTFP), cover and so on (FSI, 2013). In short-pivot rear entryway editing agroforestry frameworks (SRACS), segments of quickly developing trees substitute with portions of yields or meadow. In mild environment zones, the tree part in such frameworks is overwhelmed by poplars planted for energy purposes while normal yearly grain crops for calm farming, like wheat, maize or soybean, rule the yield part (Wolz and DeLucia, 2018). The previously mentioned division of the editing framework offers many benefits to the rancher, the agroecosystem, the environment through carbon sequestration and replacement of petroleum derivatives and furthermore the general public (IPCC, 2019). By offering different natural administrations, for example, disintegration control and decrease in soil vanishing, trees on cropland can be a transformation measure to current or future stressors in view of environmental change. (Sheppard *et al*., 2020).

Agroforestry Grow Forest crop Production

Backwoods are indispensable to our economy, too. Trees are important for woods biological systems that assume a basic part in our vocations, giving natural, financial and social qualities. Agroforestry rehearses support horticultural creation and assist with further developing stream value and atmosphere quality, earth wellbeing, untamed life natural surroundings. These functioning trees can likewise develop yarn, foodstuff and energy. The U.S. Registration Bureau prophetic a populace flood toward nine billion by 2044, woodlands and Agroforestry container assist with satisfying developing needs designed for foodstuff, place of safety, medication, and diversion. Landowner are progressively utilizing Agroforestry near attach trees just before their scene work on strength of their woods. Agroforestry permits landowner toward deliberately develop vegetation and bushes in the midst of

crops as well as mammal cultivating frameworks, which makes a further different horticultural activity furthermore assists with boosting their benefits.

Fig:1 Silvopasture

1. Silvopasture is a significant framework to assist landowners with expanding their activities and their pay. This framework joins trees with a domesticated animals activity by overseeing search, domesticated animals, and trees on a similar section of land. Silvopasture frameworks give shade and asylum to animals, while helping scavenge creation. This mix can likewise get additional pay from wood items, Christmas trees, or amusement. Holding creatures under intercropped frameworks with numerous perpetual or potentially yearly yields is a type of agroforestry called silvopasture, which regularly additionally incorporates goats, ducks and chickens. Oil palm manor cultivators can save 25-half of weeding expenses and increment the creation of new palm organic product yield by 16.7% under such a framework, research has shown. Silvopasture has likewise been found to relieve backwoods fires in Spain, with sheep and dairy cattle eating the brush that frequently lights during dry occasions. Other natural advantages of intercropping that have been distinguished are the increment of carbon stock, the improvement of disintegration control, and the diminishing of nitrogen filtering.

Fig: 2 Alley cropping

2. Alley cropping develops crop connecting developing vegetation, call rear entryways, in favor of yearly pay. Rsembling silvopasture, this framework additionally expands activities by making both yearly and long haul revenue sources. It likewise can secure yields, further develop water quality, work on supplement usage, and store carbon.

Fig: 3 Windbreaks

3. Windbreaks be plantings of particular various lines of vegetation, bushes and both with the purpose of safe house crops and soil, creatures, home and individuals commencing wind, snow, residue, scents. These frameworks accumulate liveliness and canister reduces home warming expenses. Windbreak likewise assist with mesh large gains in carbon stockpiling, further develop pay by expanding crop yields, and shield domesticated animals from warmth and cold pressure.

Fig: 4 Forest farming

4. Forest farming be developing along with ensuring towering-esteem forte yields underneath the backwoods overhang, which be acclimated in the direction of the right gloominess echelon the harvests like. This is finished with diminishing a current backwoods to abscond the greatest covering trees meant for preceded with lumber creation while making the superlative environment designed for understory harvest. Non-wood woodland items developed utilizing backwoods cultivating techniques don't simply give an extra kind of revenue; they additionally assist with saving the class during our scenes.

Fig: 5 Riparian forest buffers

1. **Riparian forest buffers** are regular set up trees, bushes, and grasses arranged close to waterways, streams and lakes that help improve and secure amphibian assets by sifting ranch spillover and forestalling disintegration. Cradle regions can likewise uphold natural life territory, produce crops, further develop water quality, and lessen flood harm.

Oil palms crops Cultuvation under Agroforestry systems:

The common views shared by oil palm farmers and companies is that the crop needs to be cultivated as a monoculture row upon row of nothing but oil palms in order to maximize yields. This is due to the belief that other species growing among the oil palms will compete for light and nutrients. Oil palm yield from intercropping and livestock integration, palm oil and cocoa production from agronomic and socioeconomic perspectives. It found no downbeat things taking place the increase of oil palms intercrops inside the original four to five years of life of the plantations. Many smallholders produce nearly 90% of the world's palm oil, have adopted intercropping in young oil palm plantations. This leaves wide "avenues" for growing other crops between the double rows. The cultivation of different combination of lofty-value crops, such while cacao, coffee, vanilla, fruit, timber trees with smallest off-putting impact on oil palm yields. Growing multiple kinds of annual and perennial crops together in a system mimicking a forest like this is called agroforestry, an agricultural technique found all over the world, with well-known examples like shade-grown coffee. This in turn can improve the livelihoods of the farmers, as they become less dependent on a single crop, in this case oil palm, and less prone to the fluctuating price of the commodity. Intercropping also saves land, because when alternative cash crops like pineapples can be planted between oil palm trees, forests surrounding the plantations don't need to be cleared.

Agroforestry systems under rubber cultivation of the production:

Elastic manors have been a super verifiable reason for tropical deforestation, and are by and large answerable for a scope of ecological and social ills. Be that as it may, elastic filled in agroforestry frameworks in blend with foods grown from the ground trees, valuable bushes, meds, and spices is shown by another report to expand environment administrations and biodiversity, while sequestering carbon and differentiating ranchers' wages. Agroforestry is

the most gainful farming method from an environment and biodiversity viewpoint: developing food, fuel, fiber, medications and more under the front of, or neighboring, woody perennials like trees and bushes bodes well for maintainability. Monocultures of item crops like oil palm, espresso, and cocoa – isn't regularly referenced in this unique situation, regardless of agroforestry's capacity to work on such harvests' maintainability and at times, yields. Presently elastic can be added to the rundown of product crops which can get a reasonable makeover through agroforestry without experiencing a misfortune in yields. "Elastic agroforestry–plausibility at scale," of the natural and social advantages of elastic agroforestry and give definite suggestions to enormous scope execution across Asia, Africa, and Latin America. Agroforestry yields are like those in elastic monocultures – particularly if a similar establishing thickness is kept up with, and high-yielding establishing material is utilized. Elastic yields in Brazil even expanded contrasted with monocultures when elastic was intercropped with beans. The results are fossil fuel byproducts, diminished carbon sequestration limit, plant and creature biodiversity misfortune, and a decrease in downstream environment administrations, for example, clean drinking water, biocontrol or fertilization. Besides, monocultures increment the danger of soil disintegration, environment effects and nuisance episodes comparative with enhanced or blended frameworks.

Fig: 6 Monoculture rubber plantation

Intercropping auxiliary plants between lines of elastic trees, regardless of whether tea, cinnamon, ginger or nitrogen-fixing bushes, builds the measure of water getting into the dirt, and lessens soil disintegration. On the jobs side, there are models from Laos, Liberia, Thailand, Sri Lanka and Nigeria where intercropping with additional food crops, or agroforestry with other money crops, further develops food security and pay. Agroforestry frameworks, from wilderness like frameworks that yield next to no elastic, to exceptionally straightforward and 'clean' intercropping frameworks. There are likewise benefits for understudied parts of biodiversity, for example, soil full scale fauna and organisms. Elastic agroforestry has been utilized to portray broadly various frameworks, from wilderness elastic with little administration to creation driven, basic between plantings of wood species (like teak [Tectona grandis] and mahogany [Swietenia mahagoni]) with clonal elastic trees, to a more intricate blend of clonal elastic with at least two annuals, lasting bushes and trees. Present moment intercropping of light-requesting crops with youthful elastic trees is a typical practice, for example maize, pineapple, and banana. Agroforestry decreases the requirement for weeding in view of expanded plant cover, and leguminous cover yields and fertilizer lessen the requirement for preparation.

Agroforestry farming Nuts crops production:

Monocultures of corn and soybeans cover 75% of the U.S. Midwest, inciting soil deterioration, water pollution, and immense ozone hurting substance radiations. Ceaseless yields possibly accommodating in agroforestry—where annuals and perennials are become together for normal benefit—consolidate chestnuts, blueberries, pawpaws and

persimmons. Various farmers here are monoculture-focused, setting up corn or soybeans. These harvests are yearly plants; each year, fields are prepared and planted, using a great deal of energy, manures, and pesticides. Excess agrochemicals spill from field to stream, dirtying streams and killing valuable frightening little creatures, while consistent wrinkling surges soil breaking down. Trees settle soils while holding and filtering water, enable biodiversity, which can reduce the prerequisite for manures and pesticides, and temper the effects of an advancing climate. Changing course from monocropping to arranged multicrop farms has several bottlenecks, yet experts are endeavoring to help farmers with agroforestry — as the blend of woody perennials like trees and brambles with yearly collects is known — and setting up nut tree markets. There are various other nearby species — dim walnut, pecan, elderberry, aronia, pawpaw, persimmon — that we haven't totally combined into our food structure.

Pineapple crops cultivation Agroforestry system:

Pineapple-based agroforestry, generally rehearsed by the ethnic "Hmar" clan in southern Assam, can be a maintainable option to jhum development for North East India. Jhum development consumption in soil ripeness, extreme soil disintegration, and low agronomic efficiency. North East India and numerous south Asian nations are moving to agroforestry and high-esteem trimming frameworks from conventional jhum rehearses over the previous many years, which are viewed as maintainable and beneficial other options. Pineapple agroforestry frameworks are prevailing area use in the Indian Eastern Himalayas and different pieces of Asia are generally developed within relationship amid multipurpose trees. It was discovered that ranchers apply customary information for tree choice through earlier information and long haul cultivating experience. Organic product trees like *Areca catechu* and *Musa* species are planted on ranch limits because subsist fences. The survive fence decreases soil disintegration furthermore goes about the same as a windbreak moreover shelterbelt. A blend of financially significant trees like Albiziaprocera, Parkiatimoriana, Aquilariamalaccensis, just as natural product trees like papaya, lemon, guava, litchi, and mango in the midst of pineapple caters in favor of in cooperation home-utilization in addition to selling lasting through the year. In the more seasoned pineapple agroforestry ranches, ranchers present elastic trees.

Conclusion

Agroforestry rehearses support horticultural creation and assist with further developing hose down quality, air quality, soil wellbeing and natural life living space. Landowners are progressively utilizing agroforestry near include trees toward their scenes work on soundness of their backwoods. Agroforestry permits landowners to deliberately develop trees and bushes with crops as well as animal cultivating frameworks, makes a more different rural activity and assists with boosting their benefits. Intercropping optional plants between lines of elastic trees, regardless of whether tea, cinnamon, ginger nitrogen-fixing bushes, builds measure of water being paid hooked on dirt, diminishes soil disintegration. Developing different sorts of yearly and perpetual harvests together in a framework impersonating a backwoods like this is called agroforestry, a farming method tracked down from one side of the planet to the other, with notable models like shade-developed espresso. A mix of financially significant trees like *Albizia procera, Parkiati moriana, Aquilaria malaccensis,* just as organic product trees like papaya, lemon, guava, litchi, and mango with pineapple provides food for both home-utilization and selling throughout the entire year. In the more established pineapple agroforestry ranches, ranchers present elastic trees.

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Genetic transformation in pigeonpea through *Agrobacterium*: An overview on *in vitro* and *in planta* transformation methods

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Abstract

Cajanus cajan L. or pigeonpea is a pulse crop popularly grown in India for its nutritious seeds. Various factors e.g. insects, pathogens, drought, waterlogging, cold etc. limit its production and productivity. The traditional breeding strategies to develop biotic and abiotic stress-resistant pigeonpea cultivars have not been successful because of low to medium level of resistance in the cultivated germplasm and incompatibility problem with wild species. In this regard, genetic transformation of pigeonpea is a promising approach for the introgression of agronomically important traits in cultivated varieties having high yield potential. This paper provides an overview of the progress made towards generation of transgenic pigeonpea plants following Agrobacterium-mediated in vitro and in planta gene transfer methods. It also highlights the importance of floral-dip method of genetic transformation.

Keywords *Agrobacterium tumefaciens, Cajanus cajan, In planta* transformation

Introduction

Cajanus cajan (L.) Millsp. or pigeonpea is a member of family Fabaceae and is a diploid pulse crop cultivated mainly in the semi-arid tropical countries, especially South Asia, America, Africa and Australia. In India, it was cultivated on 3.9 mha area with 2.8 mtons of production annually (FAOSTAT, 2016). Pigeonpea is a source of abundant protein (21%) for humans and also used as fodder for animals. It is predominantly grown with other crops as an intercrop, such as mash, cotton, groundnut etc. on marginal soils without inputs to increase soil fertility and yield. Pigeonpea production is affected worldwide by biotic stresses (such as crop damage by insects, diseases) coupled with abiotic stresses (e.g. water logging, cold and drought). The germplasm accessions of pigeonpea worldwide have low to moderate level of resistance to the biotic, abiotic stresses that reduce yield potential of the crop (Srivastava and Raghav, 2013).

Pigeonpea primary gene pool lacks desired variation for genetic enhancement. The breeders opt for wild species from 2° and 3° gene pools, however lack of proper genetic information on useful traits, e.g. insect resistance, and strong hybridization barriers between cultivated varieties and wild spp. restrict the use of latter in breeding programs (Sharma and Mathur, 2016). Further, insect-aided natural out-crossing leads to loss of a trait for which a particular variety is renowned. In this context, genetic transformation is a good alternative approach for introducing agronomically important traits in genotypes having high yield potential. Efficient regeneration and recovery of stable transformants are two basic requirements for genetic transformation in any crop.

Genetic transformation through *Agrobacterium*

Genetic transformation or modification is defined as transfer of desirable gene(s) from one organism to another via recombinant DNA tools. A variety of different methods are available for genetic transformation of plants based on use of physical and biological agents. The most common method is genetic transformation using *Agrobacterium*, which is considered a method of stable transgene integration in nuclear plant genome.

Agrobacterium tumefaciens is a ubiquitous bacterium that resides in soil and causes crown gall disease in plants. It has Ti plasmid that can mobilize its T-DNA into plant genome and lead to crown gall formation because of presence of oncogenes within T-DNA region. Ti plasmid devoid of oncogenes in T-DNA region is utilized for genetic transformation of plants. Three regions are important for T-DNA mobilization into nuclei of plant cells, first one is 25 bp direct repeat sequences that mark T-DNA border, the second is virulence region needed for T-DNA transfer, and the third region comprises bacterial chromosomal genes responsible for bacterial cell attachment to plant cell.

There are two different types of *Agrobacterium*-mediated genetic transformation; the first method utilizes *in vitro* regeneration and the second method skips *in vitro* regeneration, and is called as *in planta* transformation method (Sreevathsa, 2016). *In vitro* regeneration-based transformation method exploits the principle of plant cell totipotency where a single plant cell divides and produces a complete plant. Attempts have been made in this direction to generate pigeonpea plants from organized explants and callus cultures by altering culture medium composition.

Effect of genotype, explants and *Agrobacterium* strain on genetic transformation of pigeonpea following *Agrobacterium*-mediated *in vitro* transformation method

The major factors affecting transformation success are explant type, crop genotype and *Agrobacterium* strain. In pigeonpea, different varieties and explants have been successfully used for transformation. The reports on regeneration via callus culture (Kumar *et al.*, 1983), direct regeneration from leaf parts (Eapen and George, 1993; Geetha *et al.*, 1998), cotyledons (Mehta and Ram, 1980), cotyledonary nodes (Prakash *et al.*, 1994) and regeneration through formation of somatic embryos (Patel *et al.*, 1994; Kulkarni and Krishnamurthy, 1998) are available. Various pigeonpea genotypes have been used for genetic transformation with ICPL87 reported as most responsive and hence widely used for transformation. A list of transgenic plants developed *in vitro* using different genotypes, explants and *Agrobacterium* strains in pigeonpea is presented in Table 1A.

Among *Agrobacterium* strains, the use of EHA 105 and LBA 4404 has been widely documented. For transformation of dicotyledonous plants, addition of phenols, such as acetosyringone into cocultivation medium or bacterial cultures is a pre-requisite. Verma and Chand (2005) observed increased transformation efficiency in pigeonpea by supplementing cocultivation medium with acetosyringone. Rao *et al.* (2008) reported increased transformation efficiency using potato and tobacco leaf discs following *Agrobacterium* infection in pigeonpea. The transformation frequency is largely affected by *Agrobacterium* cell density, measured either as absorbance at 600 nanometer wavelength (A_{600}) or as optical cell density at 600 nanometer (OD_{600}). OD value of 0.6 is reported to be most favorable for conducting genetic transformation experiments (Kumar *et al*., 2004a; Surekha *et al*., 2005; Surekha *et al.*, 2007; Kaur *et al.*, 2016). Parekh et al. (2014) used different *Agrobacterium* suspensions having OD values of 0.2 to 1.0, and found that maximum frequency (27%) of putative transgenic plants was obtained at OD value of 0.6. Ghosh *et al*. (2014) achieved transformation efficiency of 9% at OD ranging from 0.8 to 1.0. One of the reasons for poor transformation frequency at higher OD values may be extensive tissue damage due to bacterial over-growth. Surekha *et al*. (2014) achieved transformation efficiency of 15.6-19.3% in different pigeonpea genotypes at an OD value of 0.8.

Poor *in vitro* regeneration leading to low frequency of transformation is the major concern in developing pigeonpea transgenic plants *in vitro*, further shoot and root formation response of this crop is also genotype specific. Although attempts have been made for developing effective protocols of gene transfer through *Agrobacterium* based on *in vitro* regeneration in pigeonpea (Lawrence and Koundal, 2001; Sharma *et al*., 2006; Surekha *et al*., 2007), however *in vitro* regeneration conditions have been worked out only for a limited number of genotypes (Mohan and Krishnamurthy, 1998). Failure to induce roots in regenerated shoots under *in vitro* conditions is a main problem for tissue culture-based transformation (Ghosh *et al*., 2014). In this direction, an effective protocol of grafting *in vitro* regenerated shoots on rootstocks to alleviate root regeneration problem in transgenic pigeonpea shoots is documented (Krishna *et al.*, 2010; Ghosh *et al.*, 2017).

During the past few years, several attempts have been made to develop transgenic pigeonpea plants carrying insect resistance, resistance to fungal diseases and improved nutrient quality (Ghosh *et al*., 2014). Research has also been conducted on development of salt-tolerant transgenic pigeonpea (Surekha *et al*., 2014). *Bacillus thuringiensis* bacterium, residing in soil, produces a protein encoded by *cry* genes that is toxic to various insects. Different genes, such as *cry1Ac*, c*ry2Aa*, chimeric *cry1Aabc*, synthetic *cry1Aa*$_3$ and *cry1AcF* (Ramu *et al*., 2011; Parekh *et al.*, 2014; Das *et al*., 2016a; Ghosh *et al*., 2017) were used for imparting insect resistance in pigeonpea. Resistance to chewing insect-pests, mainly *Helicoverpa*, has also been reported in pigeonpea plants transformed with cowpea protease inhibitor gene (Lawrence and Koundal, 2001). Transformation of this important crop has also been reported using

Agrobacterium strain C-58 containing pCAMBIA 1302 for incorporation of rice *chitinase* gene (Kumar *et al*., 2004a). Efficient *in vitro* transformation of pigeonpea via *Agrobacterium* strain LBA4404 (pCAMBIA 1301) was reported to produce salt-tolerant and drought-tolerant plants by use of a mutagenized form (*P5CSF129A*) of wild *P5CS* gene (Surekha *et al*., 2014) [Table 1].

Genetic transformation of pigeonpea through *Agrobacterium*-mediated *in planta* gene transfer method

In planta transformation has been demonstrated for several legumes, such as clover (Trieu *et al.*, 2000), groundnut (Rohini and Rao, 2000a) and pigeonpea (Rao *et al*., 2008) as an alternative, efficient method for tissue culture-independent development of transgenics in these crops. Development of transgenics through non-tissue culture-based protocol involving infection of pricked apical and inter-cotyledonary regions of embryo axes of germinating seedlings with *Agrobacterium* suspension has been documented (Rao *et al*., 2008). We used a modified *in planta* transformation method for transforming embryo axes of germinating seeds at G.S. Khush Labs, Punjab Agricultural University, Ludhiana by using acetosyringone for *vir* gene induction instead of tobacco leaf extract, and putative T_1 transgenic pigeonpea plants of *cv.* AL 15 were developed with a frequency of around 2% based on PCR (Singh, 2020). *In planta* transformation protocol has been reported for generating insect-resistant transgenic pigeonpea (Ramu *et al.*, 2011; Kaur *et al*., 2016; Singh *et al.*, 2018). Thus, *in planta* transformation provides a good option to *in vitro*-based transformation protocols, as transformants at high frequency are obtained (Sundaresha *et al.*, 2010). Genetic transformation events developed in pigeonpea through *Agrobacterium*-mediated *in planta* gene transfer method are enlisted in Table 1B.

The plants obtained from *in planta* transformation are chimeric in nature (Keshamma *et al*., 2012), thus hindering the characterization of plants in T_0 generation. Sawardekar *et al.* (2012) injected *Agrobacterium* inoculum carrying *cryIIAa* gene into mildly injured growing buds for developing pod borer resistance in pigeonpea, however no seeds could be obtained from treated flower buds. Floral-dip is a time saving method than other *in planta* methods, as seeds obtained from treated flowers are counted as T_1 generation that does not exhibit any chimerism (Clough and Bent, 1998). Thus, among different *Agrobacterium*-mediated genetic transformation methods reported in pigeonpea, emphasis should be laid on optimization and use of floral-dip method of genetic transformation for production of non-chimeric transgenic plants.

Conclusion

Pigeonpea is prone to a number of insect-pests (e.g. pod borer) and diseases that lead to low crop yield. There is lack of sufficient resistance in the cultivated primary gene pool, thus transgenic technology is considered as an alternative approach to develop resistant plants. Poor *in vitro* regeneration coupled with poor root induction leads to low transformation frequency in pigeonpea, and *in planta* transformation method via *Agrobacterium* leads to the development of chimeric plants, and characterization of plants not earlier than T_1 generation. The dipping of floral buds in *Agrobacterium* suspension has been successfully employed for transformation of *Arabidopsis*. There is a need to use this method in pigeonpea genotypes to develop non-chimeric transgenic plants.

Table 1: *Agrobacterium*-mediated genetic transformation of pigeonpea through *in vitro* and *in planta* methods

Genetic transformation of pigeonpea through *in vitro* transformation method					
Genotype/ cultivar	**Explant(s)**	**Medium**	***Agrobacterium* strain, cell density**	**Remarks**	**Reference**
Hyderabad C	Cotyledonary node, shoot tip	MS + BAP (2.0 mg/l) for shoot bud regeneration	LBA4404, A_{600} 0.6	Transformation was done with *uidA* and *nptII* genes, presence of transgenes in plants was verified by PCR.	Geetha *et al*., 1999
Pusa 855	Embryo axis, leaf	MS + IAA (0.2 mg/l) + BAP (2.0 mg/l) for shoot regeneration, MS + IBA (0.5 mg/l) for root formation	GV2260	Pigeonpea was transformed using cowpea protease inhibitor gene. PCR and Southern hybridization was used to confirm integration of transgene in plants. Northern blotting demonstrated transgene expression in plants at mRNA level.	Lawrence and Koundal, 2001
T15-15	Embryonic axis	MS + BAP (5.0 µM) + IAA (1.0 µM)	LBA4404	Transformation was done with *green fluorescent protein* (*GFP*), *β-glucuronidase* (*GUS*) genes. Histochemical assay and fluorescent microscopy were carried out for transgene confirmation in plants.	Mohan and Krishnamurthy, 2003
Hyderabad	Embryonic axis, cotyledonary node	MS + BAP (8.87 µM) for culture initiation, MS + BAP (2.22 µM) for shoot regeneration, MS + IBA (1.48 µM) for root development	EHA105, A_{600} 0.6	Gene encoding for Rinderpest virus Hemagglutinin protein was introgressed in pigeonpea. Southern blot was done for transgene confirmation. The highest expression of Hemagglutinin protein was observed in leaves.	Satyavathi *et al*., 2003
ICPL 87	Cotyledonary node	B_5 + BAP (10.0 mg/l) for induction of callus, B_5 + BAP (0.2 mg/l) for shoot differentiation	LBA4404	Transgene introgression in plants was checked by GUS assay, PCR and Southern hybridization.	Thu *et al.*, 2003

LRG 30	Cotyledonary node	MS + BA (0.2 mg/l) for shoot differentiation, MS + GA_3 (0.5 mg/l) for shoot growth, MS + IBA (1.0 mg/l) for root formation	C58, A_{600} 0.6	*Chitinase* gene of rice for resistance to fungal disease was delivered via *Agrobacterium* in pigeonpea. Transgene confirmation was done by PCR in T_0 and T_1 generations.	Kumar *et al*., 2004a
LRG30	Cotyledonary node	-	C58	Transformation was done using *Agrobacterium tumefaciens* carrying *uidA* gene.	Kumar *et al*., 2004b
Hyderabad	Cotyledonary node	MS + BAP (2.22 µM) for shoot induction, MS + IBA (1.48 µM) for root induction	GV3101, A_{600} 0.6	Transformation was done using Hemagglutinin-neuraminidase gene. Transgene presence was confirmed by PCR, and Hemagglutinin-neuraminidase activity was observed in cell extract of transgenic plants.	Prasad *et al*., 2004
ICPL 87	Embryo plumule, embryo node	MS + BAP (2.0 mg/l) for shoot regeneration, MS + BAP (1.0 mg/l) + GA_3 (3.0 mg/l) for shoot elongation, MS + NAA (0.1 mg/l) for rooting	GV2260, A_{600} 0.6	A synthetic *cry* IE-C gene was used for transformation for resistance to Spodoptera. Integration of T-DNA into nuclear genome of transformed plants was confirmed by PCR and Southern hybridization. The highest mortality of larvae in transgenic plants was found to be 80%.	Surekha *et al*., 2005
UPAS 120, Bahar	Cotyledon, mature embryonic axis, leaf disc	-	LBA4404	*cry1Ab* gene was used for transformation and efficiency of 0.20% was obtained. Transgene integration was checked by PCR, Southern hybridization in T_0, T_1 generations.	Verma and Chand, 2001
ICPL 87	Cotyledonary node	MS + BA (22.0 µM) for shoot induction and elongation	C58	*cry1Ab* gene was used for transformation, and transformants were screened by PCR. Highest Cry1Ab expression level (0.1%) was observed in pigeonpea flowers through ELISA, and the expression was stable upto T_2 generation.	Sharma *et al*., 2006

ICPL 85063, ICPL 87, LRG 30	Embryo node, embryo plumule, cotyledonary node, cotyledon	MS + BAP	LBA 4404	Transformation was done with glutamine synthase, peroxidase construct and *uidA* gene by *Agrobacterium* harboring pAD288 plasmid. Transformation efficiency for ICPL 87, ICPL 85063 and LRG 30 was observed to be 76.6%, 75% and 65%, respectively.	Surekha *et al*., 2007
ICPL 87		MS + BAP	LBA 4404	*uid A* gene was introduced by *Agrobacterium* containing pAD288 plasmid and transformation efficiency of 40 to 76.6% was recorded.	
ICPL 87, ICPL 85063		MS + BAP	GV 2260, A_{600} 0.6	Transgenic plants were generated containing *uidA* gene with maximum transformation efficiency of 76.6 and 66.6% for ICPL 87 and ICPL 85063, respectively.	
ICPL 87	Cotyledonary node	B5 + BAP (10.0 mg/l) for induction of callus, B5 + BAP (0.2 mg/l) for shoot differentiation	LBA4404	*dhdpsr1* coding region was over-expressed in pigeonpea. Transformants were evaluated by PCR in T_0 as well as T_1 generations. The lysine content in transgenic pigeonpea seeds was 1.6 to 8.5-fold higher as compared to seeds of non-transgenic plants.	Thu *et al.*, 2007
JKR105	Embryonic axis	MS + BAP (0.5 mg/l) for shoot differentiation and elongation, MS + IBA (0.5mg/l) for rooting	GV3101, A_{600} 0.6	Transformation was carried out using *cry1Ac*. Transformation efficiency of 44.61% was achieved with use of acetosyringone. PCR, Southern blot and ELISA were done for confirming transgene presence, integration and quantification. *cry1Ac* expression was more in pods and buds as compared to leaves.	Krishna *et al*., 2011
Konkan Tur-1	Embryo axis with single cotyledon	MS + BAP (2.0 mg/l)	EHA 105	*cryIIAa* gene was introduced with frequency of 4.21% and transformed plants were analyzed by PCR.	Sawardekar *et al*., 2012

ICPL87, ICPL8719	Decapitated embryonic axis	MS + NAA (0.2 mg/l) + BAP (1.0 mg/l) for shoot induction, MS + BAP (0.5 mg/l) + GA_3 (0.5 mg/l) for shoot elongation	EHA105, A_{600} 0.8-1.0	β-glucuronidase expression was obtained in pigeonpea via *Agrobacterium*-mediated gene transfer with a transformation efficiency of 9%. Transgene confirmation was done in T_0 and T_1 plants by PCR.	Ghosh *et al*., 2014
GT-102	Embryonic axis	MS + Cefotaxime (250.0 mg/l) + Kanamycin (50.0 mg/l)	LBA4404, A_{600} 0.4-0.6	Pigeonpea was transformed with synthetic *cry1Aa3* gene. Its integration in plant nuclear genome was confirmed by PCR.	Parekh *et al*., 2014
LRG 41, LRG 30, ICPL 85063	Embryonic structures	MS + BAP (4.0 mg/l) for shoot induction, MS + GA_3 (0.3 mg/l) + NAA (0.1 mg/l) for shoot elongation, MS + IBA (0.5 mg/l) for rooting	LBA4404, A_{600} 0.8	Salt tolerant pigeonpea was developed carrying *Vigna aconitifolia P5CSF129A* gene. Transgene integration and its transmission to T_2 generation was confirmed by PCR. Transgenic plants performed better than non-transgenic plants under 200 mM NaCl.	Surekha *et al*., 2014
ICPL 87119	Axillary meristem explant	MS + BAP + Kin + NAA for shoot differentiation, MS + GA_3 for shoot elongation	EHA105, A_{600} 0.6-0.8	A codon-optimized chimeric *cry1Aabc* gene for resistance to *Helicoverpa armigera* was introduced and 0.06% transformation was recorded. The transgene presence in plants was checked by PCR.	Das *et al*., 2016a
ICPL 87119	Embryonic axis	MS + B_5 + GA_3 (0.17 mg/l) for shoot elongation	LBA 4404, A_{600} 0.6-0.8	Grafting of *in vitro* regenerated transgenic shoots onto rootstocks was done with 72.6% survival efficiency. 87% T_1 plants were positive for the transgene.	Das *et al*., 2016b
ICPL 87119, ICPL 8863	Cotyledonary nodal explant	MS + BAP (2.0 mg/l) for shoot regeneration, MS + BAP (0.1 mg/l) for shoot elongation, MS + IBA (0.5 mg/l) for rooting	EHA 105, A_{600} 0.6	Pod borer resistant pigeonpea was developed using *cry1Ac* gene with 4.28% transformation. ICPL87119 and ICPL 8863 transgenic plants showed 66.61 and 68.01% inhibition of pod borer growth, respectively.	Thangella and Fakrudin, 2016
ICPL 87119 (Asha), ICPL 87	Decapitated embryonic axis	MS + NAA (0.2 mg/l) + BAP (1.0 mg/l) for shoot differentiation,	EHA 105, A_{600} 0.8-1.0	Transgenic plants were obtained through *in vitro* grafting of transgenic shoots on rootstocks.	Ganguly *et al*., 2017

UPAS 120	Embryonic axis	MS + GA_3 + BAP (0.5 mg/l) for shoot elongation	AGL-1, A_{600} 0.8-1.0	*cry1Ac* and c*ry2Aa* genes were introduced in pigeonpea for resistance to *Helicoverpa armigera*. PCR was done for selection of T_0 transformants. The level of Cry protein expression was 0.140 - 0.175% in T_1 plants.	Ghosh *et al*., 2017
ICPL 87119	Non-decapitated and decapitated plumular axes	Modified MS + NAA (0.2 mg/l) + BAP (1.0 mg/l)	EHA 105	Transformation frequency as high as 72% was achieved. PCR and Southern blotting were used to confirm transgene introgression and copy number, respectively in T_1 progenies.	Ganguly *et al*., 2018
Genetic transformation of pigeonpea through *in planta* transformation method					
Genotype/ cultivar	**Explant(s)**	***Agrobacterium* strain, cell density**	**Remarks**		**Reference**
TTB7	Seedling	LBA4404	Transformation was done using *uidA* and *nptII* genes and transformation frequency of 13% was achieved in T_1plants (based on PCR). Gene integration and transmission was analyzed by Southern blotting.		Rao *et al*., 2008
TTB7	Seedling	EHA105	A chimeric *cry1AcF* gene was expressed with 32% transformation frequency in T_1 plants through PCR. The efficacy of the transgene was upto T_3 generation. The transgenic plants showed 75-100% larval mortality.		Ramu *et al*., 2011
ICPL 87119, BSMR 736	Cotyledonary embryo axis		*cry1Ac*, *cry2Aa* and *cry1F* genes were introduced with 12%, 18% and 7% transformation efficiency in T_1 generation (based on PCR), respectively.		Manohar, 2014
PAU 881	Cotyledonary embryo axis	EHA 105, A_{600} 0.6	Transformation was done using *cry1Ac* gene. Transgene presence and its transmission to T_2 generation was confirmed by PCR. A transformation frequency of 21.4% (based on quantitative RT-PCR), and pod borer larval mortality of 97.78% was recorded in T_2 generation.		Kaur *et al*., 2016
Pusa 992	Apical meristem	EHA 105	*Agrobacterium*-mediated i*n planta* transformation was done for pod borer resistance using *cry2Aa* gene. Putative T_1 plants (0.8%) were selected on selection medium supplemented with kanamycin (70 mg/l).		Singh *et al*., 2018

AL15	Pricked embryo axis	LBA4404, A_{600} 0.6-0.7	A transformation frequency of 2.2% was obtained.	Singh, 2020

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Utilization of Rice Bran Proteins in Foods

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Abstract

Rice bran is one of the major by-products obtained from rice milling and constitutes about 10% of the total weight of the rice. Rice branhaspericarp, aleurone, sub aleurone layer and germ. It is a major source of nutrients such as proteins, vitamins, minerals, essential fatty acids, dietary fiber and other sterols. Rice bran protein which isextracted from rice bran is considered to be a potential ingredient in nutritional and functional foods. The objective of this chapter is to throw an insight on the method of extraction, nutritional and healthy properties of rice bran proteins including thehypo allergenicity, and antioxidant, antidiabetic, and anticancer activity. The physical methods (homogenisation), chemical methods (alkaline extraction) and enzymatic methods are used for assisting the protein extraction from the rice bran. Thischapter would also provide abrief insight on the rice bran protein which are rich in bioactive peptides and is used as value added products, edible films and pharmaceutical applications.

Keywords: rice bran protein, nutritional benefits, extraction, functional food

Introduction

Rice (*Oryza sativa*) is one of the major cereal crops which is widely grown in Asian countries. According to the Food and Agriculture Organisation (FAO) the production of rice in India was estimated to be 177.6 million metric tons in the year 2019. The production of rice has increased by 3.5 times in the past 60 years. Indiaranks second in rice production in the world with the global production share of more than 11% after China. The country exported 9819 thousand metric tons in the year 2019 and the increase in rice consumption in emerging and developing nations across the world may promote the rice export. The rice global rice trade in India in 2021 reached up to 44.4 million. The increase in export from India is due to the increasing demand for rice globally. It is the second major staple food which is grown across the globe (Peanparkdee and Iwamoto, 2019) and is a major source of protein, carbohydrate, vitamins and minerals (Sohail *et al*., 2017). The outer part of the rough rice is called rice bran which is one of the major underutilised by-products obtained from the rice processing industry(Sereewatthanawut*et al*., 2008). Milling of paddy results in the major product (70%) endosperm, (20%), bran (8%), germ (2%) and the by-products such as husk (Kim *et al.*, 2011). It also provides important health benefits such as hypolipidemic effects ,anticancer activity, hypoallergenicity, Antioxidant activity, and hypocholesterolemic effect (Zhou *et al*., 2012, Ni *et al*., 2003; Yeom, 2009)

Rice protein iscome under the category of plant-based proteins, which has applications in different products such as baby foods and gluten free foods. Rice bran has numeroususes in food industry for improving the nutritional characteristics of the processed foods. The therapeutic potential of rice bran plays a predominant role in the formulation of functional foods and value-added foods which are of increased demand in the present scenario. Various foods such as bread, pasta, noodles, cakes, and ice creams are supplemented with rice bran which improve the textural and functional properties of foods.

Rice bran and its composition

Rice bran comprises of about 5-8% of the weight of the total grain. The chemical composition is protein,10-20%, lipid,15-20%, moisture- 8-12%, fiber 7-14%, 31.4-52.3% carbohydrate. (Fabian and Ju, 2011). It consists of minerals such as phosphorous, magnesium, iron, etc. (Oliveira *et al*., 2011). Therice bran compositionchanges with the climatic conditions, method of processing and the variety of rice (Grist, 1985). Rice bran consist of oryzanol, tocopherols and tocotrienol (Godber and Wells, 1994), which is the major source of antioxidant. The quality of rice bran is also affected by the type of

equipment which is used for milling of rice. The composition and the yield of the bran in milling depends on the degree of milling.

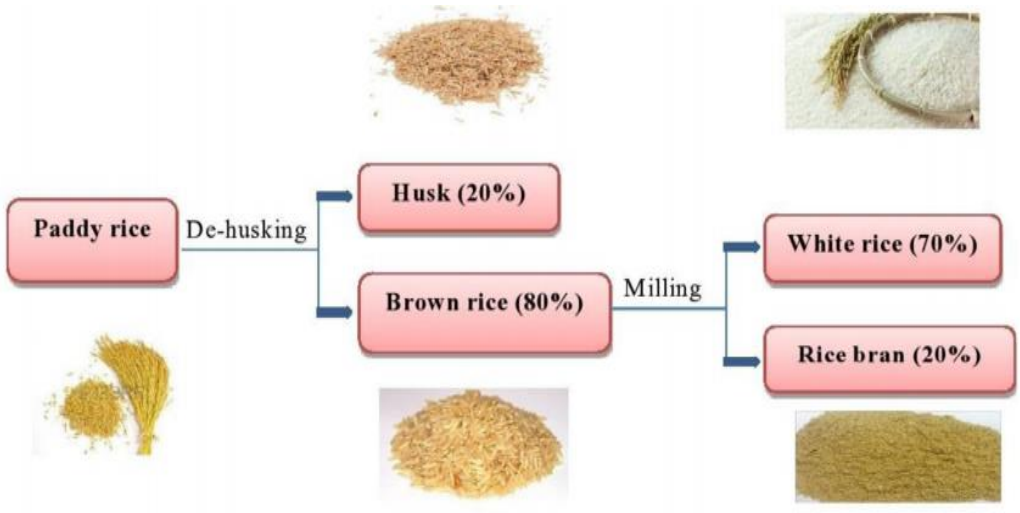

Figure 1. Rice Milling by-products

Rice bran proteins

The average protein content of rice bran protein varies from 10-20% and the digestibility and function are greater compared to other cereals such as maize, wheat, barley (Amaglian*iet al*., 2017). Rice bran protein which are globulin, glutelin, prolamine are soluble in water, alcohol and saline medium. (Amagliani *et al.*, 2017; Phongthai *et al*., 2017). The major rice protein in the whole grain, milled rice and rice polish fraction is glutelin. Other types of proteins such as albumin and globulins, are present in rice bran and rice polish. The most evenly distributed protein in prolamine.The amount of lysine content is higher in rice bran compared to the protein of wheat and maize (Amagliani *et al.*, 2017). It also has high amount of free amino acids such as glutamic acid (7–13%), serine (5–15%) and alanine (11–16%)(Liu *et al.*, 2019).

The World Health Organisation and Food and Agricultural Organisation recommended the Protein Digestibility Amino acid score (PDCAAS) to evaluate the quality of proteins (WHO Report 935, 2007). The dairy protein had higher PDCAAS value than rice protein. The rice bran protein had similar PDCAAS value of soy protein (Han *et al*., 2015). The lesser value of rice protein when compared to dairy protein was due to the deficiency of lysine content in rice protein.

In comparison with other protein sources, the foaming property, solubility and emulsification property are the main functional property of rice bran protein. The white rice proteins have the solubility fractions about 15% albumin, 20% prolamin and 65% glutelin. Bran proteins consist of are 66-98% albumin. The low amount of prolamin is the reason of higher lysine content.

Rice bran protein has higher solubility compared to brown and white rice proteins at rangeof pH 4-7 (Cao *et al.*, 2009). The availability of the concentrates and isolates of rice bran protein is not common. The decrease in availability is because of the complexity of proteins in rice bran and large amount of fibre (12%) and phytate (1-7%) and which bind to the proteins, makes the protein bodies hard to get separated from each other components. Lipases, trypsin inhibitors, haemagglutinin-lectin and phytatesare the antinutritional factors present in the bran limit its usage in as feed and food ingredient. Different treatments such as alkali, mild acid and heat treatment denatures the undesirable factors other than phytates which are present in the bran that are proteinaceous in nature (Jiaxun, 2001).Phytates generally interfere with the absorption of the minerals in the gut (Rajkishore *et al*., 2015).

Extraction of proteins from rice bran

The rice bran proteinextraction iscarried out by physical, enzymatic and alkaline and methods. The process of rice bran protein extraction is difficult because of the aggregation anddisulfide cross linking (Hamada *et al*., 1997). Enzymatic extraction is investigated to have good efficiency and its widely used but the high cost of the enzyme limits the production of bran proteinin commercial scale (Apinunjarupong *et al*., 2009; Thamnarathip *et al*., 2016). Though, the extraction efficiency by physical method is not so high, it is economical and is easy to operate. Physical techniques such as homogenisation, microwave and ultrasonic methods disrupt the cell wall easily and aids in extraction of protein (Tang *et al*., 2002).

The common method of dissolving the rice bran protein by chemical method is by alkaline extraction.This will reduce the molecular size of the protein andcleave the hydrogen and disulfide bond in rice glutelin, and thus improving the rate of extraction. (Xia *et al*., 2012).Alkaline procedure accompanied by isoelectric precipitation method with pH 4-4.5 was also utilized to obtain proteins from rice bran with a yield varied from 40 to >80% (Bandyopadhyay *et al*. 2008). High alkaline conditions can in turn affect the nutritional parameters of the protein and produce toxic products. Therefore, different methods are used to extract rice bran proteins through chemical, physical and enzymatic methods.

Alkali extraction

The rice bran protein is commonly extracted by alkali extraction. The high concentration of sodium hydroxide causes the breakdown of disulphide and amide bonds in protein structure (Phongthai *et al*., 2017). The increase in the temperature of extraction and pH, increased the yield of rice bran. (Sari *et al*., 2015, Gupta *et al*., 2008). The protein exposure to high alkali conditions and heat cause denaturation of protein, changes inflavour and colour and decrease the nutritional and functional value and also generate some toxic compounds likelysinoalanine (Kelly *et al*., 1982). The rice bran protein extraction by the isoelectric precipitation method (pH 11) resulted in the recovery of 71-86% of protein (Paraman *et al*., 2008). At lower isoelectric pH 4-4.5, the yield varied from 40-80% (Bandyopadhyay *et al*., 2008; Chandi and Sogi, 2007; Jiamyangyuen *et al*., 2005; Yadav *et al*., 2011).

The functional properties such as foaming, emulsification and film formation are affected by the process of alkali treatment.The drawbacks of alkaline extraction are that the protein yield is of low quality.The accelerated maillard reaction lead to low purity proteins products of dark color and formation compounds such as lysinoalaninewhich is toxic in nature (Fabian and Ju, 2011).

Enzyme-assisted method

The alternative method to extract rice bran protein is by enzymatic method. There are different enzymes such as carbohydrase , protease and lipase that help in the isolation of protein bybreaking the bondbetween proteins and components such asstarch, fiber etc. Proteases have better extraction rate, solubility and thus hydrolyse the rice bran protein (Tang *et al*., 2002). Hamada (1999) concludedthat the use of the enzyme protease enhanced the yield from 60% to 93% byimproving the rate of hydrolysis. Enzymatic extraction facilitates the starch -bound protein release, degradation of cell wall and thus, increased protein solubility (Fabian and Ju, 2011). The combination of phytase and xylanase was used in the extraction of protein from rice bran and concluded that the use of enzyme carbohydrases might increase the yield of the soluble protein (Wang *et al*.,1999). Wanyo *et al*., (2014) investigated the combined action of enzymes in the increased yield of protein; however, the cost of processing was highand did not result in pleasant organoleptic profile and good nutritional properties in protein.

Khan *et al*. (2011) estimated that rice bran protein was 61% for parboiled rice and 85% for unstabilised bran.The commonly used enzymes which are food grade are flavourzyme, protamex, alcalase and the enzymes such as papain (from plants) and pepsin and trypsin (from animal sources) (Samaranayaka and Chan, 2011). The protein solubility can be improved by proteinases by splitting them down into peptides and enhancing the extraction efficiency. Enzymes such as,flavourase, alcalase, chymotrypsin and papain were used to extract the protein from rice endosperm and evaluate the antioxidant capacity. The endosperm proteins were hydrolysed depending on the optimal conditions, for 4 h (Zhang *et al*., 2009).

Thedefatted rice bran hydrolysed by the enzyme flavorzyme and alcalase increased the protein yield upto 81-88% at 50°C and pH 8 (Hamada, 2000). Enzymatic hydrolysis improved the functional, sensory and physicochemical properties of proteins (Yeom *et al.,* 2009). Since, the use of commercial enzymes are costly, alkaline extraction is preferred for protein extraction.

Physical method

The different physical methods for the extraction of protein areultrasonication, colloid milling, microwave treatment etc. (Anderson and Guraya, 2001). Tang *et al*., (2002) investigated the extraction rate of different physical methods such as, freeze-thaw, high-speed blending, sonication and found that the defatted rice bran resulted in the yield of protein about 11–16%. The shear force developed by physical methods contributed to the cell disruption (Anderson and Guraya, 2001). Ultrasonication method also induce damage to the cell membrane and disrupted the molecular bonds. The shock wave generated during the process of ultrasonication also resulted in cavitation bubble collapse. Further, sonication for 5 min resulted in the yield of 15% compared to high pressure techniques which gave 11% protein yield (Tang *et al.,* 2002). Subcritical water extraction is one of themethods to extract amino acids or protein or from rice bran (Amagliani *et al*., 2017; Sereewatthanawut *et al*., 2008). Theincrease in temperature used in subcritical extraction may cause denaturation of the proteins (Fabian and Ju, 2011).

It was found that protein extractability rate increased to 15%by milling and differential sieving of rice bran (Prakash and Ramanatham, 1994). The protein recovery from physical methods was found to be economical and easier but compared to the enzymatic and chemical extractions the yield was low. So,rice protein was extracted effectively by the physical processing in combination with alkali extraction and enzymatic treatments was suggested to effectively extract protein from rice bran (Tang *et al.,* 2002).

Health benefits and functional properties of rice bran protein

Rice bran proteins and peptides consist of bioactive molecules which areinvolved in several physiological functions. These include lowering of cholesterol, antidiabetic effect, antioxidant effect etc. The use of cost-effective protein supplements is increasing in the present scenario. The rice bran is very cheap by-product and has a highamount of nutrients. The quality proteins can be utilised effectively in the production of bioactive peptides.

Table 1. Composition of Amino Acid in Rice Isolate, Soy Isolate, and Casein

Amino acid	Rice isolate (%)	Soy isolate (%)	Casein (%)
Aspartic acid	11.44	14.21	9.17
Serine	9.49	8.73	7.99
Histidine	2.05	1.93	2.25
Arginine	7.84	6.59	3.23
Proline	6.67	6.61	12.4
Isoleucine	2.07	2.37	3.54
Glutamic acid	17.82	18.51	18.8
Threonine	4.17	4.24	4.59
Glycine	8.44	8.8	4.99
Alanine	6.73	6.03	4.99

Valine	2.34	2.89	5.77
Tyrosine	3.87	2.71	3.51
Methionine	4.62	0.92	2.1
Leucine	4.55	4.88	6.41
Lysine	3.41	5.58	8.48
Phenylalanine	4.49	4.01	3.46

(Frederick *et al;* 2000)

Chittapalo and Noomhorm (2009) found that the maximum solubility (85%) was found in alkaline extraction at the pH 12 and solubility was minimum (2%) at pH 4–6. Rice bran protein exhibited higher foaming stability in comparison to casein under different range of pH (5-9). Rice bran protein has good emulsifying property and can be used as emulsifiers in the processed foods (Xia *et al*., 2012). The emulsification property of protein concentrates was in the range 24 to 74% (Chandi and Sogi, 2007). The capacity of oil absorption (OAC) values of the proteins from heat stable and unstabilised rice bran was estimated to be 3.3 and 2.4 mL/g (Khan *et al*., 2011). The rice bran protein concentrates had the excellent functional property to be used for food applications. Rice bran protein has gained much importance due to its nutraceutical properties, high protein quality and various other health benefits.

Antihypertensive effect

Rice bran protein is used as therapeutics for the prevention and treatment of hypertension. A strong angiotensin-converting enzyme (ACE) inhibitory action was found in rice bran protein with molecular weight (<4KDa) (Wang *et al.*, 2017). A higher inhibitory effect was found in prolamine and globulin compared to albumin (Uraipong and Zhao, 2016).A strong hydrogen bond was formed within the active sites of human angiotensin-converting enzymeby the tripeptide sequence Try-Ser-Lys, from rice bran protein. Hence, rice bran protein is considered to be the good source to treat hypertension (Liu *et al*., 2017).

Antioxidant Capacity

The rice endosperm protein was digested by using different enzymes with antioxidant properties such as chymotrypsin, neutrase, flavorase, alcalase, and papain. (Zhang *et al.* 2009). Under the in vitro digestion approach, the radical scavenging capacities of rice protein can reduce the oxidative stress (Liu *et al.*, 2016). The antioxidant activity of the protein hydrolysate from rice bran resulted to its hydrophobicity, structure of aminoacids and molecular weight. The bran protein rich in albumin showcased the highest reducing power followed by glutelin, prolamin and globulin fractions (Zhang *et al.*, 2014). The rats diet supplemented with the rice bran extract improved the antioxidant enzyme actionlikeglutathione peroxidase and catalasewhich reducedthe oxidative damage of lipids and proteins compared with control. There was significant reduction in the superoxide radical production with low expression of reduced NADPH (nicotinamide adenine dinucleotide phosphate oxidase),which is an enzyme which help in superoxide production in rats. In general, the oxidative stress was also found to be reduced by the rice bran protein (Fan *et al*., 2008).

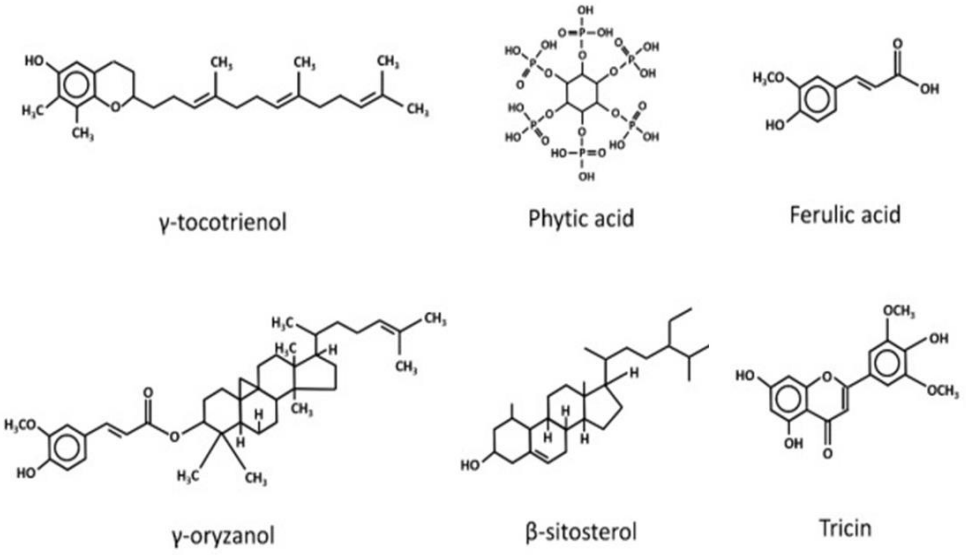

Figure 2.Phytochemicalsof anti-oxidative nature in rice bran

Antidiabetic Activity

The protein isolates of rice bran extracted by the enzymes proteases (and Protamax and Alcalase) resulted in high inhibitory action on the enzymes α-glucosidase and α-amylase.The activity of inhibition was observed to be similar with acarbose which is an antidiabetic drug that showcased that the rice bran protein hydrolysate had the potential to manage diabetes and also function as dietary or nutraceutical supplement. (Uraipong and Zhao, 2016). In the in vitro study, a strong α-glucosidase inhibitory activity was noted in the protein hydrolysate of rice bran which concluded that the antidiabetic peptides can be generated by the consumption of rice bran protein (Uraipong *et al*, 2018)

Anticancer activity

The in vitro studies of the viability and proliferation of breast, colon, and liver cells of the rice bran peptide fractions weredemonstrated. The hydrolysates and peptides of the bran protein was formulated by the enzyme alcalasetreated defatted form of rice bran.A significant growth inhibition and cytotoxic effects was noticed in breast cancer cell lines and human colon when treated at 500µg/mL (Kannan *et al.*, 2009).The characterisation and further purification of the peptidesobtained from the protein hydrolysates of rice bran resulted in sequence ofamino acid Glu-Gln-Arg-Pro-Arg of the peptide which indicated about 84% inhibition on the growth of colon cancer,80%inhibition in the breast cell growth and 84% inhibition on growth of liver cancercells (Kannan *et al.*,2010). The results suggested that the rice bran protein is a good source and can function as an antitumour agent for curing breast, colon and liver cancer.

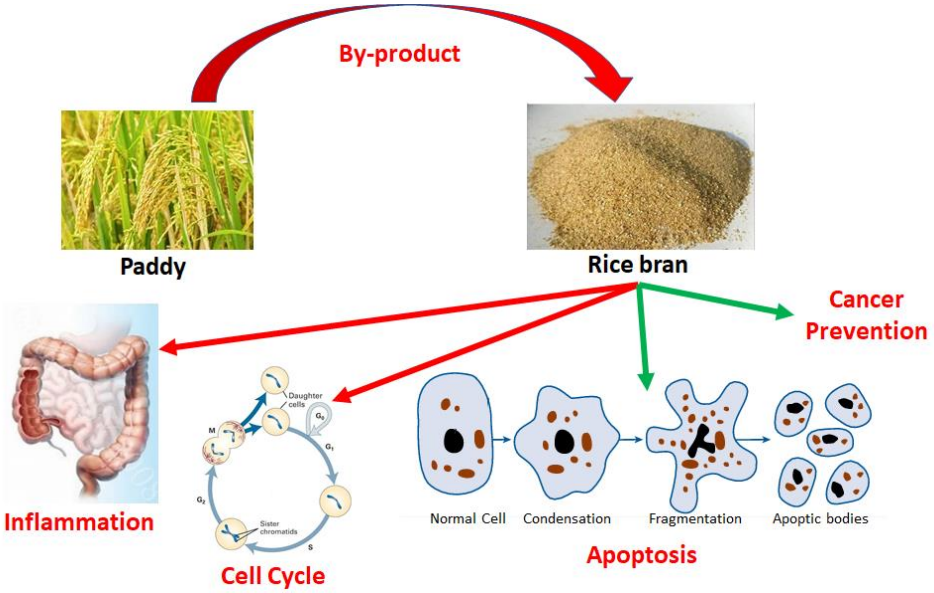

Figure 3. Anticancer activity of rice bran (Yonghui *et al*; 2019)

Anti-Cholesterol activity

The hypocholesterolaemiaproperty of rice bran is due to the binding protein ofbile acidfound in the rice bran (Wang *et al.*, 2015). Zhang *et al.* (2010) concluded that rice bran protein isolates by the action of pancreatic peptidase trypsin lowered the solubility of the cholesterol in the invitro study. The bile acids get bound with the sequestrant factors and insoluble complexes were formed which are eliminated from body. More cholesterol is converted in the liver to bile salts to compensate the excreted bile acids. This lowers the serum LDL cholesterol level and also enhances the expression of low-density lipoprotein receptor (LDL) which.

Table 2. Functional Properties of Rice Bran Protein

Properties	Benefits/ Results	References
Functional Properties		
Solubility	Maximum nitrogen solubility was 82%and 80%at differentpH 12 and 10	Wang *et al.* (1999)
	Maximum solubility of 60% at pH 11	Pinciroli *et al.* (2009)
Foaming ability	Maximum foam capacity between 40-90% and foam stability 30-90min	Yeom *et al.* (2010)
	Foam capacity was more than 60% at pH 11	Cao *et al.* (2009)
Emulsifying capacity	Maximum emulsifying capacity of 0.149 and 0.634 and emulsion stability 24.26 and 25.96 min	Zhang *et al.*(2010)
	Emulsifying capacity between 0.6-0.8 and stability 20-30min	Yeom *et al.* (2010)
Water absorption and binding capacity	Capacity of Water absorptionrate of 3.71 and 4.4g/g Highest water absorption of 3.83mL/g	Zhang *et al. (*2014) Cao *et al.* (2009)

Bio-function properties		
Reducing power activity	6964,2904,2017 and 1809 mmoL of Fe^{2+} for albumin, globulin, prolamin and glutelin.	Chanput *et al.* (2009)
ACE inhibitory activity	IC50 of 0.46mg/mL(hydrolysate)	Chen *et al.* (2013)
Inhibit lipid peroxidation	Reduce the formation of lipid hydroperoxides	Zhao *et al.* (2012)
Other bio functions	Reduces serum cholesterol concentrations	Tang *et al.* (2002)
	Potential to potentiate anti leukaemia immunity	Chen *et al.* (2010)

Applications of Rice bran protein

Rice bran is used in foods in different forms such as defatted bran, full fat bean, rice protein concentrates and bran oil. Defatted bran is used as the binder for meat and sausage product and protein supplements. The protein hydrolysates derived from rice bran has wide application as dietary supplements and functional foods.Meat products which are sensitive to lipid oxidationneed protection against the reactive species. The rice protein liberates antioxidant peptides which decrease the oxidation of meat. (Zhou, Canning, and Sun 2013). It was estimated that therice bran protein hydrolysates would increase the oxidative stability of emulsion and oil (Cheetangdee and Benjakul, 2015). The rice proteins are used as additives in sports nutrition and is also used to substitute casein, whey and soy protein.

The rice bran protein serves as a good source of nutritional supplements and as a functional food.It is used in various bakery goods, sausages etc.because of its oil and water binding properties (Tang *et al.*, 2003). It is also used as a base for desserts and cake batters. Rice bran protein can also be supplemented as an ingredient in infant foods. The rice bran protein has high amount of glutamine and asparagine which functions as the flavour enhancing ingredients in sauce, soup etc. The rice bran protein gained more attention as the ingredient of food in the preparation of products which are gluten free such as bread, cookies, pasta and noodles.The rice bran protein concentrate (2%) was added into gluten free bread which improved the retention of gas, specific volume and shelf life of rice bran protein concentrate. Also, protein supplemented pasta which is gluten free was made by using rice bran protein, whey proteinandsoy protein. The results showed the greater cooking loss andcracked and irregular surface. (Phongthai *et al*., 2017).The rice bran protein concentrate was formulated using alkaline method of extraction and had the oil adsorption capacity of 2.3 mL/g,water adsorption capacity of 2.9 mL/g and thus formulated the protein enriched biscuits which had excellent overall acceptability Yadav *et al*. (2011).The rice bran protein such as albumin and globulin incorporated into tea catechins enhanced the its stability in the process of digestion (Shi *et al*., 2017).

The rice bran proteins were also applied in the fabrication of biodegradable film(Wang *et al*., 2012). The bran protein-based film was fabricated which exhibited comparable functional proteins than the films prepared from soy proteins (Adebiyi *et al.*, 2008). Shin *et al.* (2011) fabricated a composite film of 4% rice bran proteinand 4% gelatinwhich improved the mechanical properties of the films. The Rice bran-chitosan nanoparticles exhibited slower degradability in gastric conditions and digestible completely in the small intestine with better biodegradability (Hailong *et al*., 2017).

Table 3. Applications of rice bran protein

Sample form	Product Type	Usage (%)	Properties	Results

Protein concentrate-Rice Bran	Bread	1-5	Lesser microbial load and%Weight loss than control sample	Jiamyangyuen *et al.*(2005)
Protein isolate-Rice bran	Rice noodles	10	Increase the cooking quality, nutritional quality, properties of dough, and microstructure of rice noodle	Kim *et al.*(2011)
Rice bran protein	Films	4	Used as food packaging film with enhanced properties	Shin *et al.*(2011)
Protein hydrolysate-Rice bran	Yeast culture	2	Supporting the yeast growth same as glucose	Sereewatthanawut *et al.*(2008)

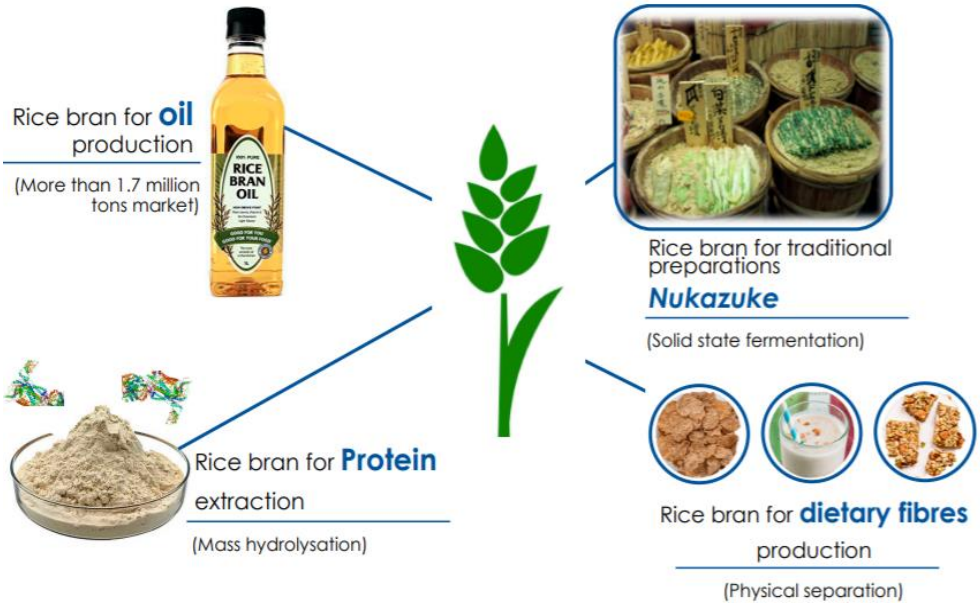

Figure 4. Applications of Rice Bran (Spaggiari *et al.*, 2021)

Conclusion

The protein from rice bran has various application in food processing industry, pharmaceutical, and cosmetic industry. Many researchworksiscarried out on the functional and nutritional properties of rice bran protein. Many experiments are to be carried out to increase the extraction of rice bran protein more economically and efficiently. The antioxidant peptides from the protein were used in food and pharmaceutical industry. Future studies are to be carried out for commercial preparation and characterisation of the antioxidant peptides. The rice bran protein has greater potential to be used as functional foods. Future research works are to be carried out to effectively utilise rice bran protein**.**

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Early shoot borer, *Chilo infuscatellus* (Snellen) incidence in sugarcane-Role of abiotic factors in subtropical climate of India

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Abstract:

Climate is a crucial factor which determines distribution and survival of many ecosystems in nature. Incidence of Crambid moth borer, Chilo infuscatellus Snell. is identified as one of the production constraints in sugarcane. Yield loss could be attributed mainly to moisture stress and ESB. Hence the attempt is made here to identify the role of weather parameters on incidence of seedling borer on sugarcane was carried out at ARS, Sankeshwar during 2017, 2018 and 2019. Results revealed that activity of pest stared from 50 days after planting and the average larval population ranged from 0.5-2.00 per cane. The peak incidence of 1.80 mean numbers of larvae per 5 canes was observed from April-May, which coincides with high maximum (36.00-38.85ºC) and minimum temperature (21.85-24.00ºC). Correlation studies revealed that minimum temperature (r = 0.434**) exhibited positive and were highly significant relationship with mean number of larvae per plant and rainfall events exceeding 50 mm/day controlled the pest during the early stages of crop growth which exhibits negative relationship (r = -0.257) with pest population.

Key words: *Chilo infuscatellus*, Correlation, Rainfall, sugarcane, temperature,

Introduction:

Weather has a highly dominant impact on the incidence of insects or diseases on all crops. The pests that are influenced by weather conditions are termed `Meteotropic' pests. Sugarcane is a long duration crop with luxuriant vegetative growth and is damaged by a number of insects. Yield loss is also increasing day by day due to monoculture. Amongst insect pests termites, borers, pyrilla, whiteflies, bugs, mites, etc. attack this crop and cause heavy losses in terms of yield and sugar recovery. The early shoot borer, *Chilo infuscatellus* Snell. is most notorious and destructive one.

In India, sugarcane is growing in two different agroecological zones the tropical peninsular zone with extreme weather conditions with 69.01 tonnes per ha productivity. Karnataka is one of the leading state in sugarcane production with 68.92 tonnes per ha productivity (Anonymous, 2017). This low yields under rainfed conditions could be attributed partly to moisture stress and partly to *Chilo infuscatellus* damage. In severe cases of infestation, the damage due to ESB could be as much as 90% (Rao et al., 1991). Several attempts were made in the past to relate the incidence of ESB to planting time (Rao et al., 1991 and Sithanantham et al., 1975); varieties/genotypes (Avasthy *et al.*, 1969; Avasthy and Tiwari, 1986); soil factors (Rao et al., 1991), sucrose content (Rao, 1962) and climatic factors like temperature (Rao and Rao 1963) and humidity (Mali, 1990). The moth of the borer being a nocturnal one, its activity is related to moonlight (Rao *et al.*, 1991).

Thermal requirements and survival threshold also influence the activity and abundance of natural enemies, which in return influence the success of biological control programs (Bernal, 1995). Climate change impacts on agriculture are being witnessed all over the world, but countries like India are more vulnerable in view of higher demographic pressure on natural resources and poor coping up mechanisms. Small change in weather factors viz., temperature; relative humidity and rainfall have great bearing on the effectiveness of incidence and abundance of insect pest. Thus investigations were carried out to know impacts of abiotic factors on abundance of pest in sugarcane ecosystem.

Materials and Methods

Monitoring studies were conducted at ARS, Sankeshwar Karnataka during 2017, 2018 and 2019. Incidence of shoot borer was monitored on planted Co-86032 with spacing of 3feet at monthly intervals.

Dead hearts and number of larvae per plant were counted and cumulative incidence of ESB was worked out.

Correlation studies between weather parameters and per cent incidence of ESB was carried out for three consecutive years. Based on correlation studies, regression equation were worked out. Observations on per cent dead heart was recorded by selecting ten plants and out after application by using the following.

$$\% \text{ Dead heart} = \frac{\text{No. of dead hearts per treatment}}{\text{Total number of tillers per treatment}} \times 100$$

Observations were analysed by using SPSS software for correlation and regression analysis with weather parameters.

Results and discussion

The mean incidence of ESB at ARS, Sankeshwar was found to be high in early stage of crop (45 and 60 DAP) and it attains peak during 90DAP. Sugarcane germinates one month after planting thereafter the shoots are exposed to the pest. As the moth takes one month to complete its life cycle, infestation is likely to commence from 45 DAP (Fig 1.). There is high inter annual variability as well as inter periodical variability in the incidence of the ESB (Fig. 2), which could be due to manifestation of several factors including weather factors. The influence of cultivar, soil type and management on ESB as demonstrated by Prasad Rao et al (1991). As present investigations were carried out with identical conditions on one cultivar throughout, hence we can only able to draw possible weather influences on occurrence of pest.

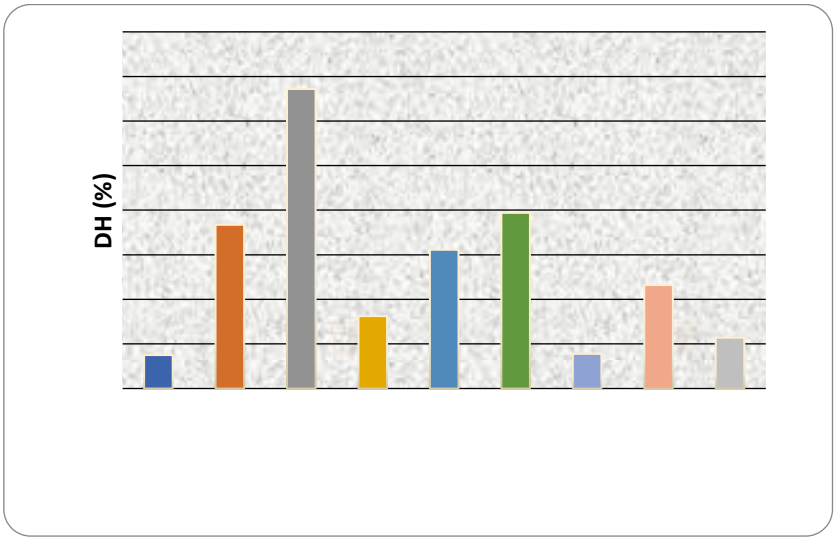

Fig 1: Mean per cent infestation of early shoot borer at different crop stages

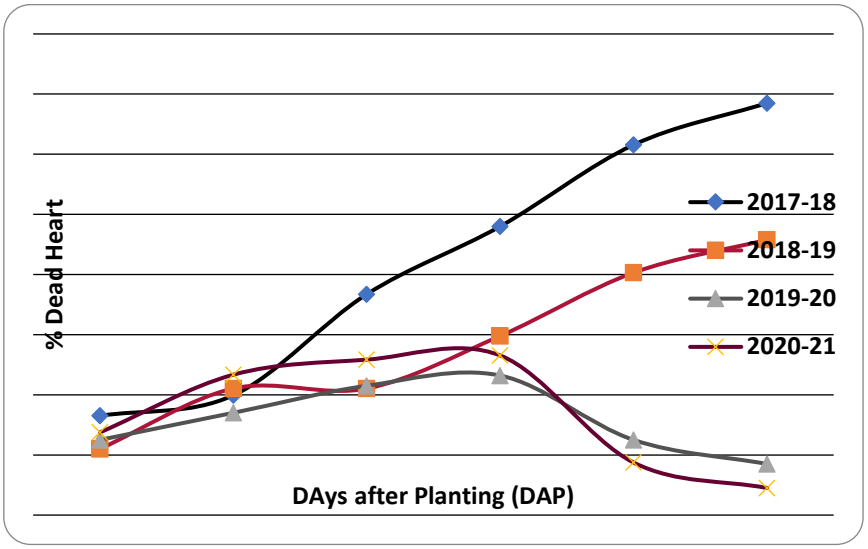

Fig 2. Iner annual variability ESB incidence

Role of weather factors on incidence of ESB in sugarcane

1. Rainfall

Rainfall during 60-90 DAP was observed to reduce borer infestation though the association was not significant. One would expect a washing down of the borer in a high rainfall event. Barring few occasions, the rainfall intensity in most of the years was below 50 mm per day, which might be the reason for the low correlation coefficients with rainfall. During the year 2019, a rainfall of 63.4 mm on 80 DAP, resulted in decline of percent incidence of ESB from 11.60 to 6.25, precipitation of 27.6 mm again on 98 DAP resulted in further decline of percent incidence of ESB from 6.25 to 0.85 by 110-115DAP (Fig 3 and 4). Except this occasion, the influence of rainfall on ESB could not be established. Sithanantham *et al.* (1975) reported that lesser rainfall appear to be favorable for the borer multiplication. Regression equation reveals that rainfall is negatively correlated with population build up (Table 1).

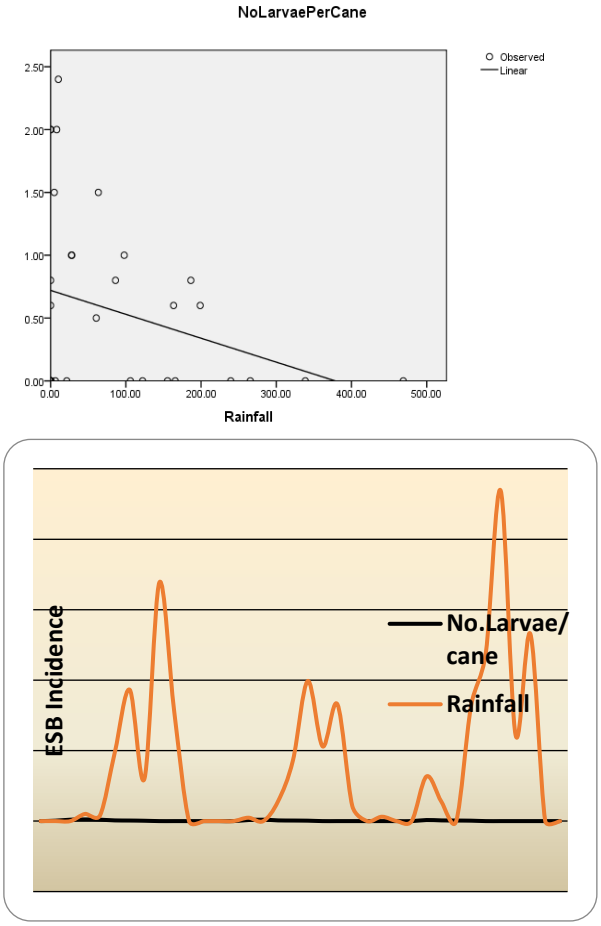

Fig 3: Relationship of Rainfall on incidence of Early shoot borer

Table 1: Interaction of rainfall with larval population

Parameter	Dead heart (X_1)	Rainfall (X_2)	R square value
No. Larvae /Cane	0.800**	-0.257	0.168
		Regression equation	$Y=0.66-0.002X_2$

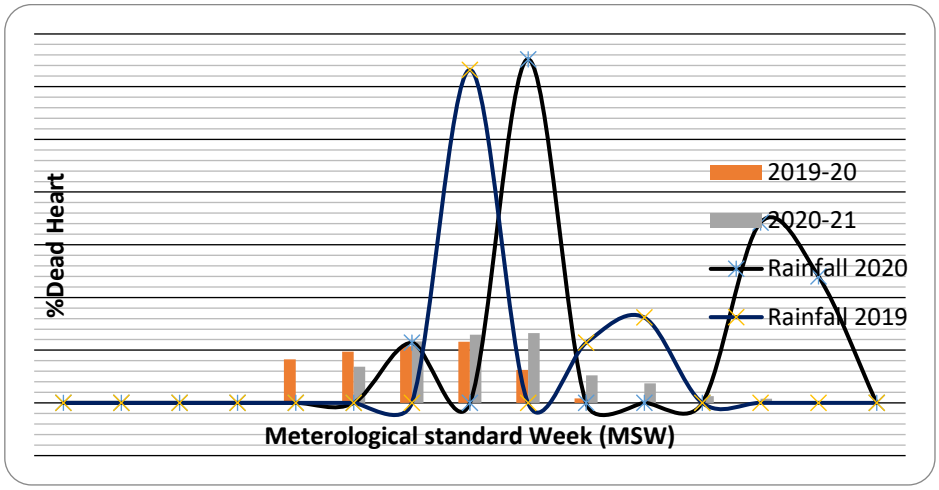

Fig 4. Effect of Rainfall on incidence of Early shoot borer (2019-20 and 2020-21)

2. Temperature

The weekly maximum temperature ranged between 33.3 and 39.6°C and minimum temperature from 17.6 to 29.5°C during peak period of ESB incidence (45-60 DAP). The correlation between maximum temperature and incidence of the pest is significant only during 60-90 DAP. Correlation and regression analysis reveals that temperature has a positive and significant relationship with number of larvae per cane and per cent dead heart (Table 2). The peak incidence of 1.80 mean numbers of larvae per 5 canes was observed from April-May, which coincides with high maximum (36.00-38.85ºC) and minimum temperature (21.85-24.00 ºC) (Fig 5).

The present findings also agree with that of Tanwar and Bajpai (1993), who reported a significant positive correlation between maximum temperature with borer incidence. However, minimum temperature showed a strong association with the pest incidence during major part of the crop season. The association between mean temperature and the pest seems to be dominated by minimum temperature. The diurnal range of temperature behaved as that of maximum temperature. In the earlier studies also (Hapse *et al.*, 1979, Avasthy and Tiwari, 1986), the conducive role of maximum temperature was noticed. In a two year study on light trap catches of ESB moths, Rao and Babu (2004) found significant positive correlation between moth catches and maximum, minimum temperatures. Early morning relative humidity in the present investigation was found to profoundly influence the borer infestation and high humidity reduced the infestation levels (Table 1). It is interesting to note the contradictory role of minimum temperature and relative humidity. For a better explanation of this abiotic factors one has to look into the insect behaviour. The moth of the pest is nocturnal and its movement and ovipositional activity is mainly during night time. High night time temperatures coupled with low humidity might have favoured the moth movement during nights. Conversely, cool nights coupled with humid weather must have curtailed the moth movement or larval hatching or both. Absence of data on night time wind speed became a handicap to draw a more meaningful role of weather on this nocturnal behaviour of the pest. Avasthy and Tiwari (1986) noted the ovipositional activity of moths after midnight. Rao et al (2013) reported that relatively warmer (> 23.8ºC) and RH < 77% favoured the incidence. But rainfall events exceeding 50 mm/day resulted in decline of the pest at early stages of crop. Correlation studies revealed that maximum (r = 0.732**) and minimum temperature (r = 0.741**) exhibited positive and were highly significant association with mean number of larvae per plant (Matti *et al.*, 2019). A firm conclusion, however, could not be drawn to predict the incidence of ESB based on weather parameters.

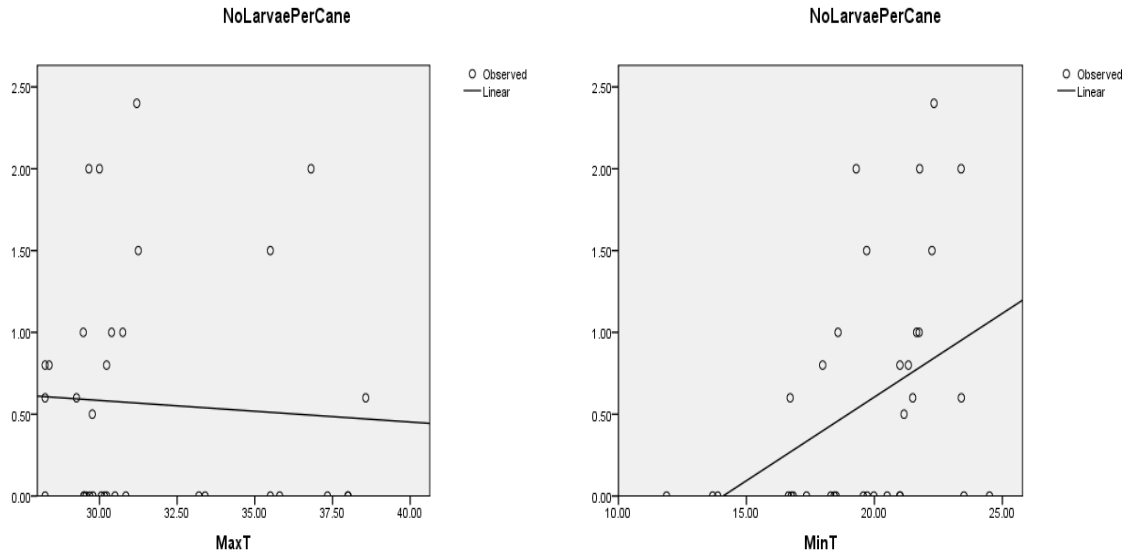

Fig 5: Incidence in relation to Temperature

Table 2: Correlation with temperature

S.No	Parameters	MaxT (X_1)	MinT (X_2)	Regression Equation	R2 value
1	**No. Larvae/5 canes**	0.732^{**}	0.741^{**}	$Y=-5.727+0.125X_1+0.154X_2$	**0.714**
2	**Deadheart (%)**	0.221	0.823**	$Y=-52.95-1.38X_1+5.58X_2$	**0.732**

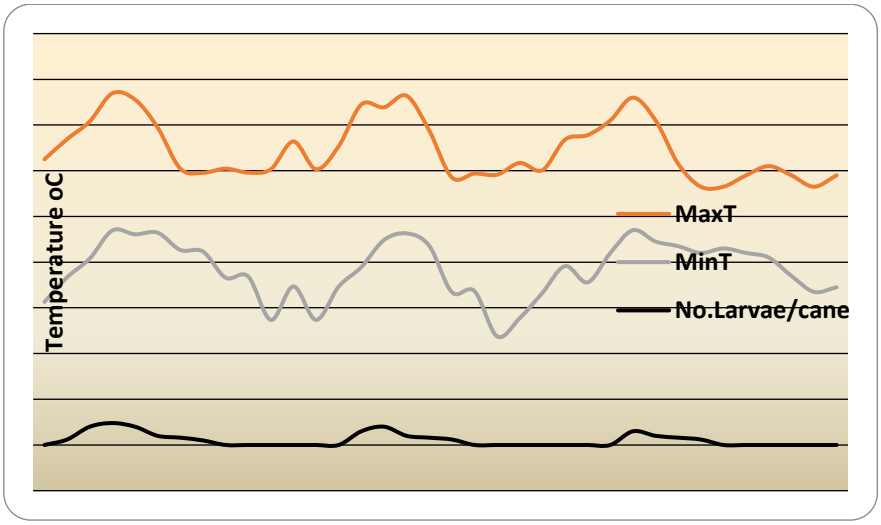

Fig 5: Influence of temperature on number of larvae per cane

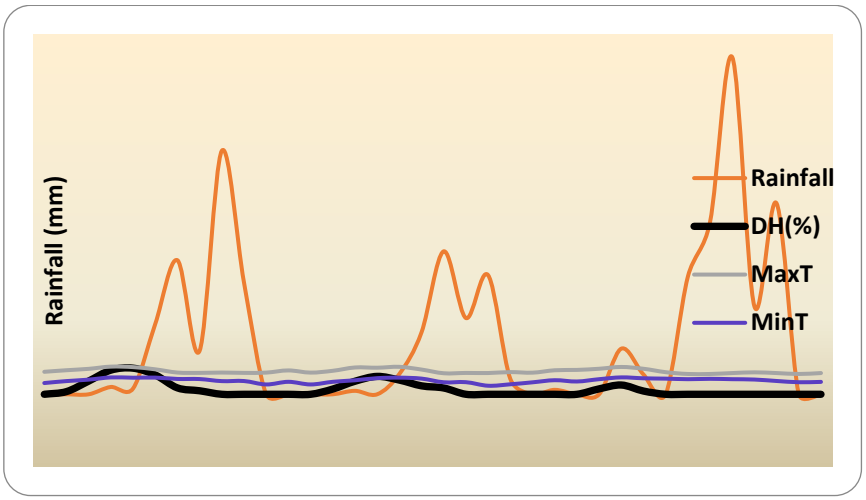

Fig 6. Impact of Weather on per cent infestation of early shoot borer

Conclusions

Climate change have major impacts on agricultural production and mainly on insect pests, but countries like India are more vulnerable in view of higher demographic pressure on natural resources and poor coping up mechanisms. Small change in weather factors have great bearing effectiveness of incidence and abundance of insect pest in many ways. Among the insect pests, early shoot borer is a serious pest in peninsular regions of India and more vital in early stages of crop growth causing economic loss. Incidence of ESB on sugarcane started from 40 DAP and the development and oviposition of ESB seems to be favoured by relatively warmer (> 23.8ºC) and RH < 77%. Declined population of ESB due to high rainfall more than 50 mm/day may control at early stages of crop growth.

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