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WILDLIFE MANAGEMENT UNDER ENCROACHMENT AREA OF SARISKA TIGER RESERVE: A FUTURE CHALLENGES AND OPPORTUNITIES

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ABSTRACT

Wild tigers experience unparalleled coercion due to habitat destruction, prey reduction and commercial poaching. The Indian tiger (*Panthera tigris tigris*) one of the world's most endangered carnivore species, and is now thought to be in the verge of extinction in the wild. Sariska National Park in India is considered to be a highly suitable habitat for the tigers. Relocation and change in habitat of these living giant creatures is a thought of study for their survival and existence in the coming future considering change in climatic conditions. But the main problem for the tigers in the new habitat will be poaching and the human-wildlife conflicts. Integrated geospatial techniques provide accurate, cost-effective as well as time-effective method for habitat evaluation. The aim of the study is current status followed by opportunities and challenges. The results point out a large and comprehensive research on each of these issues, in particular on the community involvement in wildlife management and government policies.

Keywords: Wildlife, Geospatial technology, Sariska tiger reserve, *Panthera tigris tigris*.

INTRODUCTION

India is the seventh largest country in the world and Asia's second largest nation with an area of 3,287,263 km2 , a national border of 15,200 km, and a coastline of 7516 km. The people of the Indian subcontinent were once blessed with some of the most profuse natural gifts: verdant forests, water-stocked Himalayan ranges, rich coastal fish resources, productive estuaries, grassy pastures, and bountiful river systems (Srivastava, 2009; Walston et al., 2010; Change and Wang, 2009 Gurnell *et al*., 2002). Years of mismanagement, however, have degraded our forests, wounded our coastline, and poisoned our aquifers with devastating results. Today, India contains 172 species (2.9% of the world's total number) of animals that are considered to be globally threatened by the IUCN. These include 53 species of mammals, 69 species of birds, 23 species of reptiles, and 3 species of amphibians. Establishment of protected areas (PAs) has been the most widely accepted means of biodiversity and wildlife conservation so far, supported by national and international agencies. However, alarming loss of habitat makes it difficult for wildlife to adapt. The reason for such alarming habitat loss is because international conservation policies have neglected considering niche habitats of species when setting targets for the expansion of protected areas. The global protected area network, which implies forests or other ecosystems protected under law, don't cover the environmental or climatic conditions required by a majority of 19,937 vertebrate species assessed in the study. The loss of Sariska Tigers Reserve (STR) was the most prominently broadcast wildlife story in post-Independence India (Butfiloski *et al*., 1997; Rittenhouse *et al*., 2006; Roy *et al*., 1995; Singh *et al*., 2009).

Natural forests and wild animals that live in it can still live without the help of humans. Whereas human society cannot maintain its existence for many days in the absence of them

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(Kushwaha et al., 2000; Kushwaha *et al*., 2004b; Larson *et al*., 2003; Aspinall and Veitch, 1993). As much as centuries of thought have been given to the ancient Indian society about conservation of natural resources and wildlife, it is not even in Western knowledge. But the irony is that today, foreigners are telling us about conservation of nature and wildlife, while conservation of nature is a part of our Indian way of life.

Formerly a dominant part of Sariska's ecology and culture, tigers were missing from some areas of this landscape for decades before being officially declared extirpated (locally extinct) in 2005.In July 2008, two tigers from Ranthambhore National Park were relocated to Sariska Tiger Reserve. Another female tiger was relocated in February 2009 (Sankar et al., 2010). Also, translocations will likely increase in popularity after Jhala et al. (2015) reported a 30% increase in India's tiger population, now reported at 2,226. Rising tiger populations in isolated protected areas (PAs) pose a conservation concern, as competition for resources can expedite intra-specific conflict (conflict within a species population), prompting translocations. Effective conservation of the endangered tiger depends upon reliable knowledge of factors driving genetic differentiation and population connectivity (Reddy et al 2017). As per state forest department, the major factor which tilted the scales against Sariska, despite the honest intentions with which the tiger relocation programme started here, is the presence of 29 villages inside the reserve, Sariska is on the verge of losing its tigers once again. There is a great need for conservation of wildlife while studying the causes of wildlife loss in STR. it was hypothesized that stress caused by these anthropogenic pressures in the habitat might have an influence on the reproductive potential of the introduced tigers in Sariska and a study was conducted (Bhattacharjee *et al*., 2015).

This study makes an explorative overview on two main research topics in policies of wildlife management in Sariska tiger reserve: current status followed by opportunities and challenges. The results point out a large and comprehensive research on each of these issues, in particular on the community involvement in wildlife management and government policies.

1. Wildlife and Environment

The pressure of development has led us to the brink of environmental crisis today by prudent exploitation and exploitation of natural products that now even the survival of the natural is in crisis. In such a situation, what will be the value of our priceless heritage wildlife? Lack of pure life, degradation of soil origin from contaminated water, fertilizers and synthetic chemicals, shrinking natural forests, increasing temperature due to gaseous pollution in the atmosphere, depletion of organic-borne nutrients in food grains and lack of rainfall, etc. (Bian and West, 1997; Brooks, 1997; Conner and Leopold, 1998; Craighead, 2008). Today, if there is a stroke, then a question mark is automatically put on the conservation of vegetation and wildlife. In other countries of the world, wild animals have been destroyed due to hunting and their neglect. As a result, the number of wild animals and birds has remained very low. Many breeds have perished. Therefore, in such a situation, the need was felt to protect and preserve these wild animals in national parks, protected forest areas. Therefore, like many countries, national parks, sanctuaries have also been established in India (Debeljak et al., 2001; Donovan et al., 1997; Edenius and Mikusinski, 2006)..

2. Sariska Tiger Reserve : At the verge of loos wildlife

The Sariska Tiger Reserve (STR), is compromised of the Sariska Wildlife Sanctuary and adjoining areas in the Alwar district of Rajasthan, a state in India (Kumar et al., 2018a; Kumar et al., 2018b; Kumar et al., 2014a; Kumar et al., 2014b; Kumar et al., 2012; Rani et al., 2011) STR stretching for 1200 Km^2 located between 27°13' N to 27°31' N latitudes and from 76°15' E to 76°33' E longitudes (Figure 1). It lies in Alwar district of Rajasthan state in India. Government of Rajasthan (2007) notified three distinct zones in STR viz. critical tiger habitat (881.11 Km^2), protected forest (276.14 Km^2) and reserved forest (604.97 Km^2).

Prior to the formation of the state of Rajasthan, the Reserve was a part of the Alwar princely state, managed solely to supply hunting grounds for the royals. After India's independence, Government of Rajasthan assumed control of the Reserve. The Sariska was declared as a Wild Life Reserve area in 1955 under the Rajasthan Wild Animals & Birds Protection Act of 1951. The park was brought under Project Tiger in the second phase of the Project's expansion from 1978-79. In 1982, the land was declared as Tiger Reserve. The Ministry of Environment & Forests (MoEF) issued guidelines to the state government to delineate the Critical Tiger/wildlife habitats in tiger reserves / protected areas. According to the guidelines, delineating of critical tiger habitat (CTH) is required for the sustenance of a viable population of tiger and other wild animals in tiger reserves and protected areas. For supporting viable population of tigers, a minimum inviolate space of 800-1,000 km^2 should be maintained based on tiger life history parameters, territory sizes, and population viability analysis.

According to the tiger census of October 2018 in this national park famous for tigers, there are a total of 18 tigers,

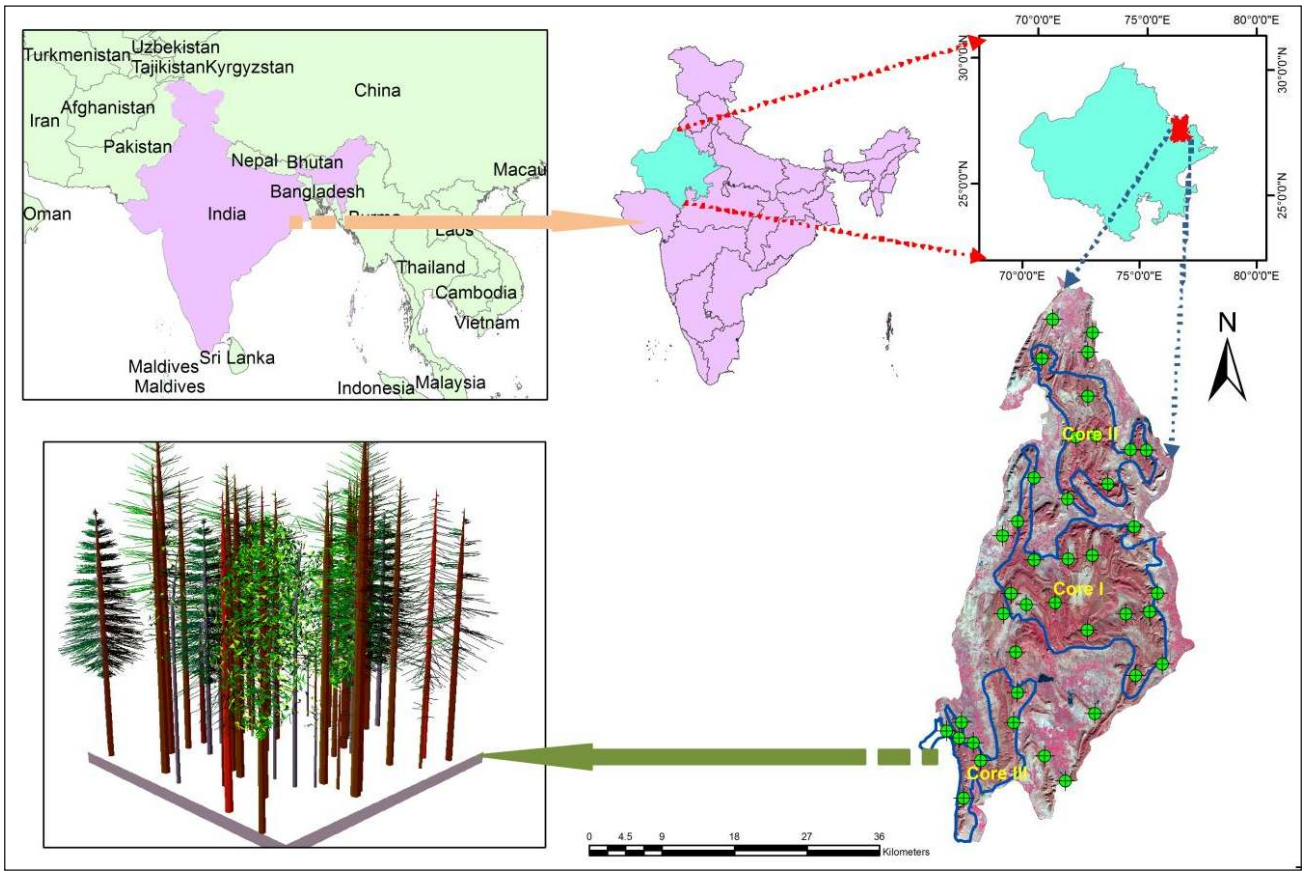

Figure1: Map of Study area

but it is not that only tigers are found here, this national park of India has always been a natural habitat of wildlife, and among these wildlife Some are predatory animals and some are prey animals. Among the wildlife found here, you get to see magnificent wildlife like wild cat, Indian leopard, golden jackal, nilgai, striped hyena, caracal, wild boar, deer, fox, sambar, chital, rhesus macaque, langur. The environment of this sanctuary is very favorable for bird habitat. In this park you can find a confluence of migratory and native birds. Indian peacock, bush quail, golden-backed woodpecker, Indian eagle-owl, gray partridge, white-throated kingfisher, Native and exotic birds such as trepees, sandgrass, crested snake eagles are seen.

Primary hetrotrophs

The herbivorous animals or the primary consumers which depends on the producers in the preceding paragraphs are Sambhar, Nilgai, Chital, Wildboar and Chausingha. They are distributed through out of area. Their distribution pattern is governed by the food habits. Deep dense woody areas mainly in the valleys like kalighati, Silibery, Jhaj, Naldeshwar Panidhal, Bandipul etc. are occupied by the Sambher while outskirts of these valleys which are having plain land with modest slop are liked by the Chital, Nilgai and Wild boar. The Langur (*Presbytis entellus* Eschsch.) is common through forest but their concentration is near temples.

Secondary hetrotrophs

The Leopard (*Panthera pardus* L.) and Tiger (*Panthera tigris* L.) are the principle predators. Important niches of tigers are Bandipur, Algwal, Tarunda, Tunda Chhalipaj, Chamoli, Slopka, Kalighati and Jahaj. The Leopard prefers outskirts of the forest tract but also in the tiger niches. Though the vegetation is not very dense here you are unlikely to spot tigers in the daytime as the tigers at Sariska are nocturnal creatures. Sariska national park is home to numerous carnivores, including Rusty Spotted Cat (*Prionailurus rubiginosus* L.), Jackal (*Canis aureus* L.), Leopard (*Panthera pardus* L.), Caracal (*Caracal caracal* Schreb.), Jungle Cat (*Felis chaus* Schreb.), Tiger (*Panthera tigris* L.), Hyena (*Hyaena hyaena*) and Wild Dog (*Cuon alpinus* Pallas.). These feed on an abundance of prey species such as Sambar (*Rusa unicolor* Kerr.), Cheetal (*Axis axis* Erxleben.), Nilgai (*Boselaphus tragocamelus* Pallas.), Chausingha (*Tetracerus quadricornis* Blainville.), Wild Boar (*Sus scrofa* L.), Civet (*Viverricula indica* Dasmerest.), Palm Civet (*Paradoxurus*

hermaphroditus Pallas.) Sariska is also well known for its large population of Rhesus Monkeys (*Macaca mulatta* Zimmermann), which are found in large numbers around Talvriksh. The avian world includes the Great Indian Horned Owl (*Bubo bubo* L.) Peafowl (*Pavo cristatus* L.), Grey Partridge (*Perdix perdix* L.), Golden backed Wood Pecker (*Dinopium benghalense* L.), Bush Quail (*Perdicula asiatica* Sykes.), Sand Grouse (*Pterocles indicus* Gmel.), Crested Serpent Eagle (*Spilornis cheela* Latham) and Tree Pie (*Dendrocitta vagabunda* Latham) (Figure 2).

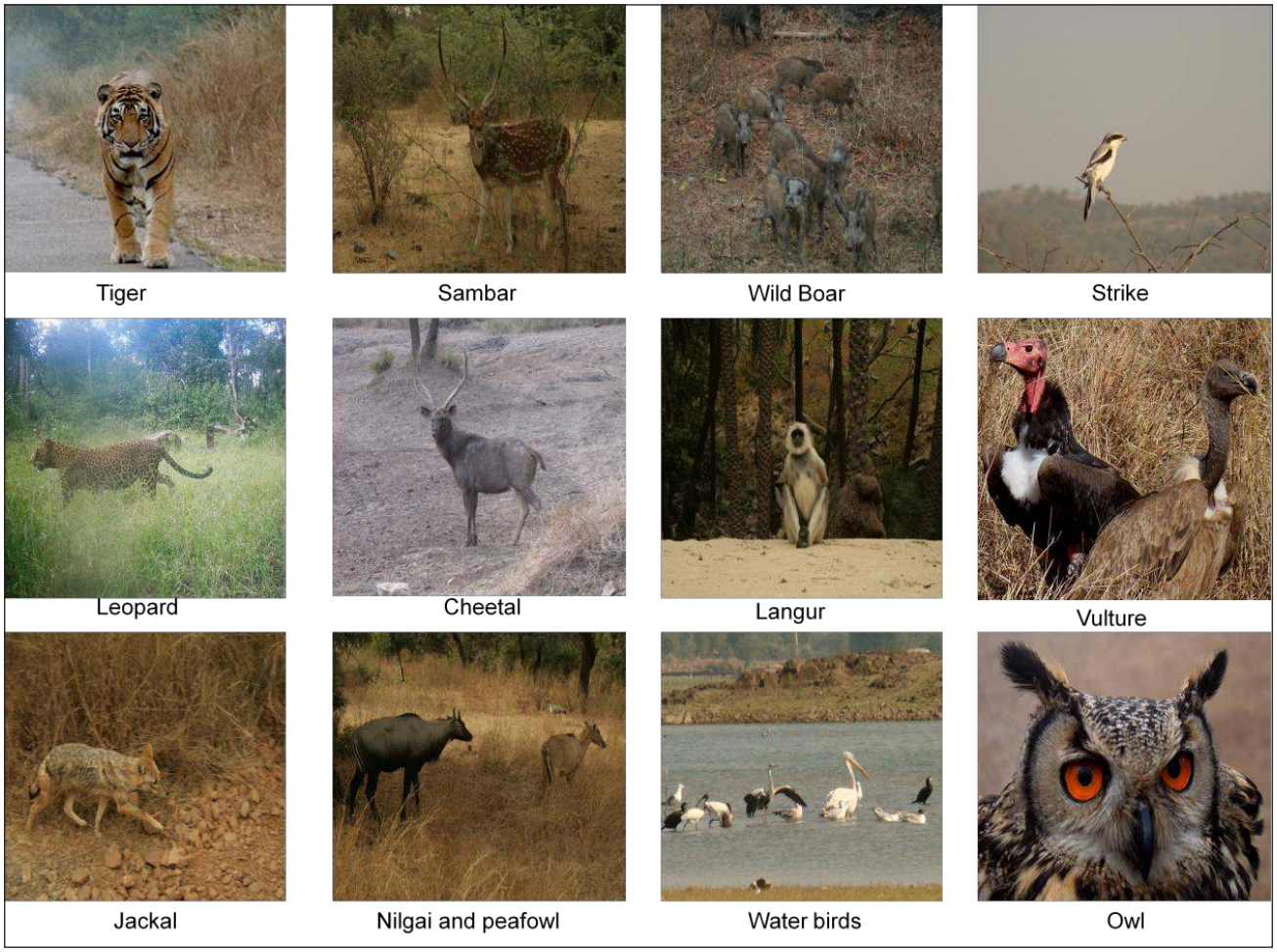

Figure 2: Faunal diversity in Sariska Tiger Reserve.

1. Community and wildlife conflict

Very high anthropogenic disturbances due to large number of villages in core and adjoining areas, organized poaching, high pilgrimage, state highways passing through the core area, low strength of frontline staff for enforcement, lack of commitment and careless approach were some of the identified factors responsible for local extinction of tigers. Local people also move in and out Sariska extensively, as selling livestock milk is the primary economic activity. Consequently, livestock graze and fodder is collected from inside Sariska and the buffer daily (Jain and Sajjad, 2016). These and other pressures (e.g., highway traffic, pilgrimage traffic, village relocation, tree felling) continue to impact Sariska (Johnsingh & Madhusudan, 2009). About 2254 families live in the core area while about 12000 families live around the Critical Tiger Habitat (Garcia and Armbruster, 1997; Gerrad et al., 2001; Gurnell et al., 2002) thus making this reserve as human dominated landscape that is subjected to immense anthropogenic pressures. Tigers only have 50 km2 of the 882 km2 reserve to themselves due to villages inside Sariska and other anthropogenic pressures (Sebastian, 2016).

Some researchers with a political ecologist view attribute the poor performance to the failure to devolve authority to local communities and unequal distribution of tangible economic benefits around PAs (Heglund, 2002; Jessup,

1998; Kamat, 1986; Keith et al., 2008). On the contrary, biologists claim that wildlife species are at risk if local people are given more priority over conservation objectives. (T Kelly and Goulden, 2008; Kushwaha et al., 2004; Kushwaha et al. 2004). But the general consensus is that such projects if successful, would lead to better support from local communities towards conservation. Non-recognition of community role by state in conservation has led to conflicts over land and poachers took the opportunity of it. Also, human-tiger conflict is a major problem. The future of conservation now depends on solutions that minimize these conflicts.

Wildlife policies and implication in India

Enforcement of various wildlife and forests laws in any protected area is the key to the protection of biological diversity of the area. Although many studies have been done in Sariska tiger reserve focusing mainly on the ecological, biological and technical aspects of floral and faunal components of the landscape (Sankar and Johnsingh, 2002: Lai et al., 2000; Lancia et al., 1982; Larson et al., 2003; Luck, 2002b; Lurz et al., 2008; Osborn et al., 2001; Ozesmi and Mitsch, 1997) or critical review of the working of forest department with local communities (Radeloff et al., 1999; Rittenhouse et al., 2006; Schamberger et al., 1982) but the studies related to wildlife law enforcement are still lacking.Despite challenges associated with patrolling vast landscapes (Schamberger and O'Neil, 1986; Senapathi et al., 2007), research suggests that frontline law enforcement presence is necessary to curb wildlife crimes (Shalaby et al., 2007).Wildlife Protection Act, 1972 is comprehensive enough to tackle the crisis with regard to wild life protection. However, it is believed that, in spite of having comprehensive legislation, there is no sign of reduction in the poaching of wild animals and other offences related to habitat destruction in many of PAs, therefore, it can be concluded that enforcement mechanism is suffering decay from within (Store and Jokimäki, 2003). Accordingly, it raises the question with regard to effective implementation of the provisions of wildlife law. The draft National Tourism Policy of 1997 in India, speaks of "maintaining a judicious balance between conservation and development". The policy addresses social and environmental impacts and also suggests guidelines for sustainable growth, but does not discuss the legal or institutional framework for activities that would contribute to sustainable development, for example wildlife tourism, or the role of local communities in tourism development. It is also seen that the enforcement mechanism of the laws in India for the conservation and protection of wildlife is also complicated in nature. The laws, on one hand, enable the forest officers to protect the forests resources, but they are not given any powers to make policies pertaining to the situation which further creates problems in the confiscation of the felled timber or the poached animal. It was concluded that the protection of forest and wildlife depends on the efficient enforcement of meticulous provisions by the enforcement agencies (Poddar, 2017). Implementation of existing forest and wildlife laws in a protected area or a tiger reserve is the determining factor for the long term conservation of biological diversity of that area.

Wildlife policies are typically motivated by the wildlife species imposing an external cost of some kind, e.g., on agricultural or forestry production, or by the species providing benefits that have public good characteristics, e.g., if the species is threatened and its preservation is considered highly valuable. There is a large body of literature on voluntary cooperation on management of natural resources, but we have not found applications to wildlife resources in this regard. However, conditions for successful voluntary cooperation are restrictive, and governmental interventions with coercive power are often needed for improved management.

CONCLUSION

Local communities are vulnerable to the establishment of protected areas, particularly in developing countries since their livelihoods are dependent on them. They pay indirectly not only by loss of access to resources like fuel wood, fodder and other non-timber forest products, but often by direct losses from crop and livestock raiding by wild animals dispersing from protected areas. Some researchers with a political ecologist view attribute the poor performance to the failure to devolve authority to local communities and unequal distribution of tangible economic benefits around protected areas. On the contrary, biologists claim that wildlife species are at risk if local people are given more priority over conservation objectives. Linking communities with conservation goal can be a viable option however, linking economic benefits to conservation is difficult where wildlife is highly endangered, pressure on biomass resources is high, and stakeholders are many.

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GROWTH PERFORMANCE OF TISSUE-CULTURED ´LAKATAN´ BANANA (*MUSA ACUMINATA*) PLANTLETS USING STIMULANTS

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ABSTRACT

The study aimed to determine the effects of stimulants on the growth performance of tissue-cultured 'lakatan' banana plantlets *(Musa acuminata)* under nursery condition; to determine the best treatment combination that will increase the growth performance of tissue-cultured 'lakatan' banana plantlets; and to determine the economic benefits of using stimulants in tissue-cultured 'lakatan' banana plantlets.

The study was laid out in a Complete Randomized Design (CRD) with seven treatments and replicated thrice with eight samples per replicate. The treatments were; T1- Control (No Application); T2- Conventional Practice (CP) (5 grams N-P-K every 15 days); T3- Bio-Forge (5 ml/ liter water foliar application); T4- CP + Bio-Forge (0.125 grams of N-P-K + 5 ml of Bio-Forge/ liter water); T5- CP + Stimulate (5 grams of N-P-K + 10 ml of Stimulate/ liter water); T6- Bio-Forge + Stimulate (5 ml of Bio-Forge/ L. of water + 10 ml of Stimulate/liter water); T7- CP+ Bio-Forge+ Stimulate (5 grams of N-P-K + 5 ml of Bio-Forge/ liter water + 10 ml of Stimulate/ liter water). All data were analyzed through Analysis of Variance (ANOVA) and differences among treatments were computed using Honest Significant (HSD) test.

The results of the study revealed that the percentage of survival and pseudostem diameter at 15 Days after planting (DAP) was not significantly affected by different treatments. Plant height, length of leaves, width of leaves, pseudostem diameter (30 & 45), number of leaves, root length and number, fresh and dry weights at 15, 30 and 45 DAP have significant effects. The T7 - (Conventional Practice + Bio-Forge + Stimulate) significantly increased plant height by 7 times, width of leaves by 3 times, stem diameter by 6 times, root number by 3 times, fresh weight by 17 times which were comparable with T6- (Bio-Forge + Stimulate), T5- (CP + Stimulate), T4- (CP + Bio-Forge) and T3- (Bio-Forge).

The T3 (Bio-Forge) had the highest leaf length increase by 10 times, root length by 100% which is comparable to T5- (CP + Stimulate), T6- (CP + Stimulate) and T7- (CP + Bio-Forge + Stimulate). While T4- (CP + Bio-Forge) had the heaviest dry weight by 28 times which is also comparable to T3 (Bio-Forge), T5 (CP + Stimulate), T6 (Bio-Forge + Stimulate) and T7- (CP + Bio-Forge + Stimulate).

Keywords: Growth Enhancer, Tissue-Cultured, ´Lakatan´ Banana, Musa acuminata, Plantlets, Stimulants.

INTRODUCTION

Banana and Plantains (*Musa sp.*) are the major staple food for many millions of people throughout the humid and sub-humid tropics. The export trade in banana amounts to about seven million tons, indicating that the crop is mainly grown as a food crop for local and export consumption. Banana is an important source of income for small farmers who constitute 75% of the banana growers among the 80 distinct Philippine banana cultivars, Lakatan, Latundan, Bungulan and Saba, which are popularly grown for the local market. Banana has a great significance; the fruit composed mainly of water as well as carbohydrates, which provides energy in human body. The unripe fruit crop contains more starch and

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less sugar as compared to the ripe fruits. It contains 11 vitamins; among them are vitamin A, B and C. Nowadays, Lakatan is considered as one of the most important banana cultivars in domestic and export market, moreover, it is the leading fruit crops in terms of volume, area and value of production with the national average yield of 9.4 tons/ha. (http://www.pcarrd.dost.gov.ph).

Edible banana do not form seeds and are propagated vegetatively. In the Philippines, farmers traditionally used sword suckers as planting materials. These planting materials are in great demand during the period when most growers replant their orchards or farm every season to adjust the harvesting period for local market or export. However, one disadvantage of this method is that the disease can be retain in the suckers, causing loss of productivity in the new banana plant. By tissue culture, plants can be free from virus, fungi, bacteria, nematodes and pathogen (Mwinga, 2006). The tissue cultured technique developed by Damasco and Barba (1984) as cited by Sim (2012) was further modified to sustain and become economically viable in vitro propagation system for banana. This technology was developed for the purpose of producing a high and disease-free plants for wide areas. Furthermore the tissue cultured derived plants performed much better in terms of growth, vigor and yield.

Banana plantlets are therefore mass produced through tissue culture. However, growth performance of these plantlets is limited by erratic environmental condition and nutritional constraints in the nursery. Bioforge up-regulates key genes associated with stress: DREB1A, Catalase, Dehydrin RAB18, RD29A. It also up-regulates genes controlling root hair growth to enhance nutrient uptake: RLS4 and reduces excess ethylene from stress but leaves ethylene required for normal functioning unaffected. Bio-Forge® enhances seedling emergence. It is a concentrated formulation of Bio-Forge with additional humic acid to ensure early root development and protection from stress on young plants, especially stress associated with cold spring soils or water logged conditions. Bio-Forge ST promotes root nodulation and nodule activity in legumes. Plus, it ensures continuous new root growth for efficient nutrient uptake, especially nitrogen. Overall, Bio-Forge ST improves plant growth hormone balance for continued cellular viability and optimal plant functioning throughout the life of the plant. While Stimulate contains a combination of 3 different phytohormones: cytokinin, gibberellic acid, and indole-3-butyric acid necessary to stimulate cell division, cell differentiation and enlargement, nutrient uptake, and nutrient utilization (www.stollerusa.com).

Hence, this study aimed to determine the effects of bio-forge and stimulate on the growth performance of tissue-cultured 'lakatan' banana plantlets under nursery condition and to determine the best treatment combination that will increase the growth performance of tissue-cultured 'lakatan' banana plantlets.

MATERIALS AND METHODS

The study was conducted at the research nursery area of the University of Southeastern Philippines, Tagum-Mabini Campus, Mabini Unit, Mampising, Mabini, Compostela Valley Province from the month of November 2014 to January 2015 (Figure 1). The study was laid out in a Complete Randomized Design (CRD) with seven (7) treatments and replicated three (3) times. Each treatment was composed of eight (8) plantlets. Note: (1st Application: 6-25 hrs. after transplant; 2nd application: 7 days after transplant) in both 5ml Bio-Forge and 10ml Stimulate).

The treatments were: T1- Control (No Application); T2- Conventional Practice (CP) (5 grams N-P-K every 15 days); T3- Bio-Forge (5 ml/ liter water foliar application); T4- CP + Bio-Forge (0.125 grams of N-P-K + 5 ml of Bio-Forge/ liter water); T5- CP + Stimulate (5 grams of N-P-K + 10 ml of Stimulate/ liter water); T6- Bio-Forge + Stimulate (5 ml of Bio-Forge/ L. of water + 10 ml of Stimulate/liter water); T7- CP+ Bio-Forge+ Stimulate (5 grams of N-P-K + 5 ml of Bio-Forge/ liter water + 10 ml of Stimulate/ liter water). All data were analyzed through Analysis of Variance (ANOVA) and differences among treatments were computed using Honest Significant (HSD) test.

A total of 180 plantlets of tissue-cultured ´lakatan´ banana plantlets ready for potting from the tissue culture laboratory of USeP-Mabini Campus was used in the study. The tools and equipment used include polyethylene bags (4x6¨), shovel, grass hook, bolo, caliper, ruler, ballpen and record book for recording the data. The nursery was prepared by clearing the area and providing a shade of net to condition the plantlets. The potting media were used was coco coir and vermicast. Both were pulverized and properly mixed during bagging operation using the standard ratio of the potting media. The potting medium was composed of 80% coco coir and 20% vermicast. This was done by rebagging the plantlets into 4x6 inches polyethylene bags. One piece of tissue-cultured ´lakatan´ banana plantlet was planted per bag and watered immediately. Basal and side-dress application of complete fertilizer was applied based on conventional practice two weeks after planting. The newly transplanted plantlets were protected from direct sunlight by shading them in a nursery net. The shade was gradually

removed until the plantlets survived in the direct sunlight. Watering was done right after planting and thereafter whenever necessary early in the morning and late in the afternoon to provide enough moisture for growth and development of plantlets. Weeding was maintained from planting to termination. It was done by hand pulling to ensure that the growth of tissue-cultured 'lakatan' banana was not disturbed.

Data Gathered

The percentage of survival was obtained by counting the number of plants that survived at 15 days from planting. The plant height was determined by measuring 5 sample plants per treatment from the base up to the growing point where the leaves intersect. Measurement was done at 15 days interval using a tape measure/ruler and expressed in centimeters (cm). The increment was computed by subtracting the final height and the initial height. The length of fully developed leaves was determined by measuring 5 representative sample leaves per treatment per replication using ruler at 15 days interval. The increment was computed by subtracting the final length and the initial length. The width of leaves was taken from the same sample leaves which were obtained in length measurement using a ruler at 15 days interval. The increment was computed by subtracting the final width and the initial width. The Pseudostem diameter was determined by measuring 5 representative samples per treatment at 15 days interval using a vernier caliper from the base of the stem. The increment was computed by subtracting the final diameter and the initial diameter. Number of Leaves was determined by counting 5 representative samples per treatment at 15 days. The fully developed leaves were being counted prior to emergence. Root Length was determined by measuring one (1) sample plant per replication per treatments using ruler from the base up to the tip of the primary roots and expressed in centimeters. Number of roots was taken from the same plant per treatment in each replication by counting the number of primary roots per plant on the same one (1) sample upon termination and was expressed by number of quantity. At the time of termination, one (1) fresh sample plant per replication per treatment was taken and weighed at random from each plot.The sample plants taken at random from each plot was oven-dried at 105^0C for a span of 16 hours to remove completely its moisture content and weighed expressed in grams.

RESULTS AND DISCUSSION

Percentage of Survival (%)

The percent of survival of tissue-cultured 'lakatan' banana plantlets at 15 days after planting (DAP) is presented in Table 1. All tissue-cultured plantlets survived in all treatments. This implies that all the sample tissue-cultured plantlets have high survival capacity. This result coincides with the statement of Abaniza (2008) as cited by Ycot (2005) that tissue-cultured plantlets provide a very high survival after planting which will result in uniform stand.

Table 1: Percentage of survival on tissue-cultured 'lakatan' banana plantlets using stimulants.

	REPLICATION			MEAN
TREATMENT	**I**	**II**	**III**	
T1- Control (Untreated)	100	100	100	100
T2- Conventional Practice	100	100	100	100
T3- Bio-Forge	100	100	100	100
T4- CP + Bio-Forge	100	100	100	100
T5- CP + Stimulate	100	100	100	100
T6- Bio-Forge + Stimulate	100	100	100	100
T7- CP + Bio-Forge + Stimulate	100	100	100	100

Plant Height Increment (cm)

Table 2 and Figure 2 presents the Plant height increment of tissue-cultured 'lakatan' banana plantlets using stimulants at 15, 30 and 45 Days after planting (DAP). The result of statistical analysis shows highly significant effects on the 15, 30 and 45 DAP. Based on the result, highest increase in plant height was observed in T7- Conventional Practice + Bio-Forge + Stimulate which is up to 7 times higher than the T1- Control (Untreated) at 15, 30 and 45 Days after Planting (DAP). T7- Conventional Practice + Bio-Forge + Stimulate has the same effects with T3- Bio-Forge and T4- CP + Bio-Forge, T5- CP + Stimulate and T6 Bio-Forge + Stimulate.

While lowest increment in height was observed in T1- Control (Untreated) and T2- Conventional Practice at 15, 30 and 45 Days After Planting (DAP). Comparable result is also observed between T2- Conventional Practice and T5- CP + Stimulate at 15 DAP; among T2- Conventional Practice, T4- CP + Bio-Forge, T5- CP + Stimulate and T6- Bio-Forge + Stimulate at 30 DAP; and between T3- Bio-Forge, and T6- Bio-Forge + Stimulate at 45 DAP. This coincides with reports that Bio-Forge tremendously performs well on the growth performance of any crops. A field trial in cotton, soybean and corn conducted by Iowa State University on March 2009 shows that application of Bio-Forge and Stimulate produces better stand growth and root vigor performance in any types of stressors. It contains micronutrients that stimulate the production of auxin, a hormone generated by the plant to trigger vegetative growth.

Table 2: Plant height increment (cm) of tissue-cultured 'lakatan' banana plantlets at 15, 30 and 45 Days after planting (DAP) using stimulants.

TREATMENT	15 DAP**	30 DAP**	45 DAP**
T1-Control (Untreated)	0.62c	0.76c	0.58c
T2-Conventional Practice	2.33b	2.84b	1.64c
T3-Bio-Forge	3.97a	4.90a	3.44ab
T4-CP + Bio-Forge	3.95a	4.13ab	4.15a
T5-CP + Stimulate	3.55ab	4.23ab	2.90b
T6-Bio-Forge + Stimulate	3.75a	4.30ab	3.39ab
T7-CP + Bio-Forge + Stimulate	4.25a	4.35a	4.26a
C.V. (%) =	14.75	15.57	14.96

**= Highly Significant
Means in column having common letter are not significantly different at 1% level of probability using HSD.

This implies that Bio-Forge and Stimulate with application of Conventional Practice ensures greater performance on the growth of the plants. Bio-Forge has the ability to unleash the power of plant on its full grown potential by gearing-up important nutrients needed by the plants. It has a patentented formulation of N,N' — diformyl urea, classified as an antioxidant, and shown to significantly improve growth in a variety of agricultural crops by working on the genetic level. Bio-Forge works by up-regulating genes from the plant's own major antioxidative pathways. As Stimulate also enhances cell division, cell differentiation, cell enlargement, root growth and nutrient utilization which resulted to Increase in yield, Improved fruit size and quality and Increased vigor (www.stoller.com).

Length Increment of Leaves (cm)

As shown in Table 3, the length increment of leaves (cm) of tissue-cultured 'lakatan' banana plantlets was significantly affected by different treatments at 15, 30 and 45 Days After Planting (DAP). Data showed that highest leaf length increase by up to 10 times more was obtained in T3- Bio-Forge treatment at 15, 30, and 45 Days After Planting (DAP). Similar results were also obtained in T4- CP + Bio-Forge, T5- CP + Stimulate, and T6- Bio-Forge + Stimulate and T7- CP + Bio-Forge + Stimulate. This data coincides with previous results conducted by North Carolina State University in March 2009 on Corn that application of Bio-Forge increases the yield by up-to 20-bushel-per-acre. Application of Bio-Forge alone enhances and promotes length of leaves of tissue-cultured 'lakatan' banana plantlets.

Bio-Forge® has a patented formulation of classified antioxidant (N, N' — diformyl urea), which significantly improves growth in a variety of agricultural crops by working on the genetic level. Bio-Forge works by up-regulating genes from the plant's own major antioxidative pathways as well as genes responsible for ethylene production and root growth (www.stollerusa.com).

Width Increment of Leaves (cm)

Table 4 presents the data on Width Increment of Leaves of tissue-cultured 'lakatan' banana plantlets using stimulants at 15, 30 and 45 DAP. Analysis of Variance showed highly significant differences among treatments.

Table 3: Length increment of leaves (cm) of tissue-cultured 'lakatan' plantlets at 15, 30, 45 Days after planting (DAP) using stimulants.

TREATMENT	15 DAP**	30 DAP**	45 DAP**
T1-Control (Untreated)	0.53[b]	0.71[c]	1.25[b]
T2-Conventional Practice	1.72[bc]	4.22[b]	3.45[ab]
T3-Bio-Forge	4.94[a]	8.32[a]	8.67[a]
T4-CP + Bio-Forge	4.88[a]	6.76[ab]	7.08[ab]
T5-CP + Stimulate	4.34[a]	6.85[ab]	6.26[ab]
T6-Bio-Forge + Stimulate	3.76[ab]	6.25[ab]	6.46[ab]
T7-CP + Bio-Forge + Stimulate	3.17[ab]	6.56[ab]	6.15[a]
C.V. (%) =	24.86	20.00	36.47

**= Highly Significant
Means in column having common letter are not significantly different at 1% level of probability using HSD.

Table 4: Width increment of leaves (cm) of tissue-cultured 'lakatan' banana plantlets at 15, 30 and 45 Days after planting (DAP) using stimulants.

TREATMENT	15 DAP**	30 DAP**	45 DAP**
T1-Control (Untreated)	0.90[b]	1.03[c]	0.31[b]
T2-Conventional Practice	1.06[b]	1.71[bc]	1.99[ab]
T3-Bio-Forge	1.81[ab]	4.66[a]	2.68[ab]
T4-CP + Bio-Forge	2.05[ab]	3.45[ab]	3.38[ab]
T5-CP + Stimulate	1.79[ab]	3.19[abc]	3.62[a]
T6-Bio-Forge + Stimulate	2.16[ab]	2.74[abc]	5.15[a]
T7-CP + Bio-Forge + Stimulate	2.81[a]	2.96[abc]	4.62[a]
C.V. (%) =	32.35	29.97	36.02

*= Significant
**= Highly Significant
Means in column having common letter are not significantly different at 5% and 1% level of probability using HSD.

Result showed that treatment with Bio-Forge and Stimulate increased the width of leaves. Highest width increments at 15 DAP was obtained in T7- CP + Bio-Forge + Stimulate which is not significantly different with T3- Bio-Forge, T4- CP + Bio-Forge, and T5- CP + Stimulate T6- Bio-Forge + Stimulate. While T3- Bio-Forge got the highest width increment at 30 DAP similar to T4- CP + Bio-Forge, T5- CP + Stimulate, T6- Bio-Forge + Stimulate and T7- CP + Bio-Forge + Stimulate. Still at 45 DAP, T5- CP + Stimulate, T6- Bio-Forge + Stimulate and T7- CP + Bio-Forge + Stimulate have the highest width increment of leaves as much as 13 times higher which is also the same with T2- Conventional Practice, T3- Bio-Forge and T4- CP + Bio-Forge.

This result supports the study conducted by Ohio State University in March 2009 that application of Bio-Forge on soybean triggers the plants natural hormone (Auxin) which may resulted to visible vegetative growth and yield (www.stoller.com). Combination of Bio-Forge and Stimulate enhances the width size of leaves in tissue-cultured 'lakatan' banana plantlets. Bio-Forge has a dreb1A

master gene for drought tolerance, salt tolerance, frost tolerance, and an increase in expression of genes related to stress tolerance. Physiological studies indicate very good suppression of crop plant ethylene production under abiotic or biotic stress conditions which may provide a healthy and good stand crop.

Pseudostem Diameter Increment (cm)

As presented in Table 5, the pseudostem diameter increment of tissue-cultured 'lakatan' banana plantlets was significantly affected by stimulants at 15, 30 and 45 Days After Planting.

Table 5: Pseudostem diameter increment (cm) of tissue-cultured 'lakatan' banana plantlets at 15, 30 and 45 Days after planting (DAP) using stimulants.

TREATMENT	15 DAP**	30 DAP**	45 DAP**
T1-Control (Untreated)	0.10	0.12^{b}	0.10^{b}
T2-Conventional Practice	0.16	0.34^{ab}	0.10^{b}
T3-Bio-Forge	0.16	0.41^{ab}	0.22^{b}
T4-CP + Bio-Forge	0.26	0.36^{ab}	0.42^{ab}
T5-CP + Stimulate	0.16	0.40^{ab}	0.41^{ab}
T6-Bio-Forge + Stimulate	0.23	0.42^{ab}	0.65^{a}
T7-CP + Bio-Forge + Stimulate	0.23	0.48^{a}	0.74^{a}
C.V. (%) =	53.90	30.21	36.98

^{ns}= Not Significant
*= Significant
**= Highly Significant
Means in column having common letter are not significantly different at 1% and 5% level of probability using HSD.

Results consistently showed that T7- CP + Bio-Forge + Stimulate increased stem diameter of tissue-cultured 'lakatan' banana plantlets at 30 and 45 Days after Planting (DAP) by up to 6 times higher. At 30 DAP, T7- CP + Bio-Forge + Stimulate is similar in T2- Conventional Practice, T3- Bio-Forge, T4- Conventional Practice + Bio-Forge, T5- CP + Stimulate and T6- Bio-Forge + Stimulate. While at 45 DAP, T7- CP + Bio-Forge + Stimulate was comparable with T4- CP + Bio-Forge, T5- CP + Stimulate and T6 Bio-Forge + Stimulate.

This supports previous results conducted by A&M University, Texas and Stoller Enterprise Inc. in 2010 that combined application Bio-Forge and Stimulate on cereal crops (wheat and barley) enhances root growth of seedlings, increases crop stem diameter, increases thickness of leaves, enhances crop canopy branching and enhances legume nodule development.

Application of both stoller products (Bio-Forge and Stimulate) or alone will enhances the growth performance of tissue-cultured 'lakatan' banana planlets including Pseudostem diameter. Bio-forge improves nutrient uptake that are essential for the synthesis of hormones, the perception of hormone levels and also impact the length and degree of hormone activity which may resulted to better growth of plants manifested on the leaves, stem, height and yield of the crop (www.stoller.com).

Number of Leaves

Table 6 and Figure 2 presents the number leaves of tissue-cultured 'lakatan' banana plantlets as affected by stimulants at 15, 30 and 45 Days after Planting (DAP). The result of statistical analysis shows highly significant effects at 15 DAP, 30 DAP and 45 DAP.

Results showed that T7- Conventional Practice (CP) + Bio-Forge + Stimulate significantly increased the number of leaves of 'lakatan' banana by 90% at 15, 30 and 45 Days after Planting (DAP). However, T7 CP + Bio-Forge + Stimulate were comparable with T3 Bio-Forge, T4 CP + Bio-Forge, T5 CP + Stimulate and T6 CP + Bio-Forge and Stimulate at 15 to 45 DAP.

T2 Conventional Practice and T1 Control (no application) also has the same result at 15 and 30 DAP while T2 Conventional practice has higher number of leaves at 45 DAP. This study coincides with the previous reports of Texas A&M University in 2009 that Bio-Forge and Stimulate enhances growth of seedlings, increases

thickness of leaves and yields of various crops such as corn and soybean (www.stoller.com).

This indicates that Bio-Forge and Stimulate alone or in combination with conventional practice (CP) will increase the number of leaves of tissue-cultured 'lakatan' banana seedlings. Bio-Forge is a patented formulation of N, N' — diformyl urea, classified as an antioxidant, and shown to significantly improve growth in a variety of agricultural crops by working on the genetic level while Stimulate contains the 3 growth hormones cytokinin, auxin, and gibberellic acid specifically designed to drive plant growth (www.stollerusa.com).

Table 6: Number of leaves of tissue-cultured 'lakatan' banana plantlets using stimulants at 15, 30 and 45 Days after planting (DAP).

TREATMENT	15 DAP**	30 DAP**	45 DAP**
T1-Control (Untreated)	2.20[b]	2.53[b]	3.04[b]
T2-Conventional Practice	2.13[b]	3.00[b]	4.24[a]
T3-Bio-Forge	2.40[ab]	5.00[a]	5.91[a]
T4-CP + Bio-Forge	2.66[ab]	4.60[a]	5.83[a]
T5-CP + Stimulate	2.20[b]	4.60[a]	5.62[a]
T6-Bio-Forge + Stimulate	2.66[ab]	4.40[a]	5.66[a]
T7-CP + Bio-Forge + Stimulate	3.00[a]	4.86[a]	5.83[a]
C.V. (%) =	19.59	9.00	6.15

**= Highly Significant
Means in column having common letter are not significantly different at 1% level of probability using HSD.

Results showed that T7- Conventional Practice (CP) + Bio-Forge + Stimulate significantly increased the number of leaves of 'lakatan' banana by 90% at 15, 30 and 45 Days after Planting (DAP). However, T7 CP + Bio-Forge + Stimulate were comparable with T3 Bio-Forge, T4 CP + Bio-Forge, T5 CP + Stimulate and T6 CP + Bio-Forge and Stimulate at 15 to 45 DAP.

T2 Conventional Practice and T1 Control (no application) also has the same result at 15 and 30 DAP while T2 Conventional practice has higher number of leaves at 45 DAP. This study coincides with the previous reports of Texas A&M University in 2009 that Bio-Forge and Stimulate enhances growth of seedlings, increases thickness of leaves and yields of various crops such as corn and soybean (www.stoller.com).

This indicates that Bio-Forge and Stimulate alone or in combination with conventional practice (CP) will increase the number of leaves of tissue-cultured 'lakatan' banana seedlings. Bio-Forge is a patented formulation of N, N' — diformyl urea, classified as an antioxidant, and shown to significantly improve growth in a variety of agricultural crops by working on the genetic level while Stimulate contains the 3 growth hormones cytokinin, auxin, and gibberellic acid specifically designed to drive plant growth (www.stollerusa.com).

Root Length (cm)

\Application of Bio-Forge significantly affected the root length of tissue-cultured 'lakatan' banana plantlets as shown in Table 7 and Figure 2.

As presented, the application of T3- Bio-Forge alone significantly had the longest root length of tissue-cultured 'lakatan' banana plantlets which is 100% longer than the control. This is significantly the same with T5- Conventional Practice + Stimulate, T6- Bio-Forge + Stimulate and T7- Conventional Practice + Bio-Forge + Stimulate. Shortest Root lengths were observed in T1- Control and T2- Conventional Practice. However, T2 has the same effect with T4- Conventional Practice + Bio-Forge, T5- Conventional Practice + Stimulate, T6- Bio-Forge + Stimulate and T7- Conventional Practice + Bio-Forge + Stimulate.

This agrees with the previous results that Bio-Forge improved the growth performance of root in various crops

such as corn and soybean. In 2010 AgriServe Company conducted a field testing study on Corn at Russellville, USA and found out that during vegetative stage a more healthy plant, larger leaves and larger root length which resulted to positive yield results from an average of 3.57 bushels increase in double crop and 1.59 bushel in full season (www.stoller.com).

This implies that Bio-Forge alone increases root length of tissue-cultured 'lakatan' banana plantlets. Bio-Forge enhances seedling emergence with concentrated formulation of additional humic acid to ensure early root development and protection from stress on young plants, especially stress associated with cold spring soils or water logged conditions. Bio-Forge also promotes root nodulation and nodule activity in legumes. Plus, it ensures continuous new root growth for efficient nutrient uptake, especially nitrogen. Overall, Bio-Forge improves plant growth hormone balance for continued cellular viability and optimal plant functioning throughout the life of the plant (www.stoller.com).

Number of Roots

Bio-Forge and Stimulate significantly affected the number of roots of tissue-cultured 'lakatan' banana plantlets as shown in Table 8 and Figure 2.

Table 7: Root length (cm) of tissue-cultured 'lakatan' banana plantlets using stimulants.

TREATMENT			**MEAN Root Length****
T1- Control (Untreated)			17.00^{c}
T2- Conventional Practice			20.33^{bc}
T3- Bio-Forge			35.83^{a}
T4- CP + Bio-Forge			21.33^{bc}
T5- CP + Stimulate			25.00^{abc}
T6- Bio-Forge + Stimulate			25.83^{abc}
T7- CP + Bio-Forge + Stimulate			31.16^{ab}
C.V. (%) =			17.50

**= Highly Significant

Means in column having common letter are not significantly different at 1% level of probability using HSD.

Table 8: Number of roots on tissue-cultured 'lakatan' banana plantlets using stimulants.

TREATMENT	**MEAN Number of Roots****
T1-Control (Untreated)	4.33^{c}
T2-Conventional Practice	9.33^{b}
T3-Bio-Forge	10.66^{ab}
T4-CP + Bio-Forge	10.66^{ab}
T5-CP + Stimulate	10.33^{b}
T6-Bio-Forge + Stimulate	10.00^{b}
T7-CP + Bio-Forge + Stimulate	13.00^{a}
C.V. (%) = 8.56	

**= Highly Significant

Means in column having common letter are not significantly different at 1% level of probability using HSD.

Result shows that T7- CP + Bio-Forge + Stimulate increased up to 3 times greater number of root of tissue-cultured 'lakatan' banana plantlets, which is significantly the same with T3- Bio-Forge and T4- CP + Bio-Forge. This is followed by T5- CP + Stimulate and T6 Bio-Forge + Stimulate which have comparable effects with T2- Conventional Practice, T3- Bio-Forge, T4- CP + Bio-Forge, T5- CP + Stimulate and T6- Bio-Forge + Stimulate. Fewer number of roots lengths were greatly observed in T1- Control and T2- Conventional Practice.

This again conforms with the previous results conducted in 2010 by AgriServe Company in Murray, USA on Soybean that Bio-Forge and Stimulate application significantly produces healthy and vigor plants especially on the root aspects which resulted to positive yield response with this application timing (www.stoller.com).

Combination of the two products (Bio-Forge or Stimulate) or alone will precisely increased the number of root of Tissue-cultured 'lakatan' banana plantlets.

Bio-Forge also promotes optimal plant root growth by regulating up- specific key genes associated with stress factor that ensures continuous new root growth for efficient nutrient uptake, especially nitrogen, while Stimulate contains a combination of 3 important phytohormones that are necessary in cell division, cell differentiation, and cell arrangement in all positions of the plant in order to maximize cell division. It is the number of cells that a plant can synthesize which will determine the plant size. The more cells that the plant synthesizes the more it will produce better root formation (www.stollerusa.com).

Fresh Weight (grams)

Fresh weight of tissue-cultured 'lakatan' banana plantlets was significantly affected by the different treatments as shown in Table 9. As shown, T7- CP + Bio-Forge + Stimulate, T3- Bio-Forge and T4 CP + Bio-Forge and T6 Bio-Forge + Stimulate got up to 17 times more fresh weight in tissue-cultured 'lakatan' banana plantlets, which is comparable to T5- CP + Stimulate. The lowest fresh weight in tissue-cultured 'lakatan' banana plantlets were observed in T1- Control followed by T2- Conventional Practice, which has the same effect with T5- CP + Stimulate.

Table 7: Root length (cm) of tissue-cultured 'lakatan' banana plantlets using stimulants.

TREATMENT			MEAN Root Length**
T1- Control (Untreated)			17.00^{c}
T2- Conventional Practice			20.33^{bc}
T3- Bio-Forge			35.83^{a}
T4- CP + Bio-Forge			21.33^{bc}
T5- CP + Stimulate			25.00^{abc}
T6- Bio-Forge + Stimulate			25.83^{abc}
T7- CP + Bio-Forge + Stimulate			31.16^{ab}
C.V. (%) =			17.50

**= Highly Significant

Means in column having common letter are not significantly different at 1% level of probability using HSD.

As presented, the application of T3- Bio-Forge alone significantly had the longest root length of tissue-cultured 'lakatan' banana plantlets which is 100% longer than the control. This is significantly the same with T5- Conventional Practice + Stimulate, T6- Bio-Forge + Stimulate and T7- Conventional Practice + Bio-Forge + Stimulate. Shortest Root lengths were observed in T1- Control and T2- Conventional Practice. However, T2 has the same effect with T4- Conventional Practice + Bio-Forge, T5- Conventional Practice + Stimulate, T6- Bio-Forge + Stimulate and T7- Conventional Practice + Bio-Forge + Stimulate.

This agrees with the previous results that Bio-Forge improved the growth performance of root in various crops such as corn and soybean. In 2010 AgriServe Company conducted a field testing study on Corn at Russellville, USA and found out that during vegetative stage a more

healthy plant, larger leaves and larger root length which resulted to positive yield results from an average of 3.57 bushels increase in double crop and 1.59 bushel in full season (www.stoller.com).

This implies that Bio-Forge alone increases root length of tissue-cultured 'lakatan' banana plantlets. Bio-Forge enhances seedling emergence with concentrated formulation of additional humic acid to ensure early root development and protection from stress on young plants, especially stress associated with cold spring soils or water logged conditions. Bio-Forge also promotes root nodulation and nodule activity in legumes. Plus, it ensures continuous new root growth for efficient nutrient uptake, especially nitrogen. Overall, Bio-Forge improves plant growth hormone balance for continued cellular viability and optimal plant functioning throughout the life of the plant (www.stoller.com).

Number of Roots

Bio-Forge and Stimulate significantly affected the number of roots of tissue-cultured 'lakatan' banana plantlets as shown in Table 8 and Figure 2.

Table 8: Number of roots on tissue-cultured 'lakatan' banana plantlets using stimulants.

TREATMENT	MEAN Number of Roots**
T1-Control (Untreated)	4.33[c]
T2-Conventional Practice	9.33[b]
T3-Bio-Forge	10.66[ab]
T4-CP + Bio-Forge	10.66[ab]
T5-CP + Stimulate	10.33[b]
T6-Bio-Forge + Stimulate	10.00[b]
T7-CP + Bio-Forge + Stimulate	13.00[a]
C.V. (%) =	8.56

**= Highly Significant

Means in column having common letter are not significantly different at 1% level of probability using HSD.

Result shows that T7- CP + Bio-Forge + Stimulate increased up to 3 times greater number of root of tissue-cultured 'lakatan' banana plantlets, which is significantly the same with T3- Bio-Forge and T4- CP + Bio-Forge. This is followed by T5- CP + Stimulate and T6 Bio-Forge + Stimulate which have comparable effects with T2- Conventional Practice, T3- Bio-Forge, T4- CP + Bio-Forge, T5- CP + Stimulate and T6- Bio-Forge + Stimulate. Fewer number of roots lengths were greatly observed in T1- Control and T2- Conventional Practice.

This again conforms with the previous results conducted in 2010 by AgriServe Company in Murray, USA on Soybean that Bio-Forge and Stimulate application significantly produces healthy and vigor plants especially on the root aspects which resulted to positive yield response with this application timing (www.stoller.com).

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Fresh Weight (grams)

Fresh weight of tissue-cultured 'lakatan' banana plantlets was significantly affected by the different treatments as shown in Table 9. As shown, T7- CP + Bio-Forge + Stimulate, T3- Bio-Forge and T4 CP + Bio-Forge and T6 Bio-Forge + Stimulate got up to 17 times more fresh weight in tissue-cultured 'lakatan' banana plantlets, which is comparable to T5- CP + Stimulate. The lowest fresh weight in tissue-cultured 'lakatan' banana plantlets were observed

in T1- Control followed by T2- Conventional Practice, which has the same effect with T5- CP + Stimulate.

Table 9: Fresh weight (grams) of tissue-cultured 'lakatan' banana plantlets using stimulants.

TREATMENT	MEAN Fresh Weight (g)**
T1-Control (Untreated)	3.03c
T2-Conventional Practice	19.66bc
T3-Bio-Forge	50.43a
T4- CP + Bio-Forge	49.46a
T5- CP + Stimulate	37.10ab
T6- Bio-Forge + Stimulate	31.66a
T7- CP + Bio-Forge + Stimulate	53.86a

C.V. (%) = 23.20

**= Highly Significant
Means in column having common letter are not significantly different at 1% level of probability using HSD.

Explanation can be pointed out by the report conducted by A&M University at Texas. Results from 3 experiments indicated that soil treatment with water containing 0.6% Bioforge previous to the drought treatment increased survival of water-deprived plants by an average of 44% this indicates that treatment applied with Bio-Forge improved water retention of the plant and resulted to bulk accumulation of water that tends to produced heavier weight of the plants (www.stoller.com).

Combined application of the two product (Bio-Forge or Stimulate) or alone will provide heavier weight on the tissue-cultured 'lakatan' banana plantlets. Bio-Forge is shown to up-regulate the four enzymes that protect plants from damaging free radicals which can occur in response to drought, injury from herbicides and pesticides, nutrient deficiencies and extreme temperatures.The four enzymes are: Mnsuperoxide dismutase Catalase (CAT3), Dehydroascorbate reductase (DHR), Thioredoxin reductase (THR). While Stimulate is a bio-stimulant enhancer uses as a supplement to foliar nutrients. Stimulate enhances cell division, cell differentiation, cell enlargement, root growth and nutrient utilization in which plants make its full grown potential in obtaining more controlled and productive growth.

Dry Weight (grams)

There were significant differences on the Dry weight of tissue-cultured 'lakatan' banana plantlets as shown in Table 10. Data showed that T4- Conventional Practice + Bio-Forge greatly increased the dry weight of tissue-cultured 'lakatan' banana plantlets by up to 28 times higher than the T1- Control (untreated). T4- Conventional Practice + Bio-Forge is comparable to T3- Bio-Forge, T5- CP + Stimulate, T6- Bio-Forge + Stimulate and T7- CP + Bio-Forge + Stimulate. Lowest dry weights were observed in T1- Control and T2- Conventional Practice which were also statistically the same with T5- CP + Stimulate and T6- Bio-Forge + Stimulate. This indicates that Bio-Forge is effective on carbon absorption which enhances fast performance on the growth of plants. Application of Bio-Forge in combination with Conventional practice will effectively triggers carbon absorption and nitrogen fixation.

Bio-Forge is a stress reducing hormone that provides proper absorption of important nutrient during the development stage of the plants. Dreb1A gene, a gene shown to play a role in drought tolerance. By inducing the regulatory master gene Dreb1A, a cascade affect in turn up-regulates other genes involved in resistance to drought and other environmental stresses (www.stoller.com).

Conclusion

The results of the study revealed that T7- (Conventional Practice + Bio-Forge + Stimulate) significantly increased plant height by 7 times, width of leaves by 3 times, stem diameter by 6 times, root number by 3 times, fresh weight by 17 times which were comparable with T6- (Bio-Forge + Stimulate), T5- (CP + Stimulate), T4- (CP + Bio-Forge) and T3- (Bio-Forge). T3 (Bio-Forge) had the highest leaf length increase by 10 times, root length by 100% which is comparable to T5- (CP + Stimulate), T6- (CP + Stimulate)

Table 10: Dry weight (grams) of tissue-cultured 'lakatan' banana plantlets using stimulants.

TREATMENT	MEAN Dry Weight (g)**
T1-Control (Untreated)	3.03[c]
T2-Conventional Practice	19.66[bc]
T3-Bio-Forge	50.43[a]
T4-CP + Bio-Forge	49.46[a]
T5-CP + Stimulate	37.10[ab]
T6-Bio-Forge + Stimulate	31.66[a]
T7-CP + Bio-Forge + Stimulate	53.86[a]

C.V. (%) = 23.20

**= Highly Significant
Means in column having common letter are not significantly different at 1% level of probability using HSD.

Figure 1: The experimental area at USEP, Mabini, ComVal Province.

and T7- (CP + Bio-Forge + Stimulate). While T4- (CP + Bio-Forge) had the heaviest dry weight by 28 times which is also comparable to T3 (Bio-Forge), T5 (CP + Stimulate), T6 (Bio-Forge + Stimulate) and T7- (CP + Bio-Forge + Stimulate).

Hence, the addition of foliar supplements like bioforge and stimulate with basal fertilizer application resulted to optimum growth of ´lakatan´ banana plantlets.

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T1- control	T2- NPK	T3- Bioforge	T4- NPK+ Bioforge	T5- NPK+ Stimulate	T6- Bioforge+ Stimulate	T7- NPK+ Bioforge+ Stimulate

Figure 1: The experimental area at USEP, Mabini, ComVal Province.

USeP-Mabini Campus, Mampising Compostela Valley Province.

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AN ASSESSMENT OF EDIBLE COATING AND ITS IMPACT ON SHELF LIFE AND POST HARVEST QUALITY OF GUAVA (*PSIDIUM GUAJAVA* L.) FRUITS – A REVIEW

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ABSTRACT

Legitimate postharvest treatment of food produces is a higher priority than the serious and broad cultivating in making sure about nourishment for a country, since misfortunes are a misuse of food as well as they speak to a comparable misuse of human exertion, ranch inputs, jobs, speculations and scant recourses, for example, water. Postharvest misfortunes of agricultural yields when all is said in done and new products of the soil (perishables) specifically are normal issues in creating nations, similar to Ethiopia, which negatively affects the food security program. This is mostly a direct result of their transitory nature, absence of information and deficiency of capital. The other explanation is that a large portion of these perishables are delivered by little scope ranchers the individuals who have restricted information and monetarily poor in the creating nations. Along these lines, assessment of postharvest misfortunes of new foods grown from the ground is exceptionally significant for mindfulness creation to deal with the produce appropriately in order to spare from waste and harms by physical and physiological methods. The destinations of this audit are, hence, to evaluate the accessible writings on the postharvest misfortunes of new foods grown from the ground trying to distinguish need zones of the issue; to distinguish the reasons for misfortunes of perishables so as to dodge the foundations for the decrease of misfortunes; and to distinguish the potential methodologies that can decrease misfortunes and keep up nature of the items during the period.

Keywords: Shelf life, Postharvest losses, Coatings, Waxing.

INTRODUCTION

Purchasers around the globe interest for food of high-caliber, without concoction additives, and an all-inclusive time span of usability (Jawadul *et al*. 2014). Postharvest misfortunes of tropical organic products are a major issue in view of quick decay during taking care of, transport and capacity (Adentuji C.O *et al*. 2012). Endeavors ought to be made to incorporate creation with postharvest the board since postharvest misfortune decrease has extensive bearing on food accessibility. Protection of organic products under encompassing conditions is important to expand their time span of usability and to improve commercialization (Krishna K. and Rao, 2014). These are responsible for its rich genetic diversity in fruit crops, resulting in good amount of divergence at different centers in respect to perennial fruit crops viz., mango, citrus, grapes, guava, banana, aonla, bael, litchi, jamun, several underutilized and temperate fruits for crop improvement (Tomar *et al*., 2020).

Guava is one of the most tasty and nutritious natural products, loved by the purchasers for its reviving taste and wonderful flavor (Adentuji C.O *et al*. 2012). Because of its climacteric nature the organic product ages quickly and subsequently exceptionally transitory, with a short timeframe of realistic usability extending from 2-3 days at room temperature. Retailing of guava natural products in India is normally done under non – refrigerated conditions. Therefore, palatable coatings dependent on normal items can

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give an extra assurance to new leafy foods. Eatable covering is a straightforward film that covers the food thing and goes about as a hindrance to stickiness and oxygen. There are a few sorts of eatable coatings, for example, sugar, protein, lipid and a blend of every one of these materials. (Wijewardane R.M.N.A 2013). *Aloevera* acceptable coatings which are gel-based, they have been seemed to hinder reduction of moistness and strength, improvement headway, control respiratory rate and lessen microorganism extension in natural items, for instance, table grapes, sweet organic products, and so on (Juan *et al.* 2005).

Xanthan gum is a polysaccharide gum which has an assortment of use in the food business as a settling, viscosifying, emulsifying, thickening and suspend operator (Aniseh and Shabanpour 2013). Thickener has been found to broaden the timeframe of realistic usability of negligibly prepared prickle pear upto 9 days (Asrar Y.I *et al.* 2013). Gum arabic, acquired from stems or parts of Acacia species, is the most regular polysaccharide utilized. It is an emulsifier and has film framing and epitome properties. It was discovered that gum arabic upgraded timeframe of realistic usability as well as kept up postharvest nature of develop green tomatoes for as long as 20 days during capacity at 20◦C. Different strategies have additionally been assessed for use of various sorts of covering materials. They are generally straightforwardly applied on the food surface by plunging, splashing or brushing. Stearin based wax covering applied by hand cleaning procedure could successfully improve the quality and time span of usability of guava (Abraham and Banarjee, 2018).

The current examination identifies with discover the pertinence of another eatable covering detailing comprising of *Aloe-vera* gel and thickener in upgrading the timeframe of realistic usability and nature of guava (Abraham and Banarjee, 2018). The novelty and appearance at the hour of obtainment choose the idea of new cut natural products (Kader, 2002). Negligible treatment of new cut normal items, which incorporates assessing, washing, orchestrating, stripping, cutting and packaging, can impact the decency of the nourishments developed from the beginning gets changed and waste of microbial that may bring about decay of the concealing, surface along with sort of natural items (Watada and Qi, 1999). The ejection of the trademark defence layer of common items does spillage of juices what's more, sugar amount out of the hurt mass coming to fruition within the natural items being significantly powerless to disease causing agent (Oms-Oliu *et al*. 2010).

Through an assessment, information could be accumulated about the effect of included substances, for instance, malignant growth avoidance operators, surface strengthener along with expert of antimicrobial in *Aloe vera* gel as an agreeable covering for fresh sliced guava (Nasution *et al*. 2015). Moreover, this assessment would incorporate to the grouping of wellsprings of consumable coatings. The normal wellsprings of consumable coatings used for freshcut characteristic items, for instance, starch, chitosan, carrageenan, casein and whey protein are not as successfully open as *Aloe vera* plants are. Starting at now, *Aloe vera* gel is usually used in the making of tablets, beverages, balms, chemical and also along with cleaning agent. As such, the very feasable usage of *Aloe vera* gel as a consumable concealing can change its utilization (Rojas-Grau *et al.* 2009a)

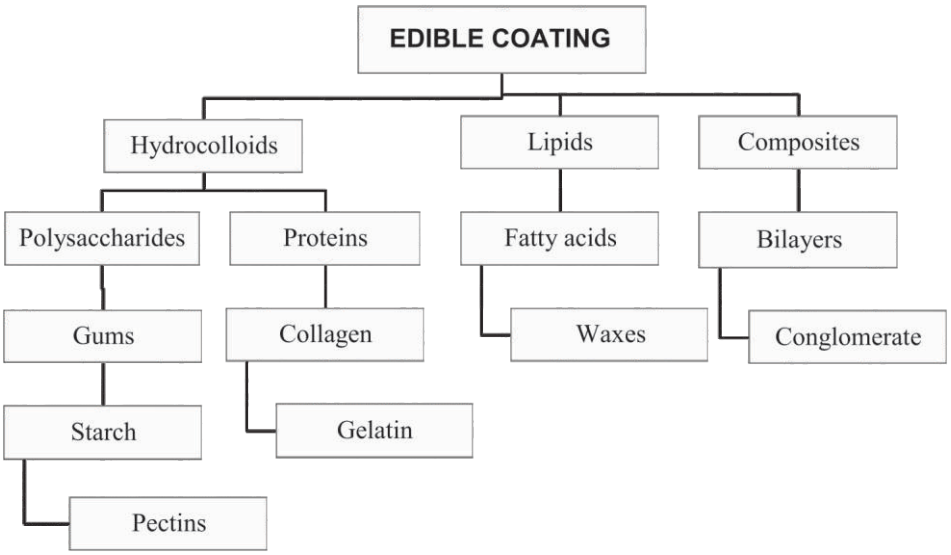

Chart: Various types of Edible Coatings (Raghav *et al.* 2016)

Aloevera: A Noble Coating

New mechanical advances in antimicrobial eatable layers for food may hold guarantee in broadening timeframe of realistic usability, decreasing bundling layers, meeting sanitation and quality prerequisites. Rising examination shows bacteriocins, polysaccharides, chemicals, fundamental oils, lipids and proteins are on the whole common coatings that have hidden potential in food safeguarding. As of late, intrigue has expanded in utilizing *Aloe vera* gel-based eatable covering material for leafy foods. *Aloe vera* gel has been demonstrated extraordinary compared to other eatable and naturally safe additive coatings for various sorts of nourishments as a result regards to its film-shaping properties, biodegradability, antimicrobial activities as well as biochemical properties. Its made predominantly out of polysaccharides and goes about as a characteristic boundary to dampness and oxygen, which are the principle operators of crumbling of products of the soil. *Aloe vera* gel can draw out time span of usability of the leafy foods by limiting the pace of breath and keeping up quality characteristics (shading, flavor and so forth). It has antifungal as well as antibacterial property, it gives a guarded obstruction against microbial defilement of leafy foods. The current survey portrays the readiness, properties and potential utilization with regards to *Aloe vera* gel coatings for upgrading postharvest life as well as nature of various sorts regards to organic products. (Misir *et al.* 2014).

The term *Aloe* is obtained from the name Arabic "Alloeh" or the Hebrew "Halal" connoting "unsavory, shinny substance"(Gage D, 1996). *Aloe vera* is even called same as "plant of never-ending status" by the Egyptians in light regards to its significant outcome on prosperity of human. It is all around accepted that the source is Sudan, Arabia, Somalia, and Oman. At present, *Aloe vera* is commonly passed on all through the subtropics and tropics (Schmelzer, 2008). *Aloe vera* is an enduring plant. It's densed, thorn edged leaves, stretching out in concealing from diminish to splendid green, give *Aloe vera* the nearness of a thorny plant. An ordinary *Aloe vera* plant delivers a couple of yellow tube shaped blooms, framed a great deal of like those of the Easter Lily, and it blooms sporadically reliably (Gage D, 1996). In the current society various people do have food hypersensitivities or individual tendencies that will shield them through ingesting these things. Henceforth, *Aloe vera* is an unfathomable dietary source used to fulfill the needs of amino acids (Misir *et al*. 2014).

Effect of coatings on attributes of guava (*Psidium guajava)*

A preliminary was coordinated to think about the reaction of gum Arabic layers on physicochemical and material attributes of guava (*Psidium guajava* L cv. Shweta) during limit at encompassing conditions for nine days. The physical and also the biochemical recognitions both were put down at three days stretch in the course of amassing. The gum arabic 10% covering basically diminished physiological hardship inside the weight (PLW) and held higher steadfastness, ascorbic destructive, strip chlorophyll content and appealing common items when diverged from control. The base PLW and the most outrageous ascorbic destructive, strip chlorophyll and faithfulness were found in GA 10% secured regular items, while uncoated natural items offered an explanation to having higher PLW (19.23%) and lower ascorbic destructive, strip chlorophyll and endurance close to the completion of the 9 days accumulating period. Most outrageous value regarding concealing (7.66) held by gum arabic (10%) followed by 5% GA (7.48) covering at the finish of the limit. The already stated suggest that usage of GA 10% covering was practical for holding physico-engineered attributes of guava as well as the defending of the substantial characteristics of the normal item during limit at ambient room temperature. (Gurjar *et al.* 2018).

In a study the fundamental oils have been assessed as defensive eatable coatings. The organic products Guava (*Psidium guajava*) and Amla (*Amblica officinalis*) were covered by dunking in fundamental oils of Tulsi, Neem and Eucalyptus and pressed in CFB, Brown paper and LDPE. The natural products were put away at room temperature and loss of weight (%), debris content (%), dampness content (%), TSS (°B), Ascorbic corrosive substance (mg/ml), absolute plate check (CFU/ml) and absolute yeast and form tally were surveyed. The coatings brought about a decrease of weight reduction, dampness content and Ascorbic corrosive substance. Guava and Amla covered with Neem oil and Packed in LDPE demonstrated decrease in microbial checks, TSS and Ash content contrasted and the control. The productivity was better than that of Tulsi and Eucalyptus treatment which were stuffed in CFB and earthy colored paper. This examination proposes that by utilizing Neem oil as a palatable covering stuffed in LDPE, the rotting of Guava and Amla can be deferred and can be protected for as long as 12 days during capacity at room temperature with no negative impacts on postharvest quality. (Sebastian *et al.* 2018).

The end result of layers with various centralizations with regards to tamarind seeds starch related along with oil of pomegranate seeds in 'Paluma' guava was explored inside the work. The regular items were procured through an estate inside the first part of the day, squeezed in compartments as of late fixed along with paper and sent to a

lab, at that place they were picked, washed, sanitized and secluded aimlessly for the utilization of every treatment. The examination design which was used, completely randomize in the 6 × 6 factorial arrangement, six layers as well as six appraisal periods, along with 3 copies involved 2 natural items. The drugs were applied under immersion of the characteristic items in the courses of action and a short time later set aside inside a chamber of refrigerated at 10±2 °C with 80±5% RH and the appraisals were experimented at time spans days to 12 extensive stretches of limit. In view of the upkeep of the idea of the natural items and without satisfactory material it was picked to expand limit time until the 21 days. Thusly, the assessments were experimented at 0, 3, 6, 9, 12 and 21 days with evaluations at 0, 3, 6, 9, 12 as well as 21 days. The treatment (3% tamarind starch + 0.24 mL/mL pomegranate seed oil) was more beneficial regarding sparkle (L*) of the nourishments developed from the beginning improvement conveyed by the assessments, similarly demonstrating the higher support in the loss of robustness lower mass adversity as well as lower dissolvable solids content, suggesting that this treatment possibly controlled the debasement of polysaccharides conceding the maturing pattern of the normal items. (Onias *et al.* 2018).

An impact on added substances into *Aloe vera* gel covering was examined on new slice guava put away at 5° C and 74–80% relative mugginess. Eight medicines were utilized including three added substances and their blends. The covered examples had less difference in the shading delicacy and yellowness contrasted with the uncoated example. Five chose covered tests and a new uncoated example were then exposed to tangible acknowledgment testing. AV + AA + PS-covered guava was the most satisfactory example. In addition, it gave the most elevated ascorbic corrosive substance (190.00 ± 14.14 mg per 100 g). With appropriate added substances, AV gel has potential as a consumable covering for new slice guava because of its capacity to drag out the time span of usability and keep up qualities of the natural product for a longer time (Nasution *et al.* 2015).

A research showed that the primary point of the examination was to evaluate the viability of various consumable covering medicines like chitosan, calcium chloride, sodium alginate and *Aloe vera gel* at different fixations on the post-collect quality traits of products of guava cultivar 'Gasp Prabhat'. After treatment, natural products were stored at surrounding condition of 27-29°C upto 12 days and broke down for different practical and tactile boundaries while the uncoated natural products fill in as control. Consequently, it was reasoned that covering treatment of 1.5% chitosan followed by Aloe vera 1:1 gel covering can be utilized for improving the timeframe of realistic usability and keeping up postharvest quality in products of guava cultivar 'Gasp Prabhat' (Kumar *et al*. 2017).

Summary

Consumable coatings are used from various years for limit of Fruits and Vegetables in food industry. Diverse covering substances are utilized for covering, for example, waxes, hydrocolloids, protein. Researchers have made new acceptable coatings, it is ensured and condition welcoming and safely eaten with Fruits and Vegetables. As per this survey, edible coatings expands timeframe of realistic usability, lessen water and dampness misfortune, postponed maturing measure and furthermore forestall microbial development explicitly in new foods grown from the ground. In eatable covering, as of late another idea has been presented and it is home grown eatable covering. It gives better outcomes and medical advantages. Natural palatable covered Fruits and Vegetables contained supplements and go about as medications.

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IMPACT OF DIFFERENT CHEMICALS ON THE POST HARVEST QUALITY OF PEAR (*Pyrus Spp* L.) FRUIT: A REVIEW

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ABSTRACT

Pear *(Pyrus Spp* L.) is one of the most harvested temperate fruit crop right after Apple. Pear belongs to the family Rosaceae along with some best known temperate tree fruits. Pear being one of the popular temperate fruit but is having a short storable life under room temperature conditions. The capability of being able to manage keeping quality of the stored fruit is must during the post harvest storage of the crops along with being able to increase the storable life of those fruits. Various post harvest technology are being used to sustain the fruit's keeping quality and also to increase those fruits storable time period. Being a crop prone to decay, diseases such as fungal diseases (mould) and short shelf life different researcher has recommended the use of different chemicals to preserve the quality as well as increase the storage lifetime of the fruits. Chemical such as Calcium have been proven to be effective in this post harvest management of fruits by having effects on the physiochemical fruits.

Keywords: Postharvest, Quality of fruits, Chemicals, storage life, Calcium, 1-MCP.

INTRODUCTION

Pear (*Pyrus Spp L.*) is one of the popular temperate fruit crop behind apple. It has a high productivity, high nutritive value and good range of adaptability under different agro climatic areas. Common Pear (*Pyrus communis*) is said to be originated from the temperate region of the Europe and Western Asia. Pear is diploid with chromosome no. 2n = 2x=34 and belong the family of Rosaceae and sub family being Amygdaloideae and Genus is Pyrus. *Pyrus communis* is a cultivated form and is derived generally from *P.caucasia* Fed. and *P. fivalis* Jaq. Pear is deciduous, rarely evergreen trees or shrubs. All species are self sterile, cross fertile and sexually diploids (2n=34) in nature. The different species are graft compatible in nature. The fruits are of pyriform pome in shape and flesh of pear fruit contains the stone cell. Pear is rich in foliate vitamin C, copper and potassium. They are also a good source of polyphenol antioxidant.

Pear being a popular crop across the world with China being the largest producer of Pear followed by countries like Argentina, Italy, USA. India currently stands in at 8th position in production. The total estimated production in world was of about 24,168,309 metric tons with contributing with approximately 16,410,000 metric tons which being about two third of the total production and 67% of total world production and India contributing with about 346,000 tones (FAO, 2017). In India Pear is cultivated in the Jammu &Kashmir, Himachal Pradesh, Uttarakhand and Punjab, Haryana, Uttar Pradesh cultivating the low chilling Pears. Jammu and Kashmir is the leading state in the production of Pear being 94.42 in 000 tones with a share of 29.23% of total production of 322.99 in 000 tones of that year. (NHB, 2015-16).

The objective of this review is to determine that whether the chemical can be used for the purpose of retaining the Qualities of the Pear fruit while also having a desirable

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impact on the storage time of the Pear fruits. This can be determine if the chemicals have good effect on the physiochemical parameters such as Fruit firmness, TSS, Core-Browning, respiration rate and other parameters.

IMPORTANCE OF CHEMICALS ON THE POST HARVEST QUALITY OF THE PEAR FRUIT

During the Post harvest of the fruits different Post harvest technologies are used for the of maintaining the quality of the fruit and use of chemical treatment is one of those technology which is advised by many researcher for the increasing the storable time period of the produces and to sustain the keeping quality of the produce.

a) Impact of Calcium in the Post harvest Quality and Physiochemical Parameters

Calcium is considered to be an essential plant nutrient which is closely linked to quality and firmness of fruits (Sams, 1999). Calcium being an important nutrient required by the cells for different role in structural area of cell wall and membranes. Its need by the cell is to act as a counter-cation for the organic and inorganic anions of cell's vacuole while also acting as a messenger within the cell in the cytosol (Marschner, 2011). Application of Calcium in the pre harvest or post harvest phase is said to be able in prevention of the physiological order and also increase the disease resistance ability of the harvest also said to be helpful in delaying of the ripening period of the fruit and along with it also helps in enhancing the quality of the fruits. (Manganaris *et al*., 2007).

The fruits were able to maximum value of mean Fruit firmness (5.78 kg/cm^2) while maintaining lower mean values of TSS to acid ratio (37.37), PME activity (2.01), Polygalacturonase (PG) (14.75) and also lowest loss in the Physiological weight of the Fruit (5.98%) when treated with 4% of $CaCl_2$ as compared to other treatment. The treatment of freshly harvested fruits treated with the 4% of $CaCl_2$ for a time period of 20 and 30 minutes were effective along with treatment of 3% of $CaCl_2$ in ambient conditions of storage. (Kumar *et al*., 2017)

The fruits of Asian pear cv. Pathernakh were treated with $CaCl_2$ at different concentration and were kept for a period of 75 days under a temperature of 0-1°C and relative humidity of 90-95%. The Mean Fruit firmness (15.82 lb) of Asian Pear was lowest in stored fruits which were dipped in the solution of $CaCl_2$ of 4% concentration. The core browning was not noticed in the $CaCl_2$ @ 2% and @4% up to 45th day of storage period and even with succession in the storage period browning was observed in just 5% of the total fruits after 60th and 75th day of storage in fruit treated with $CaCl_2$ @ 2%and @4% respectively. The fruits dipped in solution of $CaCl_2$ @ 4% concentration had maximum TSS (14°B) and total sugar (8.30%). The fruits which were treated with 4% of $CaCl_2$ had a favorable and desired impact on the physiochemical parameters during storage as compared to those untreated fruits. (Mahajan et al., 2004)

The fruits were experimented were kept in a temperature of -1°C for period of 12 weeks. The slices that were stored in Controlled atmosphere of 0.5% of O_2 of the dipping of 1% %of $CaCl_2$ were very effective in keeping the firmness and a lighter colour of those slices. On the 8th day of the storage in treatment of 1% of $CaCl_2$ at a controlled atmosphere of 0.5% O_2 had much firmed slices as compared to 1% of $CaCl_2$ at air. A 1% of $CaCl_2$ dip proved to be effective to reduce the browning and also in reducing the loss of firmness of the sliced fruits. The combination of 0.5% O_2 and 1% of $CaCl_2$ was more effective in nature and was able to control the browning and maintain the fruit firmness of the sliced fruits compared to that the treatment which was introduced to the air. (Rosen et al., 1989)

The various treatments of $CaNO_3$ and GA_3 were effective in the process of minimizing the Physiological Loss of weight, in maintaining the Firmness of the fruits, TSS, titratable acidity, total sugar content and other parameters. After the end of the storage period the most effective treatment was noted to be in the fruits which were treated with $CaNO_3$ treatment of 3% with lowest PLW of 4.81%, and highest in fruit firmness(3.88 kgf), sensory quality(7.15), TSS(12.35%), total sugar(8.45%). In case of GA_3 the minimum PLW (5.59%) was noticed in the treatment with 60 ppm concentration and maximum values of fruit firmness were noticed in those fruit which were treated with GA_3 @ 40, 60 ppm of 3.55 kgf in both cases. In case of TSS (12.31%) and Total sugar (8.38%) were highest noticed in treatment of GA_3 @60 ppm. Both $CaNO_3$ and GA_3 were both effective in reducing the PLW, maintaining the fruit firmness, TSS, Total Sugar content and other parameters with most effective being the 3% of $CaNO_3$ and in case of GA_3 the treatment of GA_3 @ 60 ppm (Kaur *et al*., 2017).

Table 1: Effect of Calcium Treatment on different Fruit Crops.

S No.	Crop	Treatment used of Calcium	Effect of Calcium treatment	Source
01	Apple	4% of CaCl2	Decrease in the weight loss, pH, TSS/acidity and increased the fruit firmness of fruits	Shirzadeh *et al*., 2011
02	Peach	6% of CaCl2	Retarded the spoilage, sustained the fruit firmness, acidity and vitamin A content	Gupta *et al*., 2011
03	Papaya	2.5% of CaCl2	Increased the storage life, control on the disease incidence and retarded the timing of ripening process	Mahmud et al., 2008
04	Apricot	1% of CaCl2	Increased the storability period of fruits.	Antunes *et al*., 2003
05	Orange	2%,4%,6% of CaCl2	Reduce the incidence of rot	El Gali, 2014
06	Loquat	2% and 3% CaCl2	Were able to sustain the highest fruit firmness, TSS, ascorbic acid content reduced browning index and weight loss	Akhtar, 2010

a) Impact of Aminoethoxyvinylglycine (AVG) and/or Oxalic acid on the Pear fruit's Quality

The treatment of AVG and/or oxalic acid were proven efficacious in decreasing the production of ethylene and the result of which was delay in the ripening of the fruits and also was able to reduced the fruit decay. Also, AVG+ oxalic acid was very successful in reducing the respiration rate, displayed a little bit of browning while the treated fruits also produced a lower amount of sugar. The loss in the weight percentage did effectively lowered in AVG treated fruit only and also it was very impactful in maintaining the firmness of fruits and by the finishing of the storage time period AVG treatment was able to preserve a higher green colour of the fruits. Additionally, fruits treated which were treated using oxalic acid single handedly retarded the decay as well as the total loss percentage following the end of cold storage conditions and also during the short period of the marketing period (Tarabih, 2014)

The fruit's ripening of 'Abbe Fetel' fruits which were treated in the pre harvest was retarded by 5 to 15 days on the basis of the harvesting dates. After the 7^{th} and 14^{th} of the cold storage period , fruits which were treated with AVG treatment retained their unripe features partially as compared to those untreated fruits. The process of flesh softening was declined due to the post harvest application of dip treatment of AVG on the fruits. (Anderotti et al., 2004)

b) Impact of Application of 1-Methylcyclopropene (1-MCP) on the storage life of the Pear fruit

The compound 1-MCP is said to being able to prevent the ill-effects of ethylene in vast range of fruits. Fruits which were treated with 1-MCP showed lesser levels of Hydrogen peroxide, Ascorbate content while also showing lowered ionic leakage in the storing period of fruits. The fruits which were treated with 1-MCP also showcased higher enzymatic antioxidant potential. The desirable impacts of 1-MCP on the ripening process were just not limited because of its influence on the ethylene but it was due to rise in the potential in the Pears fruits. (Larrigaudiere et al., 2004)

The fruits which were exposed to the 2µL L^{-1} 1-MCP while also were kept in cold conditions storage did increased the storage period with one another months as compared to fruits which were just kept under the cold storage conditions. The total sugar content of theses fruits was steady during the storage period in each and every treatment .The application of 1-MCP on the fruits and keeping them in the cold storage showcased inhibition of the sucrose loss and also in the amassing of the hexoses. The reason this was the inhibition of amassing of PpAIVI transcript and the reduction in PpSPSI transcript which resulted in the retardation of sucrose losses. Cold+ 1-MCP treatment can be proven to be beneficial to the market as it inhibit the sugar losses and maintaining the fruit firmness and appearance in the Japanese pear. (Itai et al., 2007)

The fruits which were treated with the 1-MCP @ 300 ppb had impact on the flesh firmness, texture and peel green colour had higher values and apart from that respiratory rate and ethylene production of fruits showcased lower values. Fruits treated with NO and SNP were not able to decline the fruit's rate of respiration and also the production rate of the ethylene. NO @ 20 ppm treatment of fruits was able to maintain their fruit firmness and textural features after leaving the chambers. The fruits which were treated

with SNP @1mM and NO@ 20 ppm were able to maintain their peel green color when they were compared to control fruits without having any effect on the yellowing of the treated fruits during the storage. The application of treatment of 1-MCP @ 300 ppb inhibited the buttery texture and yellowing in the fruits even during exposure to environmental conditions was done. (Hendeges *et al*., 2016)

The fruits which were treated with a treatment of 1-MCP @ 0.2 µl l^{-1} the rate of softening lowered after 7days of storage at a temperature of 20°C. The firmness of these fruits was considered acceptable for consuming even after storage time period of 14 days at the temperature of 20°C. 1-MCP @ 0.2µl l^{-1} when combine with cooling techniques can delay the ripening process as well can sustain an acceptable keeping quality of Pear fruits throughout the span of the storage. (Calvo *et al*., 2004).

Table 2: Impact of 1-MCP treatments on the different fruit's Postharvest.

S No.	Crop	Effect of 1-Methylcyclopropane on the fruit crops	Source
01	Orange	Inhibitory effect on the process of Degreening	Porat *et al.*, 1999
02	Guava	Lowered the rate of respiration and also retaining the quality	Bassetto *et al*., 2005
03	Loquat	Lowered Lipoxygenase, PPO activities while also retarding the browning resulted in quality sustaining and prolonged shelf life	Cai *et al*., 2005
04	Papaya	When fruits treated with 1-MCP the fruits show delay in the softening process. The treatment increased storage life while maintaining the quality of the Fruit	Ahmad *et al.*, 2013
05	Mango	Suppressed Anthracnose of Mango fruit in post harvest by having inhibiting impact on the spore germination and mycelial growth of C. gloeosporioides	Xu *et al*., 2016

a) Impact of Ascorbic acid and /or Calcium lactate and/or Cysteine on the Quality of Pear

The low concentration of O_2 (0.25 or 0.5 kPa), elevated amount of the CO_2 (air rich with 20,10 or 5 kPa of CO_2) or the superatmospheric O_2 (40,60 or 80 kPa concentration) controlled atmosphere single handedly did not efficaciously prohibited surface browning caused due to softening of these fresh cut pear slices. After cutting , the dip treatment of the 2%(w/v) ascorbic acid, 1%(w/v) calcium lactate and 0.5% (w/v) cysteine with a pH of 7.0 notably extended the storage life of Bartlett, by preventing the loss of firmness of flesh from the slices as well also inhibiting browning on the cut surface. There was no differentiation observed in the quality evaluation of the cut slices treated with chemical preservatives and stored over night at 0°C and the slices which were freshly cut. The 82% of total participant judged the treated slices after the interval of stored in air at 0°Cto be acceptable appearance wise after 10 days of interval of storage period whereas 70% from total participants determined the flavor to be acceptable of those treated slices. (Grony et al., 2001)

The fruits which were applied with a treatment of 0.1, 1.0 and 10 mM of Ascorbic Acid showed a reduction in Core Browning Index of 10.4%, 24.5% and 40.0% respectively after 180th day of storage. The highest TSS and firmness of the fruit which were treated with AsA @ 10 mM in comparison to that of controlled fruits. AsA treatment of fruits had retarding impact on the amassing of the malondialdehyde and Hydrogen Peroxide. Furthermore, the Ascorbic Acid treatment significantly postponed the retardation of Ascorbic Acids and glutathione levels while also it preserved activates of superperoxide dismutase catalase and Ascorbate Peroxide. (Lin *et al*., 2007).

The application of a combination that of Chitos an coating and Ascorbic acid can prolong the time period of the weight loss and sustain a higher fruit firmness , TSS as compare to that of untreated ones. There was also decrease in the respiration rate as well as the membrane permeability while also restricting the Core browning of fruits in an effective manner eve after 60 days of storage time period. This combined treatment was also able to sustain and higher amount of Ascorbic acid content and also maintaining a higher level of antioxidative enzymes activities. (Lin et al., 2008)

b) Impact of other chemicals (Hydrogen Sulfide, Nitric Oxide, GRAS chemical, Sodium Chlorite) on the keeping life of Pear

Hydrogen sulfide gas released by Sodium Hydrosulfide was able to increase the shelf life period of the fresh sliced

Pear. Additionally H_2S retained the higher reducing sugar levels as well as also the soluble proteins in those freshly cut Pear slices. The amassing of the hydrogen Peroxide, superoxide radicals also Malondualdehyde was retarded with the introduction of H_2S gas. Moreover H_2S was able to up-regulate the activity process of the antioxidant enzymes such as ascorbate peroxidase, guaiacol peroxidase and also catalase. H_2S was able to down regulate the activities of lipoxygenase, phenylalanine, and polyphenol oxidase as well as ammonia lyase. In addition to this the fumigation process of H_2S was able to efficaciously restricted the growth of 2 different types of the fungal microbe of the Pear fruits, *Asperigillus niger, Penicllium expansum.* The meaning of this was that H_2S can work as an efficacious fungicide during the Post harvest storage process. (Hu et al., 2014).

Fruits which were treated with the treatment of NO @ 10µl l^{-1} for a period of 2 hours the stage of ethylene climacteric was postponed to 4 days period of time while reducing the maximum production of the ethylene by 28% and the delay in the fruit's firmness as well change of colour by 2 days time. When the same fruits were treated with another NO @ 10µl l^{-1} after a period of 4 days there was even more reduction in the production of the fruits was noticed by 48%. While the fruits which were treated with different concentration of NO @ 10µl l^{-1} and 50 µl l^{-1} for the period of 12 hours postponed the yellowing of fruits by a time period of 2days but the rate softening rate of fruits was very much unaltered. (Sozzi et al., 2003).

The application of GARS such as Boric Acid, Sodium bicarbonate and Sodium benzoate chemical's as external treatment had a positive effect in the fruits as they retarded the TSS/acid ratio, SOD enzyme activity and decreased the pH while lowering the Reducing sugar and non reducing sugar's values as compared to that of untreated fruits. The concentration of Boric acid @ 3% treatment was proved to be much more effective than other treatments of GRAS chemical in increasing the Storage time period while also keeping the quality of fruits acceptable in the low temperature condition storage. (Kaur et al., 2019).

Sodium chlorite showed a vital prohibition in the browning of sliced fruits and also inhibited the PPO activity of the slices of Pears. Sodium chlorite treatment also was efficacious in deactivation of the Escherichia coli O157:H7 slices of Pear fruits. The combination of carboxymethyl chitosan coating and sodium chlorite on the sliced pears had a noticeable inhibitory impact on the browning reaction as well as was able to prevent the PPO activity. Furthermore the combination of Sodium chlorite with coating of either Chitosan or Carboxylmethyl chitosan was able to sustain the tissues firmness. (Xiao et al., 2011)

CONCLUSION

Application of different chemical such as Calcium based solution, Ascorbic acid, Gibberellic acid, Oxalic acid, 1-MCP and Aminoethoxyvinylglycine etc. has a positive effect on the Pear fruits as Post harvest treatment. The Calcium based solutions were able to maintain the desirable quality of the produce of fruit tree by having positive impact on the physiochemical parameters of the fruits at different concentration. Gibberllic acid, Oxalic acid and Ascorbic acid can also play an important role as post harvest treatment for the storage of the fruits. Aminoethoxyvinylglycine and oxalic acid treatment were effective in delaying the respiration rate and also the ripening of the fruits. The combination of both AVG+ Oxalic acid were deemed to be effective in the retarding the respiration rate and small to less browning and also synthesis of lower sugar in fruits.1-Methylcylopropene was able to inhibited the sucrose loss while extending the storage period in fruits also preventing the amassing of the hexoses. The chemical treatments for post harvest can be applied for the purpose of extending the shelf life while also preserving the quality of the fruits at an acceptable level.

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TISSUE CULTURE AS A PLANT PRODUCTION TECHNIQUE FOR FRUIT CROPS AND PLANT PART USED FOR PROPAGATION

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ABSTRACT

The culture of plant tissue refers to the growth and multiplication in aseptic and regulated environments of cells, tissues and plant organs on specified solid or liquid media. In the fields of agriculture, horticulture, forestry and plant breeding, plant tissue culture has been commonly employed. it is an applied biotechnology used for mass proliferation, extinction of viruses, development of secondary metabolites and In-vitro cloning of plants. Plant tissue culture, slow growth and cryopreservation, also known as long-term preservation are also known as, has been extensively used for the preservation of endangered plant species., through short and medium-term conservation. In a suitable culture medium, a whole plant may be regenerated from a small tissue or plant cell. under controlled environment. Commercial technology is mostly based on micro propagation, where rapid proliferation is performed from process cuttings, axillary buds, somatic embryos, cell clumps in suspension cultures, and bioreactors to a small degree.

Keywords: In vitro; micropropagation; fruit crops; plant part used.

INTRODUCTION

Tissue culture to be the in vitro aseptic culture of cell, tissue, organs of hole plants under controlled nutritional and environment conditions species of plant are also made the initial clones of the genotype chose are true-to type. The regulated condition provide an atmosphere to the culture that is conductive of the growth and multiplication. sufficient availability of nutrients, medium the PH, adequate temperature and the appropriate gaseous and liquid atmosphere are protected by these conditions. for large-scale plants biotechnology is commonly used sprading. the techniques of plant tissue culture have become important industrial techniques. relevance in the field of plant propagation, elimination of pathogens, plant growth and secondary metabolite processing in recent years , in addition to their use as a research method. Small tissue parts (called explants) in a period exceeding, hundreds or even thousands of plants may be grown. A one plant material could be altered in a comparatively short period of time and space into too many thousand plants under controlled conditions, on a year-round basis, independent of season and temperature. Due to improved multiplication coefficients and low requirements, endangered and uncommon species are efficiently generated and preserved by micropropagation.on the quantity of original plants and property. In addition, for developing somaclonal and gametoclonal variants, plant tissue culture is considered the most significant technique for crop development. With well-adapted high-yielding genotypes, the vitro propagation technique has a significant potential for growing excellent quality plants, isolating useful variants With increased capacity for disease resistance and stress tolerance. Types of these kinds callus cultures give rise to copies that have distinct inherited characteristics from those of parent plants as a function of the chance of somaclonal heterogeneity.(Altaf Hussain *et al*., 2011)

Production of cells,tissues, organs or entire plants in maintained environmental and nutritional production process is the in vitro aseptic production of plant tissue.

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(Thorpe 2007) Also can be generated. The resulting clones adapt to the genotype type chosen. The protected areas include an atmosphere for environment and culture favorable to their development and proliferation of growth. these criteria include specifications which include adequate nutritional supply, optimal pH temperature and ample gaseous temperatures and gaseous properties climate of liquid.the culture of plant tissues there is systematic use of technologies for massive plant multiplication scale. other than their utilisation plant tissue culture as a science medium over modern years, methods have been of strong industrial significance in the field of propagation of seeds, removal of pests, cultivation, enhancement and creation of secondary growth metabolites It is possible to use small pieces of tissue (called explants) are produced in a continuous process to generate thousands and hundreds of, thousands of plants a it is possible to multiply a single explant into many, In a relatively short time period, a thousand plants and capacity in controlled situations, irrespective of season and temperature, on a basis over round the year (Idowu,2009)

Endangered and different plants have been grown successfully and processed due to high coefficients, multiplication of micropropagation and low number requirements and area of initial plants additionally, the culture of plant tissue is known to be the most powerful cropping technology Improvement of somaclonal production by gametoclonal versions the science of micropropagation has a wide variety of Potential to generate superior quality plants Isolation of desirable variants in well-adapted forms high-yielding, better disease resistant genotypes of capacities for resistance and stress tolerance (Brown and Thorpe, 1995) any kind of sort of callus communities are the origins of clones that have hereditary attributes that are different from those due to the probability of parent plants somaclonal heterogeneity occurrence (George 1993), Which results in the growth of improved varieties, which are economically important, Industrial products by way of plants the strategies of micropropagation include some advantages over the conventional techniques of crop propagation, cutting, grafting, Air-layering etc, and that is the fast proliferation of Methods which can lead to the generation of plants free from viruses (Garcia-Gonzales et al., 2010) Coryodalis yanhusuo, a significant one somatic plants have been propagated by medicinal plants from tuber-derived callus of embryogenesis creating disease-free tubers (Sagare et al., 2000). Meristem Tip banana plant culture bunchy Top Virus (BBTV) Devoid brome mosaic The Brome mosaic virus (BMV) as well as the Brome mosaic virus was produced (El-Dougdoug and El-Shamy, 2011), pathogen free germoplasma Increase in up to 150% yield of virus-free potatoes was In controlled conditions obtained (Singh, 1992).

The plant tissue culture medium provides nutrients required for normal growth and plant growth, mainly consisting of macronutrients, vitamins, micronutrients, other organic components, growth components, in plants regulators, origins of carbon and certain gelling processes agents, if the medium is solid. Murashige the most widely used Skoog media (MS media) is widely used for vegetative reasons, in vitro production of multiple plant species it is also important to the pH of the media that impacts on plant development as well as development if regulaotrs for plant growth. It is modified according to the range from 5.4 to 5.8 Liquid media should be used for both the solid and the liquid for culture. The composition of the medium has a massive impact on the initial explant, particularly the medium nitrogen content and plant hormones. Plant Growth Regulators (PGR) plays an important role in the determination of the pathway of plant cells and growth tissues in the centre of culture. Auxins, the the most frequently contained cytokinins and gibberellins are commonly used regulators growth of plant. amount and dosage of hormones used depends largely mostly on types of plants, plant types, cultured tissues or organs and the purpose of the experiment. (Ting 1982). Auxins and the most commonly used plant growth cytokinins are plant tissue culture regulators as well as the amount depending upon the type of culture created or regenerated by the high concentration of auxin typically favours the production of root concentrations, whereas the increased levels of cytokinins facilitate the proliferation of shoots. In rebaudiana stevia, maximal root induction and proliferation have been recorded when the media is supplemented with 0.5 mg / l NAA (Rafiq et al . ,2007).

Usually, cytokinins promote cell growth and division enable the development of shoots and the proliferation of axillary shoots high cytokinin to the ratio of auxin encourages the proliferation of shoots while the ratio root formation effects from high auxin to cytokinins (Rout et al., 2004) Initiation shoot and max multiplication were reported once the black pepper callus was transported to medium augmented with BA at 0.5 mg / l of BA level. (Hussain et al., 2011).

As an alternative method of vegetative proliferation, tissue culture is becoming Including plants. plants grown in vitro are typically free of bacteria and fungi diseases. now, the most effective and widely used procedure has been improved. replication by axillary bud . Axillary buds are

found on the axis of the leaves. The dormancy of axillary buds can be a mixture of cytokinin and Auxin using optimal concentrations of cytokininin tissue culture. be ruptured they develop into shoots until the dormancy is broken. By using media containing optimal plant growth regulator concentrations, they it is possible to multiply very easily. culture of current plant tissue is performed under purified air under aseptic living conditions for plants naturally, they are affected by the atmosphere on their surfaces (and often indoors) with microorganisms, thus the beginning of surface sterilisation materials (explants) are essential (usually alcohol) in chemical solutions. Today, since it is unsafe to use and impossible to dispose of, mercuric chloride is often used as a sterilising plant agent. then, explants are usually placed on a solid culture medium 's surface, but are often placed directly into a liquid medium, particularly when cultures Suspension of cells was performed. solid and liquid media are largely made up of sodium materials, with a few nutrients, vitamins and plant hormone. that are herbal. Strong media sources are with the addition of a gelling agent, typically prepared from liquid media, purified agar. the structure of the medium, the plant in particular, hormones and origins of the influence of nitrogen (nitrate vs ammonium salts or amino acids) on tissue morphology derived from of the initial explant are important.

A abundance of Auxin, for example, will always result in a roots proliferation, while an excess of cytokinin can produce shoots. an unorganised equilibrium of both auxin and cytokinin may also produce cell growth or callus, but as culture expands, the maturation morphology may depend on both species of plants and medium composition, generally sections are broke off and distributed to new media (subcultures) to enabling development or changing the culture's morphology. The strength experience is important in tissue culturist determining which pieces to judge. culture and which one to discard.

Important history about plant tissue culture

- 1902: In vitro cell culture technology suggested by Haberland
- 1904: Hanning has developed embryos of many species,
- 1922: Culturated successfully by Kolte and Robbins on root and stem tips
- 1926: Plant growth harmone IAA indole acetic acid was first discovered.
- 1934: Implemented VITBB growth supplement media for tissue culture
- 1939: Callus culture Proliferation
- 1941: For the cell division in Datura, coconut milk was first used.
- 1946: By shoot tipi culture, Ball grew an whole lupinus plant
- 1960: Kinetin was discovered by Skoog and miller as harmone to cell division
- 1960: The enzymatic cooking of the first toisolate protoplast was cell wall degregation
- 1960: The test tube fertilisation technology was proposed by Kanta and Maheshwarararar
- 1962: Murashige and skoog accumulate high salt concentrations in medium MS.
- 1964: First haploid plant developed by Guha and Maheshwari datura (Anther Culture) from pollen grains
- 1922: Cultured by Kolte and successfully on Robinsot and stem tips
- 1955: Kinetin was defined by Skoog and miller as a cell division harmone.

Importance of tissue culture

- It is easy to produce a large number of plantlets, in a relatively small time and space. generated on the basis of a single explant.
- Taking an explant usually may not destroy the mother plant, but sometimes and endangered plants can be cloned successfully.
- Relevant characteristics could be directly taken from the culture set-up (in vitro), thereby reducing the amount of space available for field trials.
- Once established, a line of plant tissue culture may provide a continuous line
- the year-round availability of young plants
- The time needed is considerably reduced, there is no need to wait for the entire crop development life cycle. For species that have generations that are long time, lower seed production volume, or seeds that are not readily available germinate, it is possible to replicate rapidly.
- Generally free of bacterial and fungal plants, in vitro developing plants diseases. Virus eradication and virus-free management of plants state.
- This encourages the worldwide transport of plants boundaries
- Tissue banks of plants can be frozen and then regenerated by tissue culture. It retains the collections of pollen and cells from which plants are can be propagated.

Stages of tissue culture process

1. **Nutrient solution preparation:** The semi-solid solution is prepared in double distilled water containing macroelements, microelements, amino acids , vitamins, sources of calcium, sources of carbon such as sucrose, and phyto-hormones.
2. **Aseptic culture establishment:** An rapidly growing shoot tip of an axiliary or terminal bud or shoot tip of a plant is usually the starting material for the process.

   3.Inoculation: Inoculation under aseptic conditions is performed. Explants or micro shoots are passed to the sterilised nutrient medium in this process.

   4.Plant development in growth room: The bottles are sealed after inoculation of the plant tissue and moved red into the growth room to activate the development phase at 25 ± 2 o Can 50 to 60 percent relative humidity under light sources (fluorescent light 1000-2000 lux).

   5. Micro plant hardening: Due to very high humidity within the culture vessel and artificial growth conditions the plantlets are re tendered and thus not prepared for coping with the field condition.

Tissue culture of fruit crops

Traditionally, most fruit-bearing plants have been propagated using vegetative techniques to retain attractive qualities. micropropagation methods may be used to enhance, or can be employed in the future replace the methods of vegetative propagation currently in use In this is also happening in many parts of the world. for examples, the bulk of all commercially processed strawberries are grown from In some European countries, in vitro-derived plantlets.

MANGO

Clonal propagation through tissue culture facilitates rapid multiplication, easy storage and safe exchange of germplasm. While some success has been achieved with somatic embryogenesis using nucellar tissue of mango In general, the source of shot tips was 8-16 year-old genotypical trees'Alphonso ',' Banganapalli ',' Totapuri 'and' Arka Anmol '. Uniform shoots of the kind with a single leaf rosette as the lower ones were otherwise picked at the tip, rapid medium discoloration caused by cutting petioles. (Thomas, Pious, et al., 1997).

BANANA

The banana, in the world as well as in India, is the fourth most common fruit crop. In banana vitro propagation has provided outstanding benefits, including fast multiplication. level, physiological uniformity and disease-free content availability during the year, in contrast with traditional plants, higher development in the earlier stages. An effective in banana plants, the micropropagation method was developed using sword sucker explants. Shoot tips containing rhizome tissue were isolated, measuring 2.0 to 3.0 cm in size. The surface sterilized for 15 to 20 minutes with chlorine-saturated purified water and for 5 to 7 minutes with 1.0% mercuric chloride. (Govindaraju, et al.,2012)

PAPAYA;

Shoot tips have been used as plant material from fruit bearing female papaya tree (c.v. Honey Dew) Shoot tips (length of 2-3 cm).An effective protocol for papaya (Carica papaya) in vitro propagation was created. By culturing shoot tips on Murashige and Skoog (MS)-medium supplemented with 0.5 mg / l (NAA) naphthalene acetic acid + 0.5 mg / l (BA) benzyl adenine, shoot cultures were obtained. 2 mg / l NAA + 0.6 mg / l (lBA) indole butyric acid + 60 mg / l adenine sulphate was the best for shoot multiplication among the various combinations of growth regulators applied with and without adenine sulphate to culture medium. (M. M. Saker et al., 1999)

The propagation studies were conducted by improving the release of axillary buds in the CO-5 variety of Papaya. As an explant for in vitro propagation, apical buds and lateral buds from seedlings and mature plants are being used. Various treatments of plant growth compounds for culture establishment and shoot multiplication have been subjected to explants from papaya varieties and hybrid (Bindu B et al., 2015)

STRAWBERRY

Stable and high-yielding strawberry plantlets for cultivation require mass processing of standard propagules to produce basic material for the establishment of large-scale plantlets. one of the productive means of achieving this goal is micropropagation. The major effects of poor strawberry production are continuous planting of runners from old mother plants that are vulnerable to diseases & viruses for five or more years and the absence of appropriate planting materials. Efficient procedure for shoot regeneration, proliferation and rooting for nodal segments of chandler, Oso-Grande and Cama-Rosa strawberry (Fragaria x ananassa) cultivars have been produced. (Mir, J. I., et al.,2010).

A strawberry CV micropropagation protocol. Through axillary shot proliferation from runner tips, Sweet Charlie was standardised. For the induction of multiple shoots and daughter runners from runner tips, medium augmented by TDZ (1 mg / L) alone was favourable. (Rekha, R et al.,2012) An successful method of disinfection procedure and micropropagation has been developed with an enhanced survival rate of strawberry explants and decreased phenol-induced browning. three genotypes had a rate of survival

with between 89.2-100 percent. As cultured under dim light (500 lux), Shoot tip was able to grow into plantlet on a hormone-free MS medium.(Ko, C., Al-Abdulkarim et al.,2009).

POMEGRANATE

Reliable and reproducible techniques have been developed to achieve healthy and well-formed plants from nodal pomegranate (Punica granatum L) cv 'Bhagava' explants. At full strength, Murashige and Skoog (MS) and Woody Plant Medium (WPM) nodal segments have been cultured in 2 distinct media.(Patil et al.,2011).

The explants were taken from 7-year-old fruiting trees of cv. (shoot tip and nodal segment). cv. Ganesh By regular subsuting of explants to media augmented with the same hormonal concentrations, the browning of cultures was regulated.(Murkute A.A et.,2004) .

Murashige and Skoog Medium (MS medium, 1962) were mounted on Leaf, Shoot apex and Nodal segment explant of pomegranate, supplemented with various concentrations of cytokinins and auxins for callus induction. (Choudhary, R., et al.,2018)

APPLE

Effective in vitro propagation of Cvs Redspur and Goldspur parentage dwarf and semi-dwarf seedling apple trees has been achieved. The material was taken from orchard trees that were 9 years old. Shot tip explants were more readily identified in vitro when arising by freshly grafted shoots then when originally extracted from the trees of the orchard.(Jones, O. P., et al 1985)

The widely known shoot culture with the prevalence of axillary shoots is the In vitro method of propagation to preserve a clone 's genetic integrity during the last decade, such procedures have been commonly applied to apples; approximately 70 cultivars of apple rootstock and scion have now been recorded as successfully propagated by shoot culture methods (Jones, O. P et al., 1993).

By culturing axillary bud on MS basal medium, effective in vitro propagation of clone rootstock apple MM106 was achieved. (Sharma et al.,2000)

GRAPE

The objective of the present study was to establish a protocol for the in vitro propagation of deGrasset, a grape rootstock characterised by high drought and salinity. Four compositions of the medium, MS, MS shoot development from nodal explants and MS-1 were tested with 1/2 nitrates (MS-1), B5 and WPM. A significantly higher rate of shoot proliferation was provided by medium. (Mukherjee et al.,2010)

Reliable and reproducible treatment for grapevine (Vitis vinifera L.) micropropagation 'Muscat of Alexandria' cv. After surface sterilisation of the checked explants (shoot tips and internode segments) using sodium hypochlorite (NaCl) at 0.52 and 0.78 percent for 15 and 20 minutes respectively, they were formed from shoot tips and internode segments.(Abido, A. I. A., et al 2010).

For the current analysis , four grape rootstocks with various genetic backgrounds were selected: Dogridge (Vitis champini), SO4 (V. riparia x V. berlandieri), H-144 (V. vinifera x V. labrusca) and 3309 C (V. riparia x V. rupestris). Explant canes were collected from five-year - old vines at Grape Germplasm Block, Main Experimental Orchard, IARI, New Delhi, New Delhi.(Alizadeh et al.,2010)

CITRUS

MANDARIN

For Citrus megaloxycarpa Lush., a highly acidic citrus cultivar from Manipur, India, an effective micropropagation protocol was developed. explant culture through shoot tip. when cultured on agarized Murashige and Skoog medium containing 0.25 to 2 mg L-1 N6 benzyl adenine (BA) alone and in combination with 0.50 mg L-1 naphthalene acetic acid (NAA) or 0.50 mg L-1 kinetin, the shoot tip explants formed numerous shoot buds.(Haripyaree *et al*., 2010).

The current study deals with the creation of an appropriate mandarin micropropagation protocol (*Citrus reticulata* L.) by means of cotyledon and juice vesicle direct propagation as an explant. The in vitro callus induction of leaf, stem segments and cotyledon system was collected from seedlings. (Badrelden et al.,2017).

SWEETORANGE

An successful method for in vitro plant regeneration using mature tissues of sweet orange cv from thin transversal stem sections explants (1–2 mm). Pera has been created. To determine the frequency of regeneration and size of buds, explants were cultivated in various media. When the explants were grown on Murashige and Skoog medium for 2 weeks and then moved to Woody Plant medium (WPM), a high percentage of explants (54 percent with 3.1 buds / explant) developing wide buds (1-4 mm) was observed.(Kobayashi, et al 2003).

GUVA

Plantlet regeneration from In vitro germinated seedling explants. multiple shoots were produced from Psidium guajava L. in vitro germinated (50 day-old) seedlings. Cv! Cv. From Safeda. Updated Murashige & Skoog (MMS)

medium with 6-benzylaminopurine (BAP), zeatin and gibberelic acid (GA3) supplementation, Zeatin 1.0 mg / L in combination with GA3 0.5 mg / L yielded the highest result (47.6%) with (3.2) shoot regeneration per initial explant.

Micropropagation of guava from five years of bearing plants by shoot tip culture. shoot tips were cultivated on modified MS medium after sterilisation, supplemented with distinct concentrations and combinations of L-glutamin and BAP. When MS was combined with BAP 1 mg / L and 500 mg / L L-glutamine, the maximum number of shoots (72 per cent) was produced in plantlets. (Zamir et al.,2007).

CONCLUSION

One of the most significant components of applied biotechnology is tissue culture. For commercialization, effective in vitro propagation of plants is now being used. many laboratories for commercial purposes worldwide use of in vitro culture by national institutes rapid plant multiplication system, conservation of germplasm, elimination of pathogens, genetic manipulations, and for the production of secondary metabolites. yearly, millions plants are regularly processed in vitro. The great one by reducing the production cost per plant by implementing low-cost tissue culture, the ability of micropropagation for large-scale plant multiplication can be exploited. the introduction of protocols and the proper use of equipment and resources to decrease micropropagule unit costs development of plants without sacrificing quality.

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CURRENT STATUS OF TRANSGENIC COTTON-UTILITY OF GENOTYPE INDEPENDENT *IN PLANTA* APPROACHES FOR THE GENERATION OF TRANSGENICS

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ABSTRACT

Cotton (*Gossypium spp.*), is a mercantile crop plant is grown for its fluffy fiber and cotton seed oil in around 70 countries worldwide. Cotton is an economically important crop, shows erratic productivity under rain feed conditions; it is bogged down with many biotic and abiotic stresses. Due to lack of resistant germplasm, crop improvement through conventional breeding practices has been lagging. Genetic engineering offers numerous protocols to engineer plants to overcome stress. Biotechnological intervention for cotton improvement has begun three decades ago. The recalcitrance of cotton to tissue culture has been the major constraint for *in vitro* regeneration. Alternate methods that evade tissue culture regeneration steps have thus been envisaged. Till date there are very few standardized protocols that can be employed to develop transgenics in a genotype independent manner. Thus, genotype independent *in planta* transformation strategies have gained momentum in the present days, but reproducibility of reported protocols remains an amigna in many cases. *In planta* transformations holds prominence due to viability and ease in generation of transgenic cotton plants with in less time. This review focuses on grouping efforts made by different research groups in this senior. Several reports and standardizations have been focused that reports development of transgenic cotton.

Keywords: Transgenic cotton, *invitro* regeneration, genotype independent, *in planta* transformation, genetic engineering.

INTRODUCTION

The prosperity of Indian economy depends on positive strides in agricultural and industrial sectors. Sustainable agriculture is the need of the hour in the context of global and liberalization trends. Crop production encounters various biotic and abiotic stresses particularly in the arid and semiarid regions (Mohapatra and Saha, 2019). Cotton is a major fibre crop of global importance and has high commercial value. Cotton is grown in about 80 countries across the world and is planted in an average area of 329.49 lakh hectares. India holds 1st rank in cotton production and contributes to 33.23 % of total world produce followed by China contributing 16.02 %. The countries of USA, Pakistan, Uzbekistan and Brazil rank the next positions respectively (Directorate of Cotton Development Government of India, 2017), where climatic conditions suit the natural growth requirements for cotton. Cotton is the most important fibre crop and is the basic input to the textile industry. India has the largest cotton growing area in the world with about 9.6 million hectares under cultivation accounting for one-fourth of the global cotton area. Cotton cultivation surpasses periods of hot and dry weather and adequate moisture obtained through irrigation. Cotton is harvested as 'seed cotton' which is then 'ginned' to separate the seed and lint. The long 'lint' fibres are further processed by spinning to produce yarn which is knitted or woven into fabrics.

Rainfed cotton production has significant contribution towards erratic cotton productivity. It is obvious that growth and development of cotton has to face one or other stress

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entities under rainfed situation. Cotton physiology portrays unique indeterminate growth habits with longer crop duration which make cotton vulnerable to abiotic stress influences from emergence to senescence. The adverse effects on the ongoing physiological processes may affect yield projection trends leading to production lapses. The various abiotic stress factors affect cotton growth, development and yield; their occurrence may be erratic or specific and their intensity may be varying in adversity.

DNA sequence data suggests that *Gossypium* genus arose about 1020 million years ago (Wendel & Albert, 1992; Seelanan et al., 1997). However evidences do not confirm the place of its origin exactly. West-central and southern Mexican regions with 18 species, North-east Africa and Arabia with 14 species and Australia with 17 species are known to be the primary centres of diversity. Cotton species, *Gossypium arboretum* and *Gossypium herbaceum* are diploid, indigenous to Asia and Africa and are confined to the "old world cotton". American cotton *Gossypium hirsutum* and Egyptian cotton *Gossypium barbadense* are amphidiploids (tetraploid, 2n=52), with centres of variability in Mexico, Central America and South America, belong to "new world cotton". *G.hirsutum* and *G.barbadense* are the two species most commonly cultivated on commercial scale. *G.hirsutum* is also kown as upland cotton comprising of 90% of world plantings, it was introduced into India during the 18th century A.D. (Hutchinson, 1959).

Conventional cotton breeding takes advantage of desirable alleles that exist in the cultivated germplasm of the same or a different *Gossypium* species. Natural variations existing within the genus of any plant species affords for continuous genetic improvement. Although improvement of lint yield remains the top priority in cotton breeding, weed control in cotton is more difficult. Cotton yield losses due to pests, including insects, are severe. Genetic traits within cotton can provide little insect resistance, but not satisfactory for controlling them. Thus the lack of dominant gene pool in cultivated germ plasma. This has stipulated for novel traits that can be introduced from other species to cotton for effective pest control through genetic engineering.

Biotechnological invasions for cotton improvement

Transgenic crops are an illustration of trait improvement. Transgenic technology has emerged as an awful tool for crop improvement with an assortment of techniques and strategies being used for gene delivery in crop plants as well as in cotton for its improvement (Birch 1997). Genetic engineering offers the feasibility to selectively introduce one or more genes that would escort the development of transgenic cotton to overcome both biotic and abiotic stresses (Oerke 2006). The most profound and major bottlenecks for deployment of genes through transgenesis has been the amenability of the commercially important or target crops to regeneration protocols. Not all economically important crop plants are regeneration-friendly to regeneration protocols. Since the reproducibility of reported regeneration protocol becomes an amigna in the same species, the search of alternative transformation approaches have become vauge. These recalcitrant plants have been labelled as "difficult to regenerate" plants. Several techniques and strategies are being employed to accommodate foreign genes delivery into plant genome; using the only natural biological plant transformation agent *Agrobacterium tumefaciens* which can aid in gene transfer. Most conventional plant transformation protocols use, *Agrobacterium* to engineer the plant genome with either tissue culture approaches or *inplanta* approaches. For many plant species that are amenable to transformation and regeneration by *A. tumefaciens*, T-DNA-directed gene transfer remains the method of choice because of its ease, efficient transgene delivery and integration into the host genome. Compared to direct DNA transfer methods, *Agrobacterium*-mediated transformations are most sophisticated and established method of transformation for most of the higher plants. In addition, this method does not demand specialized equipment or associated consumables for transformation.

Embryogenesis and Somatic embryogenesis in cotton

The first report of development of cotton transgenics was reported in 1987. Somatic embryogenesis (SE) and embryogenesis was used for the development of cotton by regeneration based approaches. Transgenics in cotton cultivar Coker 210 (Firoozabady *et al*. 1987) and Coker 312 (Umbeck *et al*. 1987) are the initial reports of transgenics in cotton. The genotype plays a major role in regeneration of cotton. Screening of about 38 cultivars belongigng to different cotton races (*G. hirsutum, G. barbadens and G. Arborium*) with different hormonal regimes showed that only Coker 312 showed highest frequency of embryogenesis in all combinations, followed by Coker 304, Coker 315, T 25 and Coker 310 (Trolinder and Xhixian 1989). Thus Cooker 312 has become the most preferable genotype, followed by Zhongmiansuo-35 and YZ-1 for the production of transgenics through regeneration based approaches. Several research groups have proposed various media compositions and used different explants for cotton for transgenic development. Some studies have been listed below.

Table 1: List of various studies that developed transgenic cotton using different explants.

Genotype/cultivar	Explant	References
G. hirsutum	Cotyledon	Davidonis and Hamilton. 1983
G. klotzschianum Anderss	Hypocotyl	Finer and Smith. 1984
G. hirsutum L. cv. Coker, 201, 208, 310, 315, DES 56, GSA 71, Lankart 57, Paymaster 145, Quapaw, RC10-3, Stroman 254.	Hypocotyl	Shoemaker *et a*l. 1986
G. hirsutum L. cv. Coker 312, T 25, T 169, Paymaster 303,784.	Hypocotyl	Trolinder and Goodin. 1987
G. hirsutum L. cv. Coker 310	Cotyledon	Finer. 1988
G. hirsutum L. cv. Stoneville 215, 453, 506, Acala, Lu, Coker 5110, 313, 100s, 304, 315, 310, 312, 15, Paymaster 303, 784, 145. G.arboreum L. var. Jyoti	Hypocotyl	Trolinder and Xhixian. 1989
G. hirsutum L. cv. Coker 201, 310, 315, 4360, GSA 71,75,78,CSC 25, G 8160	Cotyledon, hypocotyl, leaf sections	Firoozabady and DeBoer. 1993
G. hirsutum L. cv. Coker 201, CRI 12	Cotyledon, hypocotyl	Zhang et al. 2000
G. hirsutum L. cv. Deltapine 90, GB 35, B126	Cotyledon, hypocotyl	Sakhanokho et al. 2001
G. hirsutum L. cv. Coker 312, Acala cv. Maxxa, Riata, Ultima	Hypocotyl	Mishra et al. 2003
G. hirsutum L. cv. Coker 310	Hypocotyl, cotyledon	Kumria et al. 2003
G. hirsutum L. cv. Ekang 3, 4, 6, 8, 9, 10, Emian 22, Ejing B1, B11, Coker 201	Hypocotyl	Wu et al. 2004
G. hirsutum L. cv. Nazilli M 503, Nazilli 143	Shoot apices, hypocotyl, nodes	Aydin et al. 2004
G. hirsutum L. cv. Coker 312	Hypocotyl, cotyledon	Kumar et al. 2004
G. hirsutum L. cv. SH 131, Khandwa 2.		
G. arborium L. cv. Sarvottam, Jawahartapti	Hypocotyl, cotyledon	Khan et al. 2006
G. hirsutum L. cv. Coker	Hypocotyl	Aydin et al. 2006
G. hirsutum L.	Hypocotyl, immature zygotic embryos	Hussain et al. 2009
G. hirsutum L. cv. Khandwa, RS 810, Pusa 37, Pusa 26, Stoneville, F1084, CA 1193	Cotyledonary node with shoot apex	Gupta et al. 1997
G. arborium L. cv. Shyamly, Lolnt		
G. hirsutum L. cv. Anjali and *G. hirsutum* L. cv. LRK 516	Cotyledonary node with shoot apex devoid of cotyledons	Agrawal *et al*. 1997
G. hirsutum L. cv. Stoneville 7A and Paymaster HS26	Shoot apices Secondary leaf node Cotyledonary nodes	Hemphill *et al*. 1998
NCS 3, NA 1325, NA 920, NHH 390, DCH 32, Anjali, Srisailam, PMC, LRA, MCU 5	Shoot tip	Tripathy and Reddy. 2002
G. hirsutum L. cv. MCU-5, DCH 32, Coker 310FR	Shoot tip	Satyavathi *et al*. 2002
G. hirsutum L. cv. NIAB 999	Cotyledonary node with both cotyledons	Rauf *et al*. 2005
G. hirsutum L. cv. Barac (67)B	Cotyledonary node devoid of cotyledons and apical meristems	Abdellatef and Khalafalla. 2007
G. hirsutum L. cv. Bharani, Durga, JKCH 99	Hypocotyl	Divya *et al*. 2008
G. hirsutum L. hybrid H8 *G. hirsutum* L. Khandwa 2 *G. arborium* L. cv. BD ,1BD 6, Sarvottam	Cotyledonary node	Obembe et al. 2011

G. hirsutum L. cv. MCU 11	Cotyledonary node devoid of cotyledons	Mushke *et al*. 2012
G. hirsutum L.cv. NA 1325	Embryo axis	Pathi and Tuteja. 2013
G. hirsutum L. cv. NC 601	Cotyledonary node	Chakravarthy. 2013

Source: Jutur et al., 2015.

***In planta* transformation in cotton**

Tissue culture based transformations are tedious, time consuming, lead to variations in plant morphology and poor quality of seed pool that may not be viable to carry the modified trait to the subsequent generations. Development of a large number of uniform plants in short time with less labour efforts and minimal reagent requirements is the triumph of *in planta* transformations for trait modification in recalcitrant plant species. These tissue culture-independent *in planta* techniques were first initiated in *Arabidopsis thaliana* and has thrown new insight in the scientific community to development high throughput transformation protocols with the aid of *Agrobacterium*. *In planta* transformation methods in *Arabidopsis* such as "clip 'n' squirt" and vacuum infiltration have been successfully used by many researchers.

Table 2: *In planta* approach based *Agrobacterium* mediated gene transfer for transgenic cotton developed.

Genotype/cultivar	Transformed gene(s)	Method of transformation	References
Zheda B	*hptII, uidA* and *nptII, uidA*	Ovarian injection following pollination	Bibi *et al*. 2013
Sahana and BC 68-2	*cry1Ac, cry1Ia5, cry1Aa3* and *cry1F*	Stigmatic surface treatment	Mogali *et al*. 2013
Xinluzao 019	*susy, gus* and *nptII*	Pollen mediated transformation	Zhang *et al*. 2008
NC 71	*uidA* and *nptII*	Meristem transformation	Keshamma *et al*. 2008
Khandwa 2, Anjali and Coker 310	*uidA*	Meristem transformation	Kumar *et. al*. 2013
P8-6	*hptii: gfp*	Meristem transformation	
Meristem transformation	*Kesiraju et al*., 2020		
P8-6	*nptii:CP4-EPSPS*		Karthik *et al*., 2020

Several research groups have adopted diverse *in planta* transformation strategies for the generation of transgenic cotton (Rajasekaran *et al*., 2005; Zhang *et al*. 2009; Tian *et al*., 2010; Jin *et al*., 2012; Bibi *et al*. 2013; Mogali *et al*., 2013; Pathi and Tuteja., 2013; Vajhala *et al*., 2013; Kalbande and Patil *et al*., 2016; Guo *et al*. 2018). Though apical meristem has been the preferred target in most of these studies, the mode of infection has been different. However, the hypothesis of the standardized protocols have been the introduction of transgenes into the differentiating meristematic cells leading to concomitant transgenic expression in the shoots, ultimately resulting in their inheritance (Maher *et al*., 2020).

CONCLUSIONS

Tremendous progress has been made in the development of transgenic cotton. Both *Agrobacterium* mediated and direct gene delivery methods have been developed by many research groups across the world. Despite of such advances also, genotype dependency is still considered as major constraint and splendors the potential of transformation approaches in transgenic development. Recent reports on cotton improvement have shown that apical meristem targeted *in plant*a strategy can tackle the problem of genotype dependency (Karthik et al., 2020; Kesiraju et al., 2020). As this *in planta* transformation method was previously reported by Keshamma et al. 2008 (using GUS gene as a screenable marker) in a different cultivar, the recent report of successful deployment of the same technique was reported by Karthik et al., 2020 in two different reports, where GFP was used in one study and a herbicide tolerance gene *CP4-EPSPS* was used in another study in the same genotype.

Introduction of genetic changes into meristems reported concomitant transgenic expression in the shoots which later on produced flowers and seeds, ultimately transmitting transgenes and gene edits to the next generation (Maher et al., 2020). *Agrobacterium* mediated *in planta* transformation approaches using apical meristem

has been the preferred target for successful develoyment of transgenics as reported by most of the recent studies (Maher *et al*., 2020; Tyagi *et al*., 2020; Zlobin *et al*., 2020).

In planta transformation transformation of *A. thaliana* was made feasible by vacuum filtration which was considered as a tedious task before (Clough and Bent 1998). These *in planta* methodologies are reliable and reproducible also. Moreover this apical meristem targeted *in planta* transformation has also been proved to be genotype independent. The utility of this protocol was investigated in different crops (Kesiraju *et al*., 2017) and its genotype independent nature was demonstrated crops like capsicum, pegion pea, groundnut (Karthik *et al*., 2020). Thus this protocol has the power to speed up cotton transformation programmers efficiently. Transgenic cotton for herbicide resistance and insect resistance has become a reality in achieving enormous yield gains. However, further development of insect resistant cotton with different genes and modes of actions is the need of the hour. Hope this strategy would also be useful for the demonstration and validation of RNA interference and CRISPR based genome editing strategies in cotton and aid in crop improvement.

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