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Agrobacterium Mediated Lectin Gene Transformation in *Solanum tuberosum* (Lady Rosetta)

by

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I would like to dedicate this work to ALLAH Almighty, my teachers and specially my Parents who are the most precious, honorable and loving assets of my life.

CERTIFICATE OF APPROVAL

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(Qurat Ul Ain Iqbal Malik)

Abstract

Solanum tuberosum (Lady Rosetta) is an important crop due to its high supplemented nutritional value having different macro and micronutrients. It is used as rich source of food and feed. The main objective of this study was to develop the insect resistance in potato (Lady Rosetta) by using *Agrobacterium* Mediated Lectin gene transformation protocol. *Agrobacterium* GV3101 strain is used for transformation of Lectin gene to develop resistance in potato cultivar (Lady Rosetta). This study also showed the highly efficient transformation of Lectin gene in potato (Lady Rosetta) through *Agrobacterium* mediated technique. Regeneration and transformation efficiency of calli were checked by following the optimized protocol of infection, co cultivation, pre and post selection and regeneration. Two days of co-cultivation with different concentration of potato (Lady Rosetta) with *Agrobacterium* GV3101 strain was best for transformation efficiency infection. Finally, the successful regeneration of GM plants was obtained that help the future farmer to prevent the economic losses experienced by country on annual basis.

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Abbreviations

BAP	6-benzylaminopurine
Bt	Bacillus Throringinsis
ECR	Ecdysone receptor
GA3	Gibberellic acid
GM Plants	Genetically modified plants
HCL	Hydrochloric Acid
IAA	Indole-3-acetic acid
MS	Murashige and skoog
NaCl	Sodium Hydroxide
O.D	Optical Density
PCR	Polymerase Chain Reaction
PVA	Potato Virus A
PPT	Phosphinothricin
PVS	Potato Virus S
PVX	Potato Virus X
PVY	Potato Virus Y

Symbols

μl	Micro liter
$2,4-D$	2,4-dicholorophyenoxy acetic acid
g	gram
mg/l	Milli gram per liter
pH	-logH^+

Chapter 1

Introduction

Potato (*Solanum tuberosum*) belongs to the family of Solanaceae which arise in the South America and then spread to other countries of the world. Potato have 4th position after maize, wheat and rice. The word potato is derived from patata that is used by Spanish. Tropical and subtropical zones of the world are most suitable areas for cultivation. In Bangladesh after rice and wheat, potato (*S. tuberosum*) is second biggest crop. It contributes 53% of total vegetable production in Bangladesh [1].

Depending on the variety, potato plants can grow to a height of 60 cm. They are herbaceous perennial plant, and their leaves disappear after fruiting, blooming, and tubers production. Self-fertilization is also frequent, but bumblebees and other insects that transmit pollen from other potato plants are primarily accountable for cross-pollination. Even though commercial cultivars have lessened this tendency, tubers still develop in response to shorter days [2].

Following flowering, potato plants produce tiny green fruits that imitate green cherry tomatoes and contain about 300 seeds. The deadly alkaloid solanine is present in the fruit, as well as all other sections of the plant except the tubers, making it not suitable for intake. Every new kind of potatoes are grown from seeds which are distinguished from seed tubers by the terms "true potato seed", "TPS" or "botanical seed". A method for growing healthy seed tubers in greenhouses, or planting tubers, sections of tubers cut to include at least one or two eyes, are

all options for vegetative propagation of new types created from seeds. Unlike seed-propagated plants, which generate a wide variety of kinds, tuber-propagated plants are clones of their original species.

From the southern United States to southern Chile, various wild potato species can be found [3]. Initially, it was believed that Native Americans grown the potato in different locations, but new researchers found that it originated in the present-day areas of southern Peru and extreme northwestern Bolivia. From a plant called *Solanum brevicaule*, potatoes were grown between 7,000 and 10,000 years ago [4] several potato cultivars are cultivated in the Andes region of South America, where the plant is native [3, 5, 6].

The Spanish brought potatoes from the America to Europe in the second part of the 16^{th} century. They currently make up a sizable component of the world's food supply and are a common food in many regions of the globe. After maize, wheat and rice. Potatoes were the fourth-largest food crop in the world in 2014 [7]. There are currently more than 5,000 different varieties of potatoes due to centuries of selective breeding. The vast majority of varieties that originated in the plains of south-central Chile [8]. The significance of the potato as a food source and culinary component differs by region and is ever-changing. Even now, it remains a key crop in Europe, particularly in Northern and Eastern Europe, where output is highest per capita. While China and India lead the globe in total production as of 2018, southern and eastern Asia has experienced the most rapid growth in output over the past few decades.

The toxin solanine present in the vegetative and fruiting sections of nightshades from the genus Solanum, including the potato, is poisonous to humans. Normal potato tubers produced glycoalkaloids in amounts insignificant to human health when grown and kept appropriately, but if green areas of the plant (particularly sprouts and skins) are exposed to light, the tuber can develop a high enough concentration of glycoalkaloids to impair human health [9].

Around 5,000 different potato varieties can be found worldwide. They fall under one of eight or nine species, depending on the taxonomic school. In addition to the

5,000 produced varieties, there are about 200 wild species and subspecies, many of which can breed with cultivated varieties. There have been several instances when the gene pool of wild species of potato has transferred resistance to the gene pool of farmed potato species.

S. tuberosum, a tetraploid with 48 chromosomes, is the species that is produced the most frequently worldwide. *S. stenotomum, S. phureja, S. goniocalyx* and *S. ajanhuiri* are the four-diploid species (each having 24 chromosomes). *S. chaucha* and *S. juzepczukii*, two triploid species (each with 36 chromosomes). *S. curtilobum* is the sole pentaploid species raised for food (with 60 chromosomes). *Solanum tuberosum* has two subspecies: andigena, also known as Andean, and tuberosum, also known as Chilean [10]. The Chilean potato, which is a native of the Chiloe Archipelago, has evolved to the longer days of southern Chile's higher latitudes whereas the Andean potato has adapted to the brief days of mountainous equatorial and tropical regions [11]. There are about 4,000 different potato types, each with its own set of agricultural and culinary characteristics [12]. There are roughly 80 commercially available types in the United Kingdom [13]. Based on shared traits, a few fundamental groupings are formed that include russet potatoes, red potatoes, white potatoes, yellow potatoes (also known as Yukon potatoes), and purple potatoes [14].

This indicates that they fulfill the same fundamental criteria as other types. Fresh from the field, immature potatoes, also referred to as "creamer" or "new" potatoes, are prized for their flavor. As opposed to other potatoes, their flesh has no starch and is often tiny and squishy Based on whether they are Yukon Gold or red potatoes, they are known to as gold creamers or red creamers in the US [15, 16]. In the UK, the Jersey Royal new potato cultivar is quite well [17]. As opposed to "baby," "salad," or "fingerling" potatoes, which are small and have waxy flesh but are grown to perfection and kept for months before being sold, these potatoes are larger in size and have smoother skin.

An ordinary potato has 79 percent water, 17 percent carbs, 88 percent of which are starch, 2 percent protein, and no fat (see table). A 100-gram (3+12-ounce) serving of raw potatoes has 77 kilocalories, 23 and 24 percent of the daily values of

vitamins B6 and C, respectively, and no other vitamins or minerals in appreciable levels. It is rare to eat raw potato starch because it is mainly secreted by humans. In a baked potato, the amounts of vitamins B6 and C are drastically reduced, but the amounts of other nutrients are virtually unchanged [18].

Potatoes are usually left out of the diets of people who follow a low-GI diet since they are frequently categorized as an elevated glycemic index (GI). The GI of potatoes varies widely depending on verities, growing conditions, and packing, as well as how they are cooked, whether they are eaten warm or cold, and whether they are chopped, mashed, or eaten whole (especially the adding of different high-fat or high-protein toppings) [19]. The development of resistant starch may lead to a decreased GI impact in reheated or pre-cooked and chilled potatoes [20].

The previously major Late blight infestans (late blight) is still a problem in Europe and the US [21]. Additional potato diseases include Rhizoctonia, Sclerotinia, black leg, bacterial blight, grainy scab, and leafroll virus [16].

This food is full of starch and nutrients. It consists of 79% water, 18% starch, 2% protein and 1% vitamins. It also has fats minerals and trace elements [14]. Additionally, it is carbohydrate-rich. The potato is a good source of numerous minerals, including phosphorus, potassium, and magnesium. It also contains various vitamins, including B6, B3, and B1, as well as pantothenic acid, riboflavin, and folate [22]. Additionally, potatoes include dietary fiber and antioxidants that may help prevent disorders linked to accelerated ageing [23].

Lack of high yielding varieties, biotic and abiotic stresses are the main problems for the small yield of potato in Pakistan. Due to insects and diseases invasion the crop loss occurs 30-40%. The crop is destroy during the early stages of the crop growth it may turn even 100 [24].

Among the insects that regularly cause infections or affect potatoes are the Colorado potato beetle, potato tuber moth, green peach aphid (Myzus persicae), potato aphid, Tuta absoluta, beet leafhoppers, thrips, and mites. A tiny worm known as the potato cyst nematode eats on the roots of potato plants and leads them to wilt. Crop rotation is suggested since the eggs may linger in the soil over

several years. 84 percent of the 2,216 tested potato samples showed detectable amounts of at least one pesticide, according to the Environmental Working Group's analysis of USDA and FDA pesticide residue tests done between 2000 and 2008. Among the 2,216 samples, a total of 36 distinct pesticides were found on potatoes, however no single sample had more than one. An average of 1.29 different pesticide residues were present in each sample. There were 1.29 distinct pesticide residues per sample, with an average of 6 distinct pesticide traces per sample. The 2,216 samples contained an average of 1.602 ppm of pesticide residue. Despite being relatively modest, the pesticide residue was the highest of the 50 vegetables analyzed the potato aphid (*M. euphorbiae*) originated from North America and then spread to all countries where potatoes are grown. Aphid are capable of destroying of thousands of plants species [25].

Aphid eat potato sap directly and destroy the crop by transmitting various viral infestation. Plant growth inhibit due to nonstop sucking of potato sap by aphid insect. These aphid attack when they remove sap from potato plant and inject toxin that cause leaf deformation and these weakened potatoes give third quality tubers. Aphid work as carrier because they transmit virus to plant that cause diseases. Severity of diseases depend upon the nature of virus that are transmitted in plant. Some viruses attack potato plant e.g. PLRV virus that causes tuber necrosis and leaf rolling. For example, potato leaf roll virus (PLRV) is the virus which causes leaf-rolling and also cause tuber stem necrosis in potato curling, yellowing of leaves chlorotic spotting etc. [26].

There are two main viruses that transmitted virus into potato plant cause diseases, disturb potato yield and quality, these viruses are (PLRV) and potato virus Y (PVY). Both viruses are transmitted persistent and non-persistent in the potato plant by means of different 50 types of aphid. Aphid is a vector that transmitted the virus into the potato. Aphids are polyphagous species that moves potato to other plants. Now it is essential task to overcome the problems of potato production.

For the control of these diseases use pesticides, but these pesticides create numbers of environmental and safety concerns and these problems can cause climate change.

But the problem is that some insects have resistance against these pesticides, and these pesticides failed to control the diseases. Insect pests are majorly affecting the potato crop and tuber quality. Due to insect pest average 16% crop are globally destroyed [27]. Insect pest thus reduce the quality of tubers and also disturb the potato yield from 30% to 70%. The high use of chemicals which kill the insects caused environmental and safety concerns [28].

Aphids carry viruses in one of two ways. Aphids pick up the virus by probing the diseased plant's surface, and non-persistent viruses are concentrated in the epidermis. Aphids may pick up these viruses in seconds with a single probe and then transmit them in the same amount of time to a healthy plant. Non-persistent viruses, on the other hand, are only kept alive for a short time by the aphid - usually an hour or two. Unless it eats on another infected plant after that, the aphid can no longer transmit the virus. Due to the non-persistent viruses' fast acquisition and transfer, insecticides have little or no effect on reducing aphid spread. PVY, PVA, and alfalfa mosaic are examples of non-persistent viruses carried by aphids.

One of the ten most commercially relevant plant viruses in the world, potato virus X is present everywhere, including Pakistan. The names Solanaceae virus, healthy potato virus, latent potato virus, and potato mild mosaic virus are also used to refer to PVX. Mottled, intervening, moderate, and very mild mosaics appear on inflamed vegetative leaves. Some virus lines induce leaf roughness and curl, and inflamed flowers with moderate indicators of high leaves also display common symptoms in old leaves, shadowing the top leaves. Other mechanisms of transmission in nature include the spread of infected sap, field tools, and mechanical contact with roots and leaves. Plants grown for commercial purposes are susceptible to the PVX virus.

The PVS virus, which is officially known as PVS and is a member of the Carla virus genus, was first identified in the Netherlands and has since spread to all nations that produce potatoes. Two strains, PVSA (Andean) and PVSO (ordinary), have been described in terms of biology. PVSO differs from PVSA in that it does not cause systemic infection with Chenopodium spp. The majority of the amino acids that change between PVS and PVSA are found in coat protein and 11 K proteins.

Divergence in symptomatology and aphid transmissibility could be due to this discrepancy. Pakistan's Punjab and Northern regions have both reported cases of PVS, which has a 2–12% incidence and results in yield losses of 10–20 percent.

The virus that causes leaf roll is particularly important in potato history. It was first discovered in the Netherlands in *Solanum tuberosum* sp. and is now prevalent in potato growing locations all over the world. The potato phloem necrosis virus, Solanum yellow, and Tomato yellow top strain are further names for this virus, which is a member of the genus Luteovirus. PLRV is only ever transmitted to potatoes by a bug in nature. Primary infection is indicated by the rolling of the upper leaves at the top of the plants, which keeps the plants short and upright and makes their leaves thick and pale, as opposed to other leaf types, which typically roll their leaves upward, especially at the top. The entire plant appears upright and may be smaller than healthy plants in the case of secondary illness. Older plants have rolling lower leaves and plate top leaves due to the accumulation of starch, whilst basal leaves are thick, leathery, brittle, and crackle when squeezed in the palm.

Although for the betterment of potato varieties it must be important to use conventional breeding techniques, but these techniques are difficult to handle and time consuming. Greater challenge is to pose the quantitative traits. For all these problems can be solved by using genetic transformation techniques, with the help of these techniques without changes existing characters, new traits can be introduce in the transgenic plants [29]. Thus, genetic transformation techniques help in providing new varieties with full strength against biotic and abiotic stresses which destroy the crop badly. Genetic transformation technologies not only give resistance to plants against biotic and abiotic stresses but also introduce new characters in plants which were fulfilled the demand and need of human. New characters are introducing in the transgenic plants like maximum yield, heat and cold tolerant and disease resistance and provided that the best chance for maximizing the yields with other qualities.

Gene transformation with the help of Agrobacterium is efficient method for the transmition of foreign gene into plant and then the development of transgenic plant

occurs. A huge number of crop cultivar have been transformed in other countries with the help of this method such as tomato, cotton, potato, maize, tobacco, raspberry pea and rice etc. [30]. In more than 25 countries biotech crops were grown on 125 hectares of land by 13.3 million farmers of land in 2009 and 90% of the farmers were those farmers that have not enough resources for crop production in developing countries, whose increased income from biotech crops contributed to the improvement of scarcity [31]. With the help of these techniques like DNA recombination, plant transformation and in molecular genetics development ,farmer produces better crop and earn more profit [32].

In early *Agrobacterium* mediated genetic transformation of potatoes is reported in other countries, in Bangladesh very a small number of reports are existing. According to the protocol of *Agrobacterium* genetic transformation, the gene of interest can be isolated from one plant and insert in the potato varieties which are economically very important. For virus free potato meristem culture is frequently used in a mass scale. But through in vitro methods it is not yet possible to produce fungal resistant plants. Due to this reason, different conservative breeding and biotechnological techniques are being used in diverse parts of the globe.

For this purpose, we use Lectin gene for pest control that is ecofriendly. Lectin is a hydroxyproline-rice, chitin-binding, glycoprotein which may be participated in the defense mechanism of the potato plant. Lectin have very two dissimilar domains. Lectin gene which are naturally present in the garlic and have defense mechanism against different types of insects including sap sucking and chewing insects' pests. Lectins work as actively proteins that create disorder in the insects and cause death. Lectin gene is composed of mannose binding protein called homodimeric protein that is present in garlic. This protein is isolated from garlic and inserted in to different types of plants that against insect pest attack and also increase tuber quality and crop yield [33].

1.1 Problem Statement

Potato is considered to be the staple food of many countries around the globe. It is also used as a food source but its annual production is dependent upon different biotic and abiotic factors. Among which insect attack especially by aphid is the most common biotic factor drastically effecting the overall production of potato every year.

1.2 Proposed Solution

For proposed solution of damaged induced by insect in crop, transformation technology is helpful. Transformed Lectin gene have resistance against aphid. For this purpose, lectin gene transfer from garlic to potato with the help of *Agrobacterium* Mediated Lectin gene transformation.

1.3 Objectives

- To develop progeny from explant source of potato cultivar lady Rosetta
- To co-cultivate induce transfection with *Agrobacterium* mediated transformation.
- To regenerate the transformed plants
- To confirm the transformed Lectin gene through PCR

Chapter 2

Literature Review

2.1 *Solanum tuberosum*

2.1.1 Botanical Characteristics

S. tuberosum is a perennial herbaceous plant which belongs to the family of Solanaceae. This plant has alternately arranged leaves that consisting of leaflets that are both of different shape and size. It also has branched stem. It contains tuber, peel and liquid extract which are considered as traditional medicines throughout the world. Tropical and subtropical zones of the world are most suitable areas for cultivation [34].

2.1.2 Abiotic Stress

Environmental change has presented unique demands for the production of food. The increasing abiotic and biotic stresses are associated with the altered rainfall patterns. An external factor that adversely affects the plant is a common aspect of stress. Abiotic and biotic factors both have the potential to cause strain responses that have a deleterious influence on crop yield. In conversely to "biotic stress," which is induced by things like vegetation, microbes, insect predation, fungi, bacteria and viruses' infection, "abiotic stress" is caused by things like excessive heat,

CO2 altitude stress, high salinity or mineral deficiency stress, temperature fluctuations stress, low temperature stress, air pollution, chilling and freezing, and deoxygenation [35]. The great majority of the time, indices of general growth such crop output, biomass yield, plant life, or the main processes involved uptake in CO2 and mineral uptake are used to assess the effects of stress on these variables. Strain is closely linked to the concept of "oxidative stress or abiotic stress," which describes a plant's ability to endure a demanding atmosphere. Adaptation of plants frequently refers to a genetically set resistance level obtained through a recruitment process that lasts for centuries, it can be separated from acclimatization. Unsteady plants experience harmful effects that may be catastrophic.

Worldwide potato (*Solanum tuberosum*) production is greatly hindered by abiotic circumstances as heat, dryness, and salt stress. Recently, we identified StnsLTP1, a prospective potato thermo-tolerance gene, using yeast functional testing. Here, we talk about the participation of StnsLTP1 in several abiotic stresses on potato plants as well as the structural description of this protein. Quantitative analysis of StnsLTP1 found four -helices maintained by four disulphide bonds and eight conserved cysteine sites compared to other plant LTPs. The StnsLTP1 gene's expression altered in response to stresses such heat, a lack of moisture and salt mutant potato cultivars that overexpress. In comparison to non - proliferating (UT) control plants, the StnsLTP1 gene enhanced membrane permeability under stress as seen by reduced membrane lipid peroxidation and hydrogen peroxide concentration.

Moreover, contrasted to UT plants, transgenic plants that overexpress the antioxidant enzyme StLTP1 exhibited improved oxidative stress, greater enzymatic antioxidants accumulation, and up-regulation of pressure genes such as StAPX, StCAT, StSOD, StHsfA3, StHSP70, and StsHSP20. These findings indicate that StnsLTP1 mutant plants developed their antioxidative defense systems via coordinated regulation of stress-related genes and cycle of reactive oxygen species scavenging, better resistance to a wide range of abiotic signals [36].

On transformants and wild type (WT), the visual, transcriptomic, and metabolic effects of treatment with 150, 100, and 50 NaCl ranges were resulted. While

transgenic plants exhibited considerably higher superoxide dismutase (SOD) and ascorbate peroxide (APX) than WT under salinity stress, the altered antioxidant enzyme activities and the related H_2O_2 levels had a significant impact on the subsequent cell wall lignification. The numerous genes and transposable elements directly responsible for the production of lignin were found to be markedly stimulated when transgenic lines were matched to WT. Transgenic plants also accumulated more starch, had superior growth features, and generated less reactive oxygen compounds than WT plants.

These all findings demonstrated that the expression of the SOD and APX genes in mutant potatoes may work properly as beneficial set of biological, functional, and molecular adjustments in the H_2O_2-regulated lignin biosynthesis upregulating, allowing the plants to withstand extra salty conditions [37].

In a modern project, another report exposed that the mutant sweet potato calli had amplified carotenoid content and sensitivity to salt and methyl viologen (MV) exposure due to the amplification of the sweet potato IbOr gene. In order to recovering review, the function of the IbOr gene in external species, that altered the IbOr gene into potato under the control of SWPA2, a potent incapacitating organizer under treatment with multiple ecological challenges. Thus, according our prior research on sweet potato calli, the Nova cultivar, a quasi-control, had a level of total carotenoid that was up to 2.7 times greater.

Even while only the carotenoids in the quasi control, such violaxanthin, lutien, and -carotene, were increased at a similar level to total carotenoids, the replication of the IbOr gene had no effect on the carotenoid content [38]. Transgenic tuber tissues typically had greater transcriptome than transgenic leaf tissues for the majority of genes involved in carotenogenesis in contrast to the non- mutant controls. The upper levels of carotenoid concentration in the leaves or tuber tissue of mutant plants were connected with the improved broad-mindedness activity contrary to salt- or MV-mediated oxidative stimuli and DPPH radical-scavenging activity. Initial findings indicate that any further research is essential to develop a crop by enhanced expression in the IbOr gene that is immune to salt and other environmental obstacles [39].

The potato scientific name as (*Solanum tuberosum*) is generally documented to be susceptible to water deficiency stress. Even in the short term, tuber yield and grade could suffer. In a previous article, we discussed the dramatic impact susceptibility to high sodium content and methyl viologen-mediated oxidative stress, as well as higher total carotenoids, have transgenic potato plants that express the sweet potato orange gene (IbOr) under the control of the stress-inducible SWPA2 promoter. These plants are referred to as SOR plants, to increase the yield and stress tolerance of transgenic potato plants expressing IbOr, that exposed them to water-stressed greenhouse conditions in this study. The SOR plants showed a better resistance to drought conditions when grown in a greenhouse [40]. IbOr overexpression was linked to decreased tuber output and other marginally negative features. While maintaining the same level of drought tolerance, leading to tuber levels of production that were on par with or even beyond those of wild-type plants under drought stress. In particular, GM crops surpassed non-mutant plants in terms of dryness acceptance and the yield of marketable tubers under drought pressure. These results suggest that IbOr transgene expression can better the potato's drought resistance and tuber yield, two difficult agronomically important features [41].

2.1.3 Biological Stress

It is believed that the tuber necrotic variant of Potato Virus Y (PVY) is the most dangerous kind (PVY NTN). The effects of PVY NTN on numerous metabolic processes and cytological features were investigated in three potato cultivars: Lady Rosetta, Mondial, and Santana. The frequency of the virus, the rate of infection, and the degree of the sickness were also evaluated. Potato cultivars varied in their susceptibilities to the PVY NTN infection, although multiple stages of apical necrosis with various disease severity of 30, 60, and 100% being reported for the cultivars Mondial, Santana, and Lady Rosetta, respectively [42]. The least amount of leaf pigments was found in the PVY NTN-infected potato variety Santana. PVY NTN-infected leaves acquired a substantial portion of total phenolic and

total soluble carbohydrates as relative to unaffected leaves. The concentration of sodium cation was significantly higher in potato cv.

Lady Rosetta leaves that had been treated to PVY NTN. Electron microscopy analysis of PVY NTN-infected potato cultivar cells revealed alterations in the nucleus, mitochondria, cell walls, and chloroplasts. Researchers used an inter simple sequence repetitive chain reaction (ISSR-PCR) research to identify gene variants among potato cultivars that were PVYNTN-infected. The ISSR primers generated showed (52 of the 57 bands) polymorphism (91.23 percent). According to the findings, Mondial was only slightly resistant to PVYNTN infection, whereas Lady Rosetta and Santana potato varieties were quite sensitive to it. Lady Rosetta of the frost-sensitive *Solanum tuberosum* species are unable to adapt to the cold. Hard cold can totally destroy entire crops, and even a brief exposure will significantly reduce production. The crop's resistance to mechanical damage would therefore be greatly increased even by a minor increase in chilling tolerance [43].

The capacity to withstand cold and freezing temperatures in leaf discs and entire plants from SF seedlings potato plants that express the transcription factor (SCOF-1) in soybean cold-inducible zinc finger under the regulator of the tempted oxidative sweet potato peroxidase (SWPA2) promoter was observed. Potatoes of the frost-sensitive *Solanum tuberosum* species are unable to acclimatize to the cold. Hard cold can utterly ruin entire crops, and even a brief exposure will greatly reduce production. The crop's resistance to storm damage would therefore be greatly increased even by a minimal boost in freezing sensitivity [44].

The ability to withstand cold and cold weather in leaf discs and entire plants from SF seedlings potato plants that showed the soybean cold-inducible zinc finger transcription factor (SCOF-1) under the control of the oxidative stress-inducible sweet potato peroxidase (SWPA2) promoter was explored. The appearance of SCOF-1 strongly associated with SF plants' ability to survive cold stress after already being treated to 4^{o}C for five days. Compared to non-transformed (NT) plants, SF plants under demanding conditions had higher levels of total chlorophyll and photosynthesis. During 12 hours of convalescence at 25^{o}C following the freeze-thaw procedure, SF plants' lipid peroxidation levels reverted to about pre-stress

levels, whereas they kept rising in non-transformed plants. Our experiment revealed that overexpressing SCOF-1 substantially worsens the impact of cold stress on potatoes.

2.1.3.1 Fungal Diseases in Potato

Genetically modified (GM) plants can minimize the requirement for chemical pesticides by organically defeating fungus. Researchers have created transgenic potato lines that express antifungal proteins to counteract potential production losses in potatoes (*Solanum tuberosum*) induced by sensitivity to soil yeast infections. Nevertheless, it's critical to be conscious of any possible danger that any GM crops may have unintended soil bacteria. Because they serve as dependable markers and activity of a normal root system. This study examined the consequences of potato lines over-expressing bacteriocins genes on the compost fungus *Rhizoctonia solani* (R. solani) and Arbuscular mycorrhizal fungi. When exposed to a hypervirulent infection of R. solani, the six tested GM potato lines (AG-1, AG-3, RC-1, RC-5, AGRC-8, and AGRC-12) shown a greater decrease in infection indices than conventional plants. The virus had no impact on the proliferation of the RC-1, RC-5, and AGRC-12 lines, which suggested its capability to prevent R. solani infestation.

The levels of spore germination by the Arbuscular Mycorrhizal (AM) mushroom Rizophagus intraradices in in-situ and microcosm settings did not seem to change (pure in vitro isolated). In contrast to adding residues from the wild type potato line, it was clear that introducing potato biomass residues from these GM lines promoted mycorrhization [45]. The virus's capacity to control R. solani infestation was demonstrated by the fact that it had no impact on the growth of the RC-1, RC-5, and AGRC-12 lines. The AM fungus Rhizophagus intraradices appeared to generate similar amounts of sporulation in both in vitro and miniature conditions (pure in vitro isolated).

Microcosm assays using samples of mud from areas where potato crops have been farmed for at least one century in addition to the R. intraradices testing. In the roots of the AGRC-12 GM line, native mycorrhization and arbuscule growth were

substantially more apparent. Potato lines found to tolerate R. intraradices pure inoculum less favorably than AM taxa from the wild inoculation. GM potato lines demonstrated no discernible detrimental effects on the infiltration of AM fungus [46].

More than 80% of terrestrial plants have roots connected by arbuscular mycorrhizal fungus (AMF), which are critical elements of ecosystems. In these interactions, AMF exchange photosynthates, that inspire plant better growth and help plants cope with environmental pressure, for essential nutrients, mostly phosphorus, from their hosts plant; soil surface relationships with insect herbivores can be indirectly changed by the under interactions between plants and AMF. Impulsive symbioses between AMF and potatoes (*Solanum tuberosum*), one of the most useful vegetable crops in the globe [47]. It is unknown, though, how the connection between potatoes and AMF may cause impact on insects that feed on leaves while herbivorous. The expression of a generalist AM fungus on potatoes, a generalist Lepidopteran larval of the cabbage looper (Trichoplusia ni), and potatoes themselves were inspected in this study.

The study's objectives were to: a) ascertain how a trilateral interface between a potato AM fungus, cauliflower loopers, and potatoes affected each organism; b) look at how a group of plant genes include in defensive strategy were expressed during a tripartite interaction; and c) evaluate in potato physiology during the multiparty relationship between the potato AM fungus and cauliflower loopers. The results demonstrate that eating on mycorrhizal potato plants at the low level of G. intraradices root colonisation in the first experiment negatively impacted larval growth (20–40 percent colonised at the time of insect exposure). Larvae gained much less weight after seven days of feeding on mycorrhizal plants with low levels of G. intraradices colonisation than those that dined on plants with high levels of colonisation.

The AM symbiotic had a detrimental effect on insects but didn't significantly change the metabolism of potato plants, according to the results of the experiment. Overall, this study revealed that potato root colonisation by G. intraradices circuitously effected cabbage looper growth (as measured by weight), but the effect

depended on the stage. At the little degree of G. intraradices colonisation, potato plant physiology was unaltered; however, insects once more developed fewer mass after eating on mycorrhizal plants. At the high G. intraradices colonisation levels, potato shoots bulked out more, but insects that fed mycorrhizal plants also grew bigger. These results suggest that potatoes may adjustment from insect resistance to tolerance as G. intraradices root colonisation rises from little to high levels [48].

2.1.3.2 Insect Attack

Aphids, the most popular forms of nectar sucking insects, substantially decrease the yield of grain production every year. The excessive use of insecticides for this bug elimination from the crop to has an adverse impact on the environment and threatens world health. In this work, potato plants were made utilizing six various approaches, employing the CaMV 35S and ST-LS1 regulators to express the *Galanthus nivalis agglutinin* (GNA) gene. The GNA gene was expressed in the foliage, stems, and roots of mutant plants when the CaMV 35S promoter was used, but solely in the foliage and stems when the ST-LS1 promoter was in control, as per conventional numbers of polymerase chain reaction (qRT-PCR) research. After 5 days of inoculation, the percentage of aphid fatalities in the tested mutant plants ranged from 20 to 53.3%. The aphid population in mutant plants fluctuated from 17.0-1.43 percent (ST2) to 36.6-0.99 percent (35S3) at 15 days that followed the inoculated, or 24.9-53.5 percent of the aphid population in non-mutant plants. The outcomes of our investigations reveal that GNA accumulation may give mutant potato plants some resistance to aphid assault, pointing to a possible replacement for insecticide use in the term [49].

As a consequence of intensive Bt growing crops for over 2 decades, the most of commonly produced insect resistant genetically engineered crops contain CRY gene(s) derived from Bacillus thuringiensis, raising doubts about their sustainability and endurance in insect control. In the current work, DNA sequencing (RNAi) was used to silence the highly specialised Ecdysone receptor (EcR) gene of the Colorado potato beetle (CPB), which is involved in moulting. Using the appropriate markers in both sense and anti-sense configurations, the EcR gene of the CPB's

full cDNA was duplicated, and the result was cloned in the pRNAi-GG envelope encircled by an intronic tract.

The CaMV 35S activator was used to transplant the *Agrobacterium tumefaciens* strain LBA4404 into blade and internodal micropropagation of the potato cultivars Agria and Lady Olympia bearing composites. Conventional microscopic examination of primary transformants showed that T-DNA had been correctly integrated into the plant genome. The vulnerability of the transgenic organisms to CPB larvae in their first, second, and third instars was assessed in both cultivars. The findings of the studies on leaves showed that 15–80% of CPBs suffered and perished. Gm crops exhibited a considerably smaller flip (0.87-4.14) in juvenile mass when comparison to flies fed on control plants (1.87-6.53). The lower EcR transcription levels seen in CPB caterpillars fed on transgenic plants provide additional proof of the efficacy of dsRNA EcR in suppressing EcR gene expression. This work provides as a superb illustration of how to deal with outbreaks of potato bugs, which cause enormous losses to the world's potato crop production [50].

The Colorado potato beetle is among the most troublesome pest species that damage potatoes. It connects with a variety of bacterial partners that can change the interactions among various plant species and omnivorous insects. Originally known as Enterobacter BC-8, the most prevalent *L. decemlineata* microsymbiont was identified in both the anterior and rear portions of beetle stomach. Using antibiotic-treated larvae and a model system that replicates beetle assaults, the impact of *E. coli* BC-8 on changing plant defences was investigated (wounding plants treated with Enterobacter BC-8 suspension). A development of phenolic content and hydrogen peroxide, as well as the functioning of peroxidases and trypsin inhibitors, were all suppressed by the symbiotic bacteria, as already reported. Significantly, the effects of insect symbiotic organisms on potato plants triggered genes sensitive to salicylic and the marker for the salicylate signalling pathway. Contributors genes, which produce a restricted number of antibodies that fight against predators, exhibited significant transformation of genes. In order to reduce the defence mechanisms of plants, facultative anaerobic BC-8 is responsible for controlling salicylate/jasmonate crosstalks [51].

2.1.3.3 Insect as Major Cause of Viral Infection

The potato is one of the most significant cash crop for farmers and contributes significantly to Pakistan's GDP despite being grown both domestically and as a garden food. Potatoes are planted as a summer crop in high altitude valleys, whereas 3 consecutive crops can be produced in the plains and low ravines each year. While Pakistan's overall potato crop yield is low in comparison to other grain countries, it nevertheless yields 12–15 times more and provides more calories than crops like wheat, maize, and rice. High yielding foreign potato cultivars were introduced by agricultural owners, but they also brought with them viral issues and significantly enhanced potato crop production. Among them, yield losses of 83 percent in the early, mid, along with Potato Virus A (PVA), Potato Virus S (PVS), Potato Virus M (PVM), Potato Virus Y (PVY), Potato Virus X (PVX), Potato Leaf Roll Virus (PLRV), and Potato Microfiber disease have all been detected in early summertime (PMTV).

In Pakistan, a considerable number of potato progenies were certified using the Enzyme Linked Intended to safeguard Assay (ELISA), but new molecular technologies, such as the (PCR) assay, were introduced for quick, sensitive, and more accurate pathogen validation [52]. PVA and PVM percentage incidences were reported from Pakistan's main potato-growing areas, but no molecular testing or nucleotide proof of these viruses was reported from Pakistan. Despite the fact that no genetic proof of PVS has yet been published from isolated strains from Pakistan, the coating protein (CP) oligonucleotides of PVX, PVY, and PLRV from Pakistani samples have been released in the centralized database of the National Center for Biotechnology Information. PCR tests were developed for the molecular identification of PVS PVM, PMTX, PVS, and PLRY. The CP gene genetic material reveals a unique variation in Pakistani potatoes, and the country's potato blight is becoming extremely difficult as a consequence of the growing prevalence of genotypes [53].

Marmor Solani also called as Potato Virus A, Potato moderate mosaic virus, potato virus and Solanum virus 3, in addition to the commonly used abbreviation PVA.

A member of the potyviridae family, potato virus A is a good attitude RNA virus. In Pakistan, PVA was originally cut off from Punjab in 1978. The symptoms initially appear on terminals leaves as blotchy mottling, light mottling, modest crinkling, mosaic, necrosis, and severe crinkling when PVX or PVY are present [30]. When the source plant additionally possesses PVA, Myzus persica can spread the potato aucuba mosaic virus. PVA is sap transmissible and stylet transmitted in a persistent manner by an aphididae-family mosquito vector. Punjab had a PVA incidence of 40.7% percent, whereas the northern regions had a PVA incidence of 31.55 percent. In spite of the lack of a molecular approach (PCR) and nucleotide proof, serum verification (ELISA) of PVA was utilised for filtration in potato progenies, and the existence of PVA in culturing potato plants of Cardinal, Desiree, and Diamond was also stated from Pakistan [54].

One scientist who originally reported that this pathogen had been found in Pakistan. It is known as PVM and is a member of the Carlavirus genus. Other names for this virus include Potato virus X, E. Solanum virus 11 as well as Kartoffel K virus, and Solanum virus 7, Potato Rollmosaik virus, Potato Paracrinkle virus, Potato Interveinal mosaic virus, Potato Leaf Rolling Mosaic virus, and Solanum virus 11. Depending on the viral disease, potato cultivar, and environmental factors, potato crop symptoms may range from being quite moderate to being extremely severe. Compared to older seedlings, which may not show symptoms, young plants are more prone to do so. Interveinal mosaics with clearing of the veins are a normal effect, though necrotic patches with brown streaks on the veins of the underside, petioles, and stem may also develop on the top leaf surface. PVM can be disseminated mechanically, although it is mostly carried in nature by Aphididae family insect vectors. Transmission of seeds and pollen is not known to happen. The average prevalence of PVM during the 2011–12 academic year was 24 percent in Rawalpindi, Islamabad, Faisalabad, and Sahiwal, although Pakistan has not provided any molecular or nucleotide data [55].

The virus was first identified in the Netherlands and is thought to have propagated from there to all countries that cultivate potatoes. It is a member of the Carlavirus genus and is referred to by the official name PVS. There are two variants known:

PVSA (andean) and PVSO (ordinary). PVSO differs from PVSA in physiology by not systematically infecting Capsicum species. The bulk of the amino acids that change between PVSO and PVSA are found in the coat protein and the 11 K proteins. This variance may be the reason for the difference in symptomatology and aphid transmissibility [56].

This pathogen is widespread around the world and is one of the 10 plant viruses with the biggest economic effect, along with Pakistan. Other names for PVX include the potato dormant bugs, Potato robust bug, and Potato mild mosaic viral. Mottling, interveinal, mild, and super-mild mosaic can be seen on the leaves of infected plants. Certain viral strains cause the foliage to become rugose and crinkly, and diseased plants with slight effects in the higher plants leaves may have common features in the grownup plants leaves that are shadowed by the top leaves [57]. In the natural environment, infection spreads quickly and spontaneously through contaminated tubers. Infectious sap, farm equipment, and mechanical contact between roots or leaves are other channels of transmission. The average PVX incidence rate in Punjab's seven principal potato-growing areas was 13.18 percent. In Pakistan's upper Kaghan Valley, widely cultivated potatoes are infected with PVX. Punjab has the greatest levels of infection, with a frequency of PVX infection in Pakistan's potato-growing regions ranging from 1.5 to 6.2 percent. Additionally, foreign seeds have shown PVX insertion rates ranging from 0.7% to 30%.

While Desiree variations reported occurrences of 8.33 percent and 20.8 percent from Faisalabad, Sahiwal, and Pak Pattan, respectively, Cardinal genotypes showed a minimal PVX rate of infection of 4.16 percent. There have been reports of PVX occurrence in many Pakistani cities, including [3]. PVX illness intensity increased over 28^{o}C and dropped below 25^{o}C in relation to environment [58]. Although a Pakistani isolate's 613 bp CP gene segments were produced using primers specific for the coat protein gene (GGCGCAACTCCTGCCACAGC) and antisense (TTGTTGTTCCAGTGATACGA), nucleotide sequence analysis shows that this isolate has the most genetic similarity with a USSR segregate of PVX. serological PVX confirmation was utilized for screening [59].

PVY is one of the most prevalent and carefully useful plant viruses in the biosphere, ranking among the top five in terms of economic impact. Furthermore, it holds a hopeful position among the harmful limitations on the potato yield. The Tobacco venial necrotic viral disease, the Potato acropetal virus, the Potato leaf drop strip virus, the Potato virus 20, and the Potato virus 20 are some of its substitute names. It is the type member of the RNA virus genus. Helper elements are commonly divided into several viral strain groups, and the virus generated a range of symptoms depending on the type [60]. Up to 9 significant plant species, containing major crops like the potato, tobacco, pepper, tomato, pepper, and tomato, as well as solanaceous weeds, are included in the PVY spectrum of action. It is made up of a range of potato strains, including PVYNTN, PVYNW, and PVYN:O, as well as PVYO, PVYC, PVYN, PVYE, and PVYZ.

Scientist published the first report of it in Pakistan, this virus was one of the main potato infections in the low lands of Punjab [61] estimated that this virus had a prevalence of 2 to 25% across the country, and that damages in Pakistan from PVY were predicted to range from 58 to 83 percent. In Pakistan, tobacco, tomatoes, and chilies are furthermore PVY's natural hosts [4] ELISA was used to determine the prevalence of PVY in Jhang (28.20%), Toba Tek Singh (52.77%), Sialkot (27.83%), Gujranwala (14.37%), Chiniot (18.72%), Okara (12.72%), and Sahiwal (6.81 percent).

The varied natural hosts of PVY include a number of key crops and a few weeds. Only the PVYO strain has been seropositive documented, although the rising PVY prevalence is frighteningly high in Pakistan's main potato-growing districts. A previously unknown novel strain of PVY has been found in Pakistan, according to nucleotide analysis. Forward and backward primers unique to the CP gene were developed, and Pakistani researchers published the genetic code and molecular confirmation of the CP particular gene [62].

This pathogen has a considerable historical significance for the potato because it was originally discovered in *Solanum tuberosum sp.* in the Netherlands and is now widespread [63]. Other names for this disease include Potato phloem necrotic virus, Cinnamomum yellow, and Tomato yellow top strain. The standard acronym for

this virus's genus is PLRV. Only an insect vector can persistently transmit PLRV to potatoes in the field [64].

2.1.4 Use of *Agrobacterium* Mediated Transformation

The potato R genome and the Phytophthora infestans Avr a virulence gene are the genetic halves of the nucleotide sequences exchange that results in resistance in host plants. Utilizing this promoter immunity, exceptional resistance to late blight in potatoes has recently been achieved by gene manipulations. How soon this severe resilience endures will probably affect the selection of R Genetic variants, the quantity of R genes that make up a R gene stacking, and the pathogen Avr gene variety. Here, we present the results of a comparative evaluation between the Avr-vnt1 gene, which was made from two strains of *P. infestans* pertaining to the EC-1 branch, and the Rpi-vnt1.1 gene, which was obtained from *Solanum venturii* and inserted into the potato variant Blanche using genetic manipulation. It has previously been proven that EC-1 is harmful in Rpi-vnt1.1 mutant plants, despite not possessing the Avr-vnt1 gene.

It's fascinating to note that 52 polymorphic occurrences came back positive for tolerance to two strains of *P. infestans*, POX067 and POX109, both of which are EC-1 members of the family. Researchers showed that the Avr-vnt1.1 gene was mildly transcribed in both genotypes. It has previously been established that EC-1 is harmful in Rpi-vnt1.1 transgenic plants, although not possessing the Avr-vnt1 gene. It's fascinating to note that 52 recombinant occurrences were resistant to isolate bacteria of *P. infestans*, POX067 and POX109, both of which are EC-1 relatives, almost across the entire antimicrobial susceptibility. It has been reported that both strains had mild Avr-vnt1.1 gene expression. Two genes were generated by *Arabidopsis thaliana* (AT1G12660 and AT1G12663) [65]. The antifungal effectiveness of the synthesised thionin protein is demonstrated towards pathogenic fungi that harm potatoes, such as *Alternaria alternata* and *Rhizoctonia solani*. Each of these two thionin genes was included in turn within the pEGAD vector. Then, using the multimodal cutting technique of tissue culture, the two assessed

potato varieties (lady and spunta) were transferred into chitosan nanoparticles. Thionin transgene expression in the mutant plants was established by RT-PCR. The impact of bacterial suspension on plant organs and the inhibition action of thionin peptides on fungal growth were the two types of test used to determine the contribution of thionins (in the transgenic potatoes) in vulnerability to the damaging fungus. The altered potato plant is safe from pathogenic infiltration because it has active defence system and can reduced pathogen infiltration by secreting a variety of small antimicrobial proteins, including thionins. Utilizing spore suspension on leaf and micro-tuber to screen for fungal infection, transgenic potato genotypes were created. The findings demonstrated that the novel varieties had much exposure to health resistance than the non-transformed plants. The other experiment investigated how the mutant plant's produced thionin proteins affected both *A. alternata* and *R. Solani's* radial development and diameter reduction [66]. A novel and important element of anthocyanin synthesis in the root system of sweet potatoes is the R2R3-type protein IbMYB1. IbMYB1 amplification has been found to enhance anthocyanin coloration in tobacco leaf, Arabidopsis, and storage roots of sweet potatoes in the past. For this study, we created mutant potato plants that express the IbMYB1 genes. These plants generated significant levels of anthocyanins, perhaps under the control of the sweet potato patatin (PAT) promoter or the oxidative stress-inducible peroxidase anionic 2 (SWPA2) activator. The PAT-MYB1 mutant plants have better anthocyanin tuber heights than either the empty vector control (EV) or SWPA2-MYB1 plants. In light of all of our investigation, it is rich that overexpressing IbMYB1 is a highly actual way to produce inherited disparity with greater tissue-specific anthocyanin synthesis [67].

Vitamin B6 is a crucial enzyme required by all plants as a cofactor in different internal biochemical pathways. The production of proteins that scavenge cellular reactive oxygen species is regulated by this potent antioxidant molecule, which is another name for it. Since it is good recognized that both animals and plants can have synthesized B6, all animals, including humans, must obtain it from plant food sources. However, the delicious portions of the widely consumed vegetables lack adequate vitamin absorption to supply the life sustaining doses. The quantity of vitamin B6 in the model species has been helped increase through to the utilization

of genetic editing. A transgenic variety of potatoes was made using the essential vitamin B6 pathway gene PDXII which was obtained from the Arabidopsis thaliana plant *(Solanum tuberosum* cv. *Kufri chipsona*). The prototype plants' vitamin B6 concentration has been effectively increased using genetic modification methods. The current work describes the creation of a mutant potato (*Solanum tuberosum* cv. *Kufri chipsona*) higher maternal the essential gene PDXII, which was taken from A. thaliana and was regulated by the CaMV 35S to support the company. PCR, Southern blot, and RT-PCR tests showed the consistent integration and expression of the transgene in the transgene. When contrasted to their untreated equivalents, genetically modified tubers substantially increased their ability to absorb B6 Vitamins (up to 107–150 percent). The increased levels of vitamin B6 and the enhanced mRNA translation of the PDXII gene were linked. The transgenic plants were able to endure a range of greater degrees of abiotic and biotic stresses brought on by salinity (NaCl) or methyl viologen because the mutant potato's noticeable rise in B6 content was also connected to its ability to survive under abiotic stressors (MV). Thus, we showed that constitutive promoter-driven overexpression of the PDXII gene increased vitamin B6 production, which also increased potatoes' sensitivity to a variety of biogenic stresses (*Solanum tuberosum*) [68].

2.1.5 Different Methods of Transformation of Genes in Plants

2.1.5.1 Micro Projectile System for the Transformation of Gene in Plants

Species of plants, including a few that have earlier rejected efforts to employ *A. tumefaciens* and embryogenic based approaches, have been successfully transformed utilising the recently invented microprojectile is the method for the transfer of gene into competent cells. Furthermore, microprojectile bombardment, which has already shown its particular suitability for these uses, has enabled it to immediately convert organelle genomes and understand the strengths the transient

transfection of genetic constructs put into cells of intact tissues. Here, many transient expression acceleration devices and the methods necessary to design an effective microprojectile-mediated transformation system for any plant species are described.

The need of optimizing DNA transportation to cells and modifying procedures for growing plants that have undergone stable conversion depending on the properties of the tumor site, how it behaves in cultured cells, and the existence of specific genes. There are also descriptions of other options, such organelle transformation. In stable nuclear transformants produced employing microprojectiles, trends of transformation and expression of inserted genes are gathered. In order to realize the full potential of microprojectile-mediated gene transfer as a virtually universal gene transfer technique with intriguing applications in fundamental crop molecular biology and practical plant improvement, we highlight a range of innovative barriers that must be overcome [69].

2.1.5.2 Gene Transformation Through Gene Gun and *Agrobacterium*

The two most popular techniques for plant genetic manipulation are direct gene transfer via the gene Cannon and agrobacterium-mediated conversion. Although the Agrobacterium approach has been effectively utilized for many years in dicotyledons, effective procedures for grasslands have only lately been discovered. Microspheres blasting is a frequently used approach in plant biology for introducing foreign nucleic acids into plant genomes. Here, the effectiveness of the two strategies for transforming tall grasses are evaluated in terms of transgene incorporation and expression. The transgenic tall grasses techniques result in the creation of several fertile, independent transgenic lines [70].

2.2 Lectin Gene Transformation

For the commercially important vegetations, lapping insect pests like aphid, jassid, whitefly, and mealybug stance a serious hazard. Bug pests that drain fluid

from phloem invade and feed on it. All important elements, including as glucose, carbohydrates, and amino acids, are stored in high ranges in the phloem [71]. Mealybug is one of the major insects sucking pests that devours phloem matters. Mealybug has arisen in recent years as a momentous pest species of numerous key farming crops, including tobacco, ladyfinger, tomato, cotton, and many more. Mealybug infestations on cotton and other important crops were first noted in Pakistan in 2005. Mealybug suckles sap from fragile leaves as it creeps and gets to the developing ends of plants. It is a polyphagous bug that consumes a variety of plants, notably fruits, vegetables, and field crops.

The Leguminaceae, Malvaceae, and Solanceae groups of flowering shrubs are also attacked by Mealybugs. In addition to sucking hyphae sap, mealybugs also secrete honeydew that causes fungal pollutions (black sooty mould) and interferes with photosynthesis in plants. It uses different donors to thrive during the off-season. Mealybugs severely reduce agricultural output by damaging, bending, and drying leaves. It is possible for these bugs to establish communities inside shoots and apexes. Mealybugs contain waxy secretions on their body surfaces, making foliar pesticide sprays particularly difficult to manage. Mealybugs have natural enemies and competitors; therefore, major issues haven't been recorded in the nations where they invented. When mealybugs were carried to new sites without their natural challengers, severe outbreaks were regularly experimental. These bugs not only damage crops, but they also serve as transporters of several plant viruses. While some bioengineering methods, such as Bt, have proven effective in controlling insects that sucks nectar like mealybug and chewing pests like bollworms, none have been originate for chewing pests' comparable bollworms. Challenging different B.t, d-endotoxins, VIPs, proteinase inhibitors, lectins, etc. for their toxic effects to insect infestations is urgently necessary [72]. Novel insecticidal toxin genes have been exploited for insect control method. Due to their insecticidal action and complementarity with proteolytic enzymes and Bt toxins, the Mannose binding plant lectins have been identified as potential toxicants to manage predatory insects of the Hymenoptera order. Lectins are proteins that bind carbohydrates (well-defined sugars) and have a strong affinity for attaching glycolipids, glycans of glycoproteins, or polysaccharides.

Lectins most likely stop the absorption of nutrients by coming into touch with the gastrointestinal glycolipids of insects and other flesh-eaters. Plant lectins are thought to be real against pests that feedstuff on sap. The operation of fitting pest control methods [73] can benefit from the implementation of lectin-expressing transgenic plants, even if they only exhibition limited encounter against a sap-sucking insect pest. Transgenic plants that have had their lectins altered become satisfactorily sensitive to sap-sucking insect pests. The agriculture industry would accept such resistance in transgenic plants against sucking insects and could use it in integrated pest organization. Conferring to reports, plant lectins are toxic to insects to a variety of insect orders, including the Coleoptera, Diptera, Homoptera, and Lepidoptera. A number of plant lectin are strong poisons (perhaps anti-insect chemicals) that attach to an insect's midgut surface, traverse epithelium, and enter the hemolymphs of many insects. Among other lectins, Cowpea aphid (Ahis craccivora)-targeting ASAL, a mannose-binding lectin from *Allium sativum*, was found to be predominantly competent. The *A. sativum* plant agglutinin (ASAL) lectin gene from garlicky plants has been used to assess the susceptibility of the feeding predatory insects such as the white flat - bottom plant hopper (WBPH), brown plant hopper (BPH), and green leaf hopper (GLH) [74]. The *Triticum sativum* leaf antigen (ASAL) gene was triggered, which decreased the number of the sap-sucking mustard aphid (*Lipaphis erysimi*). A genetic material trying to express the genes for the monocot mannose-binding lectins *Allium sativum L leaf agglutinin* (ASAL), *Glanthus nivalis L agglutinin* (GNA), onion *Allium cepa L agglutinin* (ACA), and a nuclear fission lectin of ASAL and ACA was tested against by the mustard aphid, a substantial nectar insect of oilseed crops.

The resistance to aphids displayed by transgenic mustard plants with ectopic expressions of ACA and fusion proteins was found to be greater than that of ASAL and GNA. *Galanthus nivalis agglutinin*, or GNA, was the initial lectin to be discovered to be effective against sucking pests during formative phases. Ectopically produced GNA is toxic to leafhoppers, planthoppers, and aphids, according to studies on mutant potato, tobacco, tomato and wheat plants. Despite being naturally found in a wide range of agricultural plants, including wheat, rice, potatoes, tomatoes, soybeans, grass, and beans, lectins only expression at low levels when

their native promoters are present [75]. By producing them with constitutive double promoters, such as 2XCaMV35S, and introducing them into crucial crops, their production can be improved. In light of these goals, the current study was created to evaluate the possibility of creating bugs tolerance in comercially important genetically engineered crops like cotton and the toxicity of the *Allium cepa agglutinin* (ACA) and (LfA) *Leptochloa fusca agglutinin* lectin genes in tobacco plants underneath the regulation of the 2XCaMV35S marker [76].

Bananas and plantains are main economic crops for Africa in addition to being important main crops in Asia, Latin America, and Caribbean islands. Major parasites that seriously affect the produce and lower revenues include various types of banana parasites and the rhizome weevil. Pesticides and biological management are used to manage pests; however, pesticide have a damaging effect on the ecology. Traditional breeding is a difficult and time-consuming process because of the limited sources of resistance, sterility of cultivated banana kinds, polyploidy levels, extended crop cycles, and lack of rapid screening methods [77].

Gene manipulation is one of the safer and most ecofriendly methods of pest control. This report examines the degree of the problem, the types and sources of pest tolerance, and the necessary procedure. The possibility for some genes to control nematodes and weevils is investigated. Also investigated that natural resistance genes and RNAi-based protections that may be utilized in a transgenic strategy to control nematodes and banana weevil. In light of biodefense concerns, nematode-resistant mutant banana cultivars expressing rice or maize cystatin genes and peptides are being investigated, as well as fungus gnat cultivars made using papaya cystatin gene with higher inhibitory capacity [78].

2.2.1 Lectin Gene Transformation in different plants

2.2.1.1 Lectin Gene Transformation in BT Cotton against Insect

The pesticidal potential of Bt -endotoxins is being reduced due to overuse in the field. 2nd Bt Vip3Aa may be a suitable replacement since it doesn't share

midgut receptor sites with any known CRY proteins [74]. Mosquito larvae repellent activity of plant lectins is still unknown. Inside this study, the primer synthetic Bt Vip3Aa gene under the CaMV35S regulators and the Allium sativum leaves antiglobulin gene under the endophyte stimulator were introduced into a native cotton crop. Initial testing of potential transgenic cotton plants was carried out using immunostrip assay, histopathological labelling, and multiplication [79]. In transgenic plants cotton line L6P3, the expression of the Vip3Aa gene was 9 times greater than in the quasi control, whereas the expression of the ASAL gene was reported to be five times higher in transgenic line L34P2. The transgenic line L6P3 was found to contain the largest density of VIP3Aa. The genetic engineered plants have L6P3 has one copy in hemizygous form at chromosome number 10 and make pairs in homogeneity form at chromosome number 9, according to fluorescent in vitro experiments. While physiological aspects of plants and fiber qualities (as determined by scanning electron microscopy) were equal in mutant and non-mutant cotton lines, significant variance remained seen in transgenic cotton lines for morphological traits [80].

All transgenic plants demonstrated substantial resistance to *Helicoverpa armigera*, with mortality rates ranging from 78% to 100%, according to a leaves assay. Comparing transgenic plants lines to non-transgenic control lines, it was found that up to 95% of fungus died in transgenic plants lines [81].

2.2.1.2 Lectin Gene Transformation in *Brassica Juncea* (Green Mustard)

Brassica juncea comprises one of the key rapeseed crops used economically around the world. It is preferred for its exceptional oil quality and its high oil content and is grown in a variety of agricultural and agri regions. The one of the main elements that worldwide inhibits plant development and production is microbial shock. Even while significant advancement has been made in the development and genetic modification of crops to impose tolerance, the job is still difficult. The lectin from lentil and the enzyme inhibitors from chickpea, two important plant defence genes with pesticidal action against herbivorous predatory insects,

have been extracted and stacked into one ORF by overlapping extension PCR in this work. A fused gene construction has also been created [71]. The phyto promoter rolC was used to mobilise the plasmid into Agrobacterium tumefaciens strain GV3101 for stem - cell activation of this fusion protein. Additionally, *B. juncea* cv. Varuna underwent transgenes using an Agrobacterium-mediated fusion gene. Hygromycin selection was used to examine the presumed altered plants' potential for regrowth [82].

By using PCR and Southern hybridized, fused gene incorporation in a subset of chosen at random transgenic plants results in an overall mutation rate of 17%. A quantitative real-time PCR study revealed that the fusion gene's documents on behalf ranged between 1.8 and 2.8 fold [76]. In the third row, the fusion gene's activity was noticeably more prominent (2.8-fold). The results of the aphid susceptibility testing indicated that GM crops had less severe blade destruction than quasi control plants, with a reduction of larvae viability of roughly 40% [83]. This work sheds light on the inter strategy created by overlapping extended PCR in *B. Juncea* and might help advance genetic engineering for the possible enhancement of aphids' tolerance [84].

2.2.1.3 Lectin Gene Transformation in Rice

The brown planthopper (BPH), which causes losses of billions of dollars annually 1,2, is a significant threat to rice production and the most dangerous pest of rice (*Oryza sativa*). The fast loss of BPH resistance is presently impeding the development of resistant cultivars [85]. Therefore, it is crucial to find more potent BPH-resistant genes. we present the molecular cloning and classification of Bph3, the rice which present in locus that confers resistance to BPH and was first discovered more than 30 years ago [86]. They demonstrated that the Bph3 family proteins includes 3 lectin receptor kinases that are located on the cell membrane (OsLecRK1-OsLecRK3) [87]. By using genetic or marker-assisted selection method to introduce Bph3 into vulnerable rice cultivars, fight to both the white back planthopper and BPH was dramatically high. According to our findings, these glycoprotein receptors phosphatase genes cooperate to give broad-spectrum

and long-lasting resistance to insects, serving as a source for breeding programs of arthropod rice varieties [88].

2.2.1.4 Lectin Gene Transformation in Tobacco

To evaluate the chronological and statistical control provided by surrounding regions, In order to transform cannabis plants, a variety of crosses bearing the soybean lectin gene (Le1) were used. The largest lectin variant contained approximately 3,00 nucleotide sequences (bp) of the Le1 5' bordering area and 1,500 bp of the Le1 3' bordering area [89]. The lowest design has 194 bp of the 3' flanking area but no 5' bordering region. The majority of constructions were inherited as one of a kind insert events, according to Southern blot analysis and ELISA tests of lectin in individual tobacco seeds [90]. More than 338 bp of the 5' region was necessary for Le1 to produce to its fullest extent, demonstrating that factors upstream play a significant role in the numerical regulation of lectin production. Lectin production decreased by more than 80% between deletions that had 5' flanking sequences of 1,700 vs 338 bp. In comparison, Le1 inserts with just 5' sequence of 190 bp were able to keep the temporal regulation of lectin expression in place [91]. The lectin promoter presents a possible method for directing high amounts of gene expression to the growing embryos of dicotyledons, such as soybeans [92].

2.3 Lectin Gene Transformation in Potato against Aphid

In vitro assays, snowdrop lectin called as GNA (*Galanthus nivalis agglutinin*) was added to a synthetic meal at a single concentration of 0.1 percent to see how it impacted the Greenhouse Condition Potato Aphid (Aulacothum solani). The results showed that GNA in the meal all through insect's life span reduced adult aphid productivity, as assessed by juvenile production, significantly up to 65%, but frequently just caused a modest reduction in aphid survival by 10% [93]. When bugs were raised on a diet supplemented and then switched to a diet that contains

GNA when they were fully grown, the harmful effects of GNA were significantly lessened. Using image processing tools to measure growth, it was also found that GNA had an impact on the growth of larval stage aphids. In this test, the inclusion of GNA to the diet culminated in high mortality rates as well as a 40% reduction in the aphids' rate of growth in both length and breadth. As just a result of conversion using a design comprising a target gene the protein controlled by the CaMV 35S promoter, GNA was produced in the plant of potato (*Solanum tuberosum*) cv. Desirée. Using polyclonal antibodies produced it against protein, immunodot blot analysis was used to regulate the quantities of GNA production in mutant potato leaves. These levels were determined to be among 0.3 and 0.4 percent of the total soluble protein [94]. The number of larvae produced on these transgenes was considerably (P 0.01) decreased as compared to that on non-transgenic control plants, according to vitro assays performed in controlled environments utilizing clip cages. Comparable to the bioassays using artificial diets, a decline in fertility was noted in this assay. Large-scale glasshouse experiments revealed that the plants saw a rate of population build-up that was around four times smaller than that of the plants treated. The tests show that the pesticidal effects of GNA found in synthetic meals can be replicated in planta, both in the glasshouse and under scientific bioassay settings [33].

Lectins are hypothetical enzymes that specifically bind to and from cross-links with glucose in order to target specific glycans found on the viral cell membrane through a process known as lectin stimulation. They support the defense mechanism of plants. In this study, scientist conducted an in-vitro experiment to evaluate tree lectin's anti-PVY (potato virus Y) activity [95]. The anti-PVY activity of the CVL lectin was evaluated by an ordinal numbers PCR test. At dosages of 30, 60, and 90 g/ml, it was shown that CVL was efficient against by the PVY target gene, and that all examined specimen doses led to up to 100% inhibition of the PVY mRNA in contrast to control. Our results unequivocally indicate that the CVL gene may be used as a useful tool in the potato conversion efforts to combat the potato virus Y [96]. Aphids are insect that act as vectors and have ability to piercing–sucking mouthparts that help in introducing interaction between virus–vector [97].

Aphids have two types of viruses; first type of the viruses is present in hindgut or midgut; this type of viruses is called circulative viruses. There are many proteins some are from animals and almost all viruses have binding sites, these sites are carbohydrate binding sites and carbohydrate [98]. The specificity of lectins genes is different, most of genes are able to bind with glycoproteins that are viral. This study gives huge explanation about how aphid involves in transmission of viruses in plants and cause diseases [99].

Sitobion avenae (Aphididae) are aphids feed with Pisum sativum Lectin (PSL) transferred one of the viruses that is Barley yellow dwarf virus that have very low efficiency rate, almost (four-fold r). Another virus called Pea mosaic virus (PMV) was considerably decrease in (Aphididae) *Acyrthosiphon pisum harris* aphids feed with the *Lectin Concanavalin* A [100]. In comparison, the diffusion of Potato Virus Y (PVY) was particularly decreased when *Myzus persicaesultzer* (Aphididae) aphids were feed with Pisum sativum Lectin PSL. Therefore, Lectin help in preventing viral diseases and used as a barrier of plant viruses, and also give alternative ways for the protection of crop [101].

Two major defense genes that originate from the plant, first gene originate from the lentil plant and second gene is originated from the chickpea that is use as protease inhibitors gene [93] this gene have major insecticidal property against phytophagous pests insect have been stacked and isolated into one open reading frame by the fusion of gene construction and extension of PCR [102].

Specific promoter of phloem rolE was used for checking of fusion gene tissue expression, with the help of strain GV3101 *Agrobacterium tumefaciens*. Further *Agrobacterium* mediated gene transformation is used for the genetic transformation of cv. *Varuna*, *B. juncea*. Hygromycin are used for the screening of regenerative transformed putative plants selection [103]. Through PCR and southern hybridization, the fusion of gene addition in at random selection transformed plants give 17% of alteration efficiency. The level of transcription of the fusion gene was noted which are within 1.8–2.8-fold with the help of quantitative real time PCR analysis. In this study scientist examined that the larval inhibition survivability was 40% and also examined the comparison between transgenic and control plant and

the damage of arial parts was measured and also diminish in the transgenic plants as compared to non-transformed control plants. This article also told us about different toxins through the extension of PCR in the species B. juncea which help in enhancing resistance against aphids [95].

Lectin activation pathway, in Plant lectins gene are present, Lectin is the latent proteins that cross linked and bound with target specific glycans and carbohydrates present on the surface of cells viruses via a specific path, this pathway are known as Lectin activation pathway [104]. This lectin activation pathway helps in enhancing immune system and help in giving resistance against many viral diseases. This study revealed that, the estimation of anti-PVY (potato virus Y) activity in plant that resultant Lectin in in-vitro assay. In-silico research shown constant interaction between targeted protein of PVY and Crocus Vernus Lectin (CVL) Cytotoxicity tests are used on the cells of HepG2 that give IC50 value was 770 μg/ml for CVL. The value of IC50 showed the non-cytotoxic and the effect of safe limit of the CVL extract and the significance for more testing. The activity of CVL of hemagglutination that act against the erythrocytes of rabbit was 100 μg/ml. The activity of anti-PVY activity was displayed in the CVL Lectin that evaluated through quantitative real time PCR assay [98].

The targeted genes of PVY at 90, 30, 60 μg/ml of concentration were affected against CVL and PVY mRNA was achieved almost 100% inhibition in this concentration of three test as compared to control group. In the future it was cleared that CVL gene will be effective tool for transformation in potato against potato virus. Rot Ricin-B-lectins are found in numerous organisms, including coleopterans insects, such as the Colorado potato beetle *Leptinotarsa decemlineata* (LdRBLs). We predicted that several LdRBLs, such as (LdRBLK), are involved in immunological responses to fungal infection. Scientist conducted a theoretical investigation of this protein structure. In addition, the LdRBlk gene expression level in *L. decemlineata* was measured in response to infections with the fungus *Beauveria bassiana* and *Metarhizium robertsii*. The LdRBlk expression level in the L. decemlineata fat body and cuticle was enhanced as a result of infection with *Beauveria bassiana* and *Metarhizium robertsii* [105].

The induction of LdRBIK determined the sensitivity of larvae to fungus. It was also discovered that the LdRBlk gene protects against additional stressors, particularly thermal burns. LdRBlk expression was frequently increased as a result of the expression of the antimicrobial peptide attacin, but it was not linked to hsp90 regulation. It was discovered that the -lectin of ricin, which is produced by Ricinuscommunis, hindered the germination of fungal conidia. The LdRBLk may aid in antifungal immune response in the Colorado potato beetle because they modulate the immune response and have fungicidal characteristics [106].

One of the Lectin termed lectin receptor-like kinases (LecRLKs) aids in the response to biotic and pathogenic stressors. Until date, the genome of the potato (*Solanum tuberosum*) has been severely constrained. There were 113 potato LecRLKs (StLecRLKs). There are additionally 2 C-type, 26 L-type, and 85 G-type members. StLecRLKs were further split into 7 clades based on phylogenetic analysis, with 26 L-type and 2 C-type clades, comprising C-type, L-type, G-I, G-III, G-II, G-IV, and G-V [107]. Although segmental duplication events whole-genome duplication (WGD) were discovered, gene duplication and chromosomal distribution revealed the increase of StLecRLKs present primarily by tandem duplication. Signal defense and stressors such as abiotic, biotic, and phytohormone are responded to by Cis-elements in the StLecRLKs promoter region. Furthermore, investigative expression revealed that members of the L-type, G- I, G-IV, and G-V families were offering anti-response to both fungal bacterial and infection. The findings of this study are significant for the LecRLKs are gene family present in potato genome, and this study establish a platform for more research into the useful analysis [76].

The wheat aphid pest attacked the wheat plant during the seed filling stage, causing damage to the crop. Wheat aphids, for example, are sap-sucking pests that are poisonous to plant lectins. *Pinelliapedatisecta agglutinin* (ppa) was cloned in this study, and it has 94 percent amino acid similarity, 92.69 percent nucleotide similarity, and 94 percent amino acid similarity with *Pinelliaternata agglutinin* (pta). The pBAC-RbcS-ppa vector expression is the promoter of ppa gene, which were relocate into the wheat cultivar [108]. T0 transgenic plants (about 54) were

created. RT-PCR and PCR were used to confirm the expression and inheritance of the gene ppa, and the results were obtained using seven homozygous transgenic lines [101].

Seven ppa transgenic wheat lines exhibit better inhibition rates and lower aphid growth rates than BF104, according to aphid bioassay on separate leaf segments. And also, two-year aphid bioassays in separate fields revealed a considerable reduction in the number of transgenic lines compared to wild type BF104. As a result, ppa may be a more powerful biotechnology gene that aids in the production of aphid-resistant wheat. Lectins are a category of proteins that bind to specific carbohydrates and carbohydrate binding sites and are found in all kingdoms [109]. In plants, Lectin plays a vital part in the defense mechanism against biotic and abiotic stress signals. Despite the publication of the genomic sequence of Glycine max, no one is similar to the expression patterns of Lectin genes in soybean. Using hidden Markov model and BLAST, a total of 359 potential Lectin genes were discovered. Furthermore, these sequences have been used to identify 9 out of a total of 12 plants.

After further investigation, it was discovered that Lectin genes code for chimero lectins, which have one or more Lectin domains coupled with other known protein domains. Duplication can be segmental or tandem. The expansion of the lectin gene family has been aided by both segmental and tandem duplication events. These findings provide a deep insight of the lectin gene family domain architecture and molecular evolution in soybeans [110].

Chapter 3

Methodology

3.1 Explant Source

The experiment was conducted with healthy, disease-free ex plants of the potato variety "Lady Rosetta". This cultivar was collected from the tissue culture lab of National Institute for Genomics and Advanced Biotechnology (NIGAB), National Agricultural Research Center in Islamabad's potato research program.

3.2 Surface Sterilization

Potato tubers were repeatedly cleaned with detergent and rinsed with distilled water. After that, the tubers were thoroughly dried and stored in a dark area for two to three weeks until sprouting began. The Lady Rosetta's healthy, disease-free tubers received a fungicide treatment (0.05 percent) for 10 to 15 minutes, after which they were dried. By soaking the potato tubers for 30 minutes and used 50mg/L GA3 to disrupt the dormancy. Potato tubers that had been cut were laid out on wet sand, where they were allowed to grow. Cut portions of the sprouting tubers' epical meristem were employed to initiate in vitro culture.

After three rounds of autoclaved distilled water washing, various Cholorox concentrations (50, 60, and 70%) were applied to potato explants for 10 minutes each.

3.3 Multiplication

The MS medium supplemented with sucrose and different concentration of GA3 having Ph 5.8 was used for establishing potato cultures to optimize the most suitable media (media composition shown in table 3.1) for multiplication of potato (Lady Rosetta). Sterilized sprout was cultured on the multiplication media. Buds became full-length plantlets with 6 to 8 nodes after 3 to 4 weeks. From the sprouts that were later employed in the experiment, full growth of potato plantlets starts to happen after two to three weeks.

TABLE 3.1: Composition of multiplication media

Ingredients	**Concentrations**
Sucrose	30g
MS salt	4.43g
GA3	50μl
Gellan gum	2.59g
pH	5.5

3.4 Transformation Vector

The cloning vector pCAMBIA 1201 used in the present study was provided by NIGAB NARC (Islamabad) pCAMBIA vector had a lectic gene and plant selectable marker PPT (Phosphinothricin).

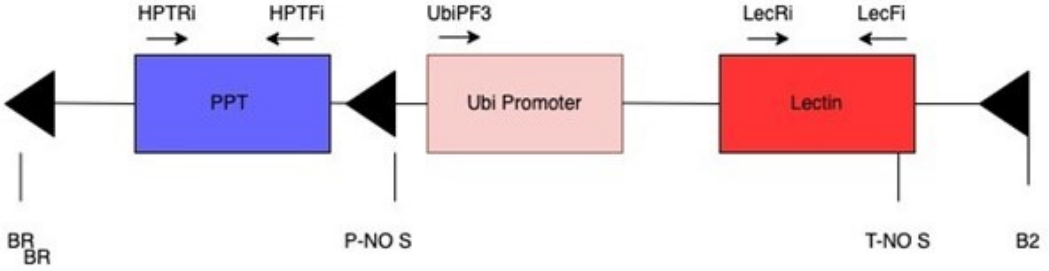


FIGURE 3.1: Vector Diagram

3.5 Preparation of Bacterial Culture

The bacteria culture was first flourished from glycerol stocks. For this purpose, 1μL culture was spread on solid LB plates having selectable marker Carbanciline. The plate was then incubated at 28^{o}C for 48 hours. When colonies of *Agrobacterium* were observed then it was stored at 4^{o}C.

Single rounded colony was picked from pure culture plates and cultured in 20ml liquid LB solution having carbanciline and Rifamsin in autoclaved falcon. It was then placed on shaking for 24 to 48 hours. It is now ready to conduct transformation experiment.

3.6 Infection

The internodal parts of the explants were transformed with desired gene of interest. For this purpose, the bacterial culture having optimized density was centrifuge at 5000 rpm for 10 to 15 minutes. The supernatant was discarded and pellet was resuspended with plane MS media. This culture infected the explant for 10 to 15 minutes in a laminar air flow cabinet. After infection the explant was dried sterile filter paper and shifted to co cultivation media.

3.7 Co Cultivation

Because T-DNA is inserted into the host plant's genome during the co-cultivation phase of an Agrobacterium-mediated transformation, this phase is crucial. To optimize the co cultivation media (composition of co cultivation media shown in table 3.2), different durations of this period along with different concentrations of proline were observed. Co-cultivation media contains MS salt with vitamins,2-4D, proline and gellen gum. The pH of media was established at 5.5.

TABLE 3.2: Composition of co cultivation media

Ingredients	**Concentrations**
Sucrose	2.5g
MS salt	1.05259g
Vitamins	60g
Gellan gum	2.59g
BAP	1mg
NAA	0.1mg
GA3	0.1mg
pH	5.5

3.8 Pre-Selection

After co cultivation the plants were washed twice with MS media and cefotaxime then the explants were rinsed with distilled water. Once the washing is done the explants were transferred to pre-selection media for different photo duration. Pre-selection media is antibiotic free media and plants found very supportive environment of growth. This selection media contains MS salt including vitamins and different concentration of BAP (6-benyLaminoPurine). (composition of pre-selection media shown in table 3.3).

TABLE 3.3: Composition of Pre-selection media

Ingredients	**Concentrations**
Sucrose	7.5g
MS salt	1.0825g
NAA	25μl
BAP	250μl
Gellan Gum	0.75g
Cefotaxime	250μl
500X vitamins	500μl
Ph	5.8

3.9 Lethal Dose

To access the lethal dose of ppt for transformation experiment, various concentrations of PPT (Phosphinothricin) (250,350,500μl/l) were evaluated culturing calli as explant. The non-transformed calli were grown on media supplemented with different PPT concentrations. when the calli die that concentrations were used as lethal dose.

3.10 Selection

The explants were transferred at selection media. Selection media contain MS salt, sucrose, BAP, NAA, GA3 and vitamins. The pH of media was established at 5.8. Added PPT antibiotic in autoclaved selection media. In the presences of antibiotics plants founds very healthy environment of growth. In selection media different concentration of PPT (250, 350, and 500) were evaluated. Selection was evaluated 15 days after inoculation (media composition shown in table 3.4).

TABLE 3.4: Composition of Selection media

Ingredients	**Concentrations**
MS+ Zeatin	1mg
2-4, D	2mg
Meronen	125mg+ selection marker

The transformation frequency was calculated against each cultivar as:

$$Plant\ transformation\ frquency(Percentage) = \frac{Number\ of\ plants\ obtained}{Number\ of\ calli\ inoculated} \times 100$$

3.11 Regeneration

Flourishing explants were shifted to regeneration media which had different concentrations of BAP and GA3. In next step, different concentrations of BAP were

tested. The optimum concentrations of BAP concentrations were identified. Regeneration frequency was calculated from the following formula and the best responsive treatment was sorted out for calli were grown at 25^{o}C, with 16 hours light and 8 hours darks cycle. (media composition shown in table 3.5)

TABLE 3.5: Composition of Regeneration media

Ingredients	**Concentrations**
MS+BAP	1mg
GA3	0.1mg
Meronen	125mg

$$Plant\ regeneration\ freq = \frac{Number\ of\ calli\ regenerated\ plantlet}{Number\ of\ calliinoculated\ for\ regeneration} \times 100$$

The plants that were transformed with lectin gene through Agrobacterium mediated gene transformation were further grown hydroponically for two weeks in order to increase the shoot length.

The DNA of these plants were extracted from leaf tissues through CTAB method for transgenic confirmation through PCR.

3.12 DNA Extraction through CTAB Method

DNA of transformed plants was isolated through CTAB method [111]. The chemicals and apparatus used in this process were prepared and autoclaved before the start of extraction procedure. For the preparation of 2× CTAB, 0.5 MEDTA, 5 NaCl and 1 M Tris HCL were prepared. The stocks were stored at room temperature. After preparing the stock, 2% CTAB was prepared.

For the DNA extraction, 0.5 g leaves sample was grinded in liquid nitrogen in pestle mortar till it is converted into fine powder. This powder was incorporated in autoclaved eppendorf tube and 700μl preheated 2% CTAB was added. The

addition of preheated CTAB catalyzed the reaction. Then, it was incubated at 65^{o}C for a period of 30 minutes. This was followed by an addition of 700μl of chloroform: isoamyl alcohol in 24:1. This solution was centrifuged at 10,000 rpm for 15 minutes which resulted in the formation of three layers. Only upper most layer (700μl) were taken in another autoclaved eppendorf tube and equal amount of chilled isopropanol (700μl) was added. It was once again centrifuged at same speed and time to obtain pellet. This pellet was air dried after being cleaned with 70% ethanol. Finally, it was dissolved in 50 ddH_2O and the DNA was kept at 20^{o}C.

3.13 PCR Analysis

Internal gene primers for Lectin were designed by using primers3 software. Fermentas Taq polymerase was used for 20μl reaction.

Table 3.6: Primers used for the Amplification of Lectin

OLIGO	**Length**
ATGGGTCCTACTACTTCATCTCCT	24bp
TCAAGCAGCACCGGTGCCAACCT	24bp

The PCR profile was set as:

Denaturation of DNA at 94^{o}C for 3 minutes. 35 cycles for the Lectin amplification of product size 546 bp was made. Denaturation of DNA at 94^{o}C for 30 seconds, annealing primer at 52^{o}C for 30 seconds and extend off the annealed primer at 72^{o}C for 40 seconds. Final extension at 73^{o}C for 20 minutes.

3.14 Gel Electrophoresis

The PCR product was run on 1% agarose gel along with 1kb ladder for confirmation of PCR product.

Chapter 4

Result and Discussion

4.1 Source for Explant

The experiment was conducted with healthy, disease-free ex plants of the potato variety "Lady Rosetta". This cultivar was collected from the tissue culture lab of National Institute for Genomics and Advanced Biotechnology (NIGAB), National Agricultural Research Center in Islamabad's potato research program.

FIGURE 4.1: Explant of Lady Rosetta

4.2 Surface Sterilization

Pre-treatment of the potato explants with antibiotics and fungicide combinations of various agents before the surface sterilization was effective. Potato explants were sprouted in 10 days. Nodes that were cultured showed full elongation and reached the length of 3-4 cm within 21 days. In vitro propagation the nutrient medium supplemented with plant growth hormones was the most significant factor affecting efficacy. Result shown in Table 4.1 showed the lowest rate of contamination was reported on T1 (Chlorox 70%) surface sterilization treatment than other T_2 (Chlorox 60%) and T_3 (Chlorox 50%). Maximum number of survival rate were also reported in T_3 treatment followed by T_2 and T_3 as shown in figure 4.2.

TABLE 4.1: ANOVA for the effect of Chlorox on Invitro micropropagation of potato (Lady Rosetta)

Source	DF	SS	MS	F	P
Replication	2	12.706	6.353		
Treatments	2	368.798	184.399	11.39	0.0223
Error	4	64.779	16.195		
Total	8	446.283			

Grand mean = 88.651

CV 4.54

LSD = 9.1228

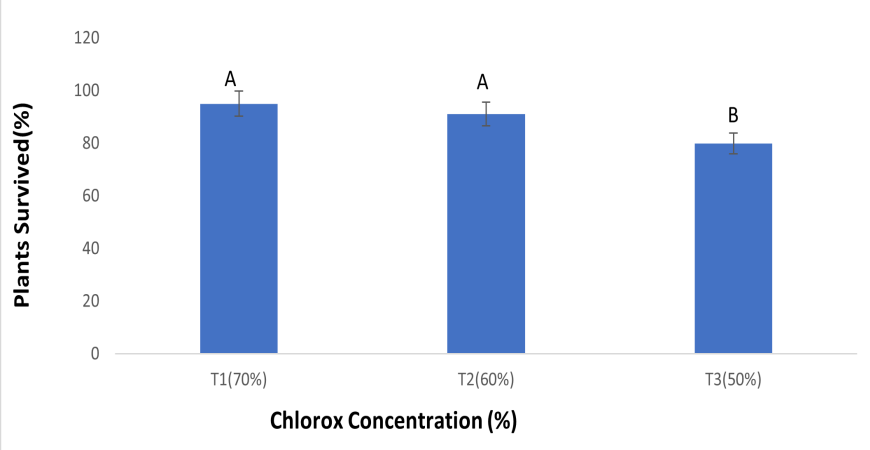


FIGURE 4.2: Effect of different cholorox concentration on Explants

(a) (b)

FIGURE 4.3: Effect of Chlorox concentration (%) on explants

4.3 Multiplication of Explants

The MS medium supplemented with sucrose and different concentration of GA3 having pH 5.8 was used for establishing potato cultures to optimize the most suitable media for multiplication of Lady Rosetta. Result showed in table 4.2 the Sterilized sprout was cultured on the multiplication media. As seedling grows their cells differentiate into shoot and root cells. Different concentration of GA3 was tested to determine the level that maximizes. The growth of explant of potato (lady Rosetta). Statiscal analysis revealed that multiplication of explant was significantly dependent on GA3 concentration. The highest growth was recorded at 0.5mg/L GA3 concentration, having mean value 41.667 followed by 0.4 mg/L and 0.6 mg/L of GA3 respectively. It was observed that the multiplication rate of explants decreases with increased concentration of GA3 as shown in figure 4.4.

The impact of various GA3 levels in combination with MS medium on the number of nodes was insignificant. This experiment employed a greater dose of GA3, which did not alter the number of nodes, although a prior research found that GA3 at 0.01 mg/L [112].

It has also been shown that a higher concentration of GA3 in conjunction with NAA (1.0 mg L-1) and vitamin supplements increases the number of nodes. This demonstrates that in order to boost node formation, larger concentrations of GA3 need be combined with other plant hormones (such BAP and NAA) and vitamins [113].

According to statistical analysis, T5 was the greatest combination for in vitro multiple shoot induction since it produced the most shoots (14) overall. T4 and T3 were the second- and third-best combinations, respectively. Results from T1, T2, and T6 are not significant [114].

TABLE 4.2: ANOVA for the effect GA3 on Multiplication of Potato (Lady Rosetta) explants

Source	DF	SS	MS	F	P
Replication	2	73.556	36.778		
Treatment	2	417.556	208.778	63.69	0.0009
Error	4	13.111	3.278		
Total	8	504.222			

Grand mean = 33.556

CV 5.40

LSD = 4.1042

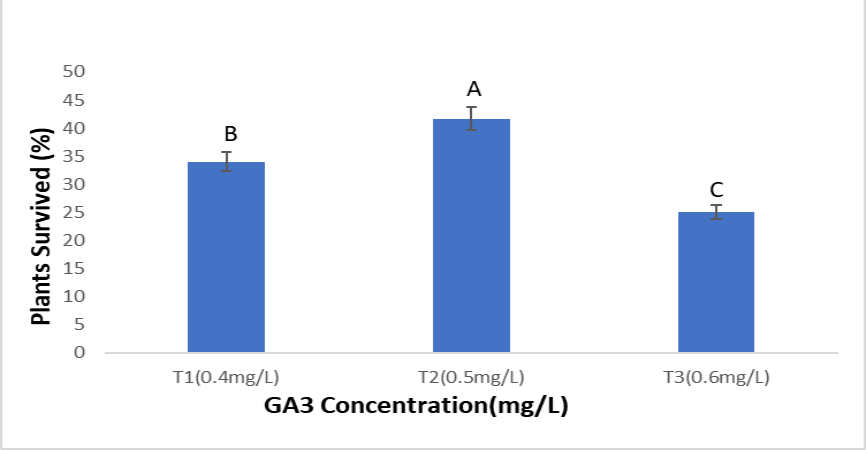
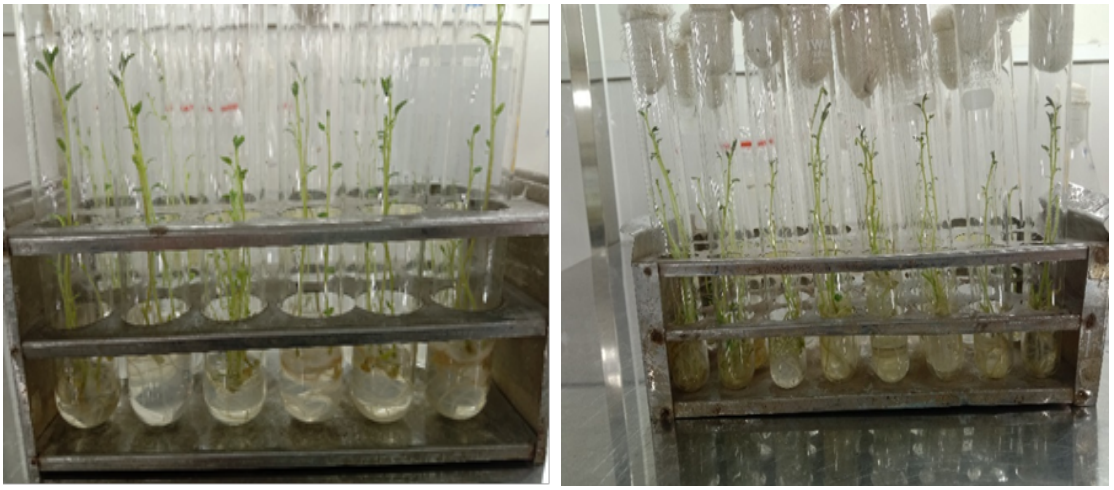


FIGURE 4.4: Effect of GA3 hormone on the growth of Explants



(a) (b)

FIGURE 4.5: Effect of GA3 on the Multiplication of Explants

4.4 Infection

The intermodal parts of the explants were transformed with desired gene of interest. For this purpose, the bacterial culture having optimized density was centrifuge at 5000 rpm for 10 to 15 minutes. The supernatant was discarded and pellet was resuspended with plane MS media. This culture infected the explant for 10 to 15 minutes in a laminar air flow cabinet. After infection the explant was dried sterile filter paper and shifted to co cultivation media.

Table 4.3 showed the results of Multiplied plants that were further used to optimized bacterial density. Three different optical densities 0.2, 0.25 and 0.3 where checked on potato (lady Rosetta) According to statistical analysis, treatments as well as their interaction were highly significant. The maximum transformation efficiency was recorded at 0.3 having mean value is 60.33 followed by 0.25 and 0.2 respectively as shown in figure 4.6.

The Bacterial cultures of different optical densities, including 1.0, 0.75, 0.5, 0.25 and 0.0 at 600 nm, were utilized to investigate their impact on the competence of wheat. At Optical Density 600nm is equal to 0.5, the highest rate of decomposition (12.5%) was noted. The findings contrasted sharply with those of our study [115].

Highest modification efficiency while using EHA105, whose O.D. at 600 nm is 0.75 found that utilizing optical density 600nm is equal to 1.0 yielded the highest therapeutic efficacy. A. tumefaciens with an O.D.600nm = 1.5 converted young wheat and barley embryos, and an O.D.600nm = 2.0 bacterial culture density changed wheat inflorescence tissue. These all reports stand in stark contrast to the existing research [116].

Optimal conversion as determined by the GUS assay was reached at a late log phase with an OD600 value of 0.6, with EHA 105 having a higher effectiveness (40%) than LBA 4404 (36%). The effectiveness of the conversion decreased with changes in the OD value. At OD values above 0.8, conversion was not possible, and at OD values above 1.0, bacterial proliferation resulted in significant tissue injury [117].

TABLE 4.3: Assessment of various optical density on transformation efficiency in potato (Lady Rosetta)

Source	DF	SS	MS	F	P
Replication	2	69.556	34.778		
Treatment	2	683.556	341.778	18.53	0.0095
Error	4	73.778	18.444		
Total	8	826.889			

Grand mean = 49.889, CV 8.61, LSD = 9.7359

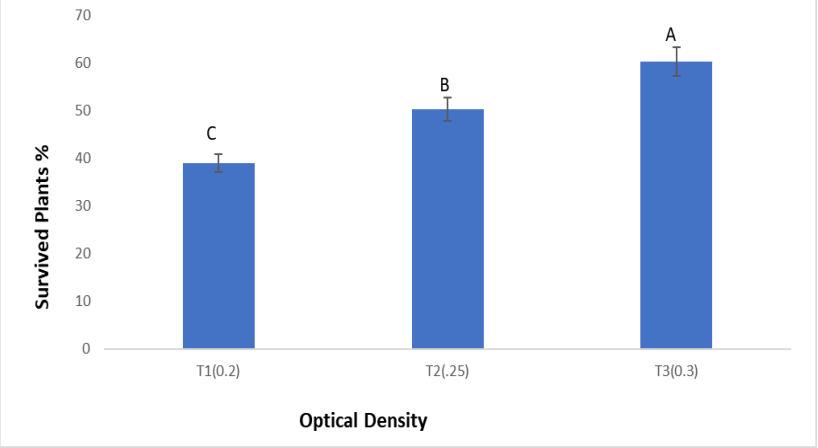


FIGURE 4.6: Optimum optical density for better growth of bacteria

4.5 Co Cultivation

T-DNA is inserted into the host plant's genome during the co-cultivation phase of an *Agrobacterium*-mediated transformation, this phase is crucial. To optimizes the co cultivation media, different durations of this period along with different concentrations of proline were observed. co cultivation media contains MS salt with vitamins,2-4D, proline and gellen gum. The pH of media was established at 5.8.

4.5.1 Proline Content

During co cultivation explants were treated of different concentration of proline 0.08, 0.11 and 0.13 g/L. the interaction between the treatment and the selection of explant was highly significant. According to statistical analysis the maximum selection value was recorded when explants were treated with 0.11 g/L having mean value 52.333 followed by 0.08 and 0.13 having mean value 35.000 and 31.667 respectively as shown in figure 4.7.

A recent study looked into the impact of proline on cultures of ripe rice seeds going through in vitro callusing. On medium without proline, average callusing was lowered to 70.4%, whereas they utilized 0.5g/L proline and found callusing of 85.3%. Similar to how proline was added, callus raw values increase [118].

Proline's effects on GUS expression in maize multi-shoot cultures were described in a research. They showed that 0.7 g/L proline added to the solution will increase the frequency of GUS gene delivery into corn tissue. However, in the corn tissue at a proline concentration of 3 g/L or higher [119].

The effects of proline supplement with doses from 10 g/L up to 32 g/L on switch-grass transformation. Based on the results, supplement of 20 g/L proline was chosen for further transformation experiments [120].

TABLE 4.4: ANOVA of the Effect of varying level of Proline on transformation efficiency in potato (LR)

Source	DF	SS	MS	F	P
Replication	2	82.667	41.333		
Treatment	2	738.667	369.333	40.29	0.0022
Error	4	36.667	9.167		
Total	8	858.000			

Grand means = 39.667, CV= 7.36, LSD = 6.8636

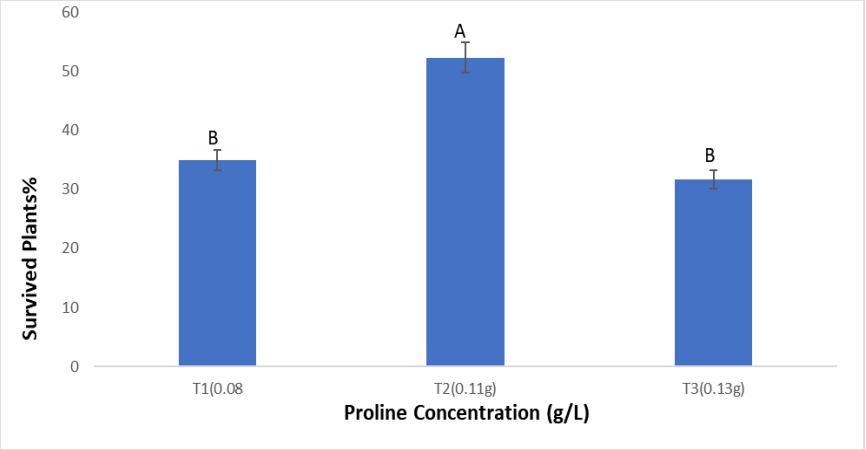
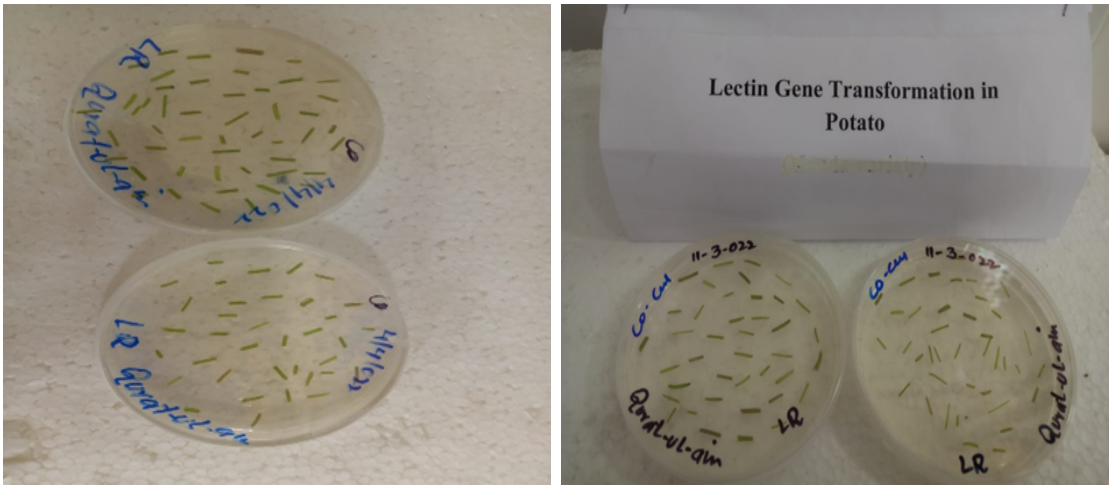


FIGURE 4.7: Effect of different proline concentration on infected Explants



(a) (b)

FIGURE 4.8: Effect of Proline Concentration on Co Cultivated Explants

4.5.2 Time Duration (Hours)

Co cultivation time was given on potato (LR) with Agrobacterium Mediated Lectin gene transformation, for 24, 48 and 72 hours. Maximum selection was observed when explants were co cultivated for 48 hours having mean value 54.333 followed by 24 hours and 72 hours having means values 37.000 and 33.667 respectively (Table 4.5).

A successful transformation depends on several factors. Optimum co cultivation time is the most important factor. A large number of researchers stated tat co cultivation time significantly effects the regeneration of transformants and hence transformation efficiency of several plant species. The success or failure of transformation can also depend on co cultivation. Therefore, it is inevitable to determine optimum co cultivation time to have maximum transformation efficiency [121].

These findings are not in accordance with the results already reported. Narcissus tazzeta transformation in which optimum co cultivation time was done for three different plant species like barley calli with bar gene and A gene [122].

Another finding contradicted the above mentioned result in which the co cultivation conditions for successful transformation of rice was found best after 3 days co cultivation and it produced higher number of transformants [123].

TABLE 4.5: ANOVA table of Assessment of co cultivation time periods on transformation efficiency in potato Lady Rosetta

Source	DF	SS	MS	F	P
Replication	2	82.667	41.333		
Treatment	2	738.667	369.333	40.29	0.0022
Error	4	36.667	9.167		
Total	8	858.000			

Grand mean = 41.667

CV 7.27

LSD = 5.8325

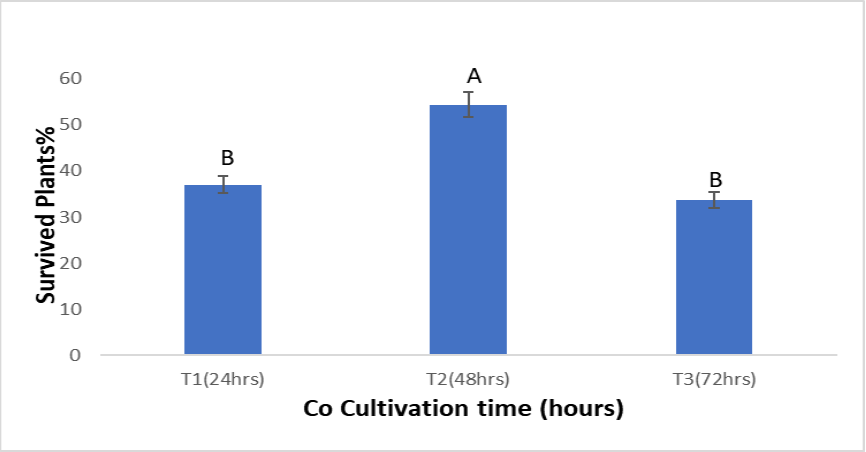


FIGURE 4.9: Effect of different cholorox concentration on Explants

4.6 Selection

To assess the lethal dose of phosphinothricin(PPT) for transformation experiment, various concentration of PPT (8mg, 10mg and 12mg) were evaluated on explant. The statistical analysis demonstrated that highly significant difference was found among all three treatments (T_1, T_2 and T_3. The interaction among variety and treatment was highly significant. It was revealed from the data that survival rate of transformed explants is inversely proportion to PPT concentration as shown in figure 4.10. The highest survival rate of transformed plants was recorded at low dose of PPT concentration 10mg/L with mean value 34 followed by 12mg/L and 8mg/L. So, the lethal dose for potato(LR) was 10mg/L as shown in table 4.6.

Recent finding on the effect of PPT on embryo axis supported the above data in which cultivating wounded embryonic spindles on shoot regenerating solution containing 2, 5, 10, or 20 mg/L PPT, it was possible to assess the reactions of plant cells to PPT and the ideal selection pressure. On media having 10 mg/L PPT, it was shown that shoot growth from developed embryo cells were inhibited [124].

Another study also contradicted the effect of PPT on the transformation efficiency of Bottle gourd. Explants were cultivated in 30 ml of a sprout induction. Complete medium with a range of PPT doses(0, 0.5, 1, 2, 5 and 10 milligrams per liter were

used to find the best dosage of PPT for the selection of mutant shoots and the best results were noted at 2.0mg/L [125].

The effects of increasing PPT doses in vitro produced shoots and fetal tips. As a result, the PPT concentration levels progressively rose to 0.5, 0.75, and 1.0 mg/l during the selection procedure. A larger number of resistant embryonic tips were produced at 1.0mg/l and improved regeneration frequency [126].

Another recent study looked into PPT's impact on shoot initiation. To the shoot initiation media were added five different doses of PPT (0.0, 0.25, 5, 7.5, and 10mg/l) were added. According to the findings, 5 mg/l was the least quantity that might prevent non-transformed plants from growing [126].

The Indica rice variety ADT 43 was genetically altered with the PCAMBIA 1301-carrying *Agrobacterium* strain EHA 105. According to research, non-transformed plants are distinguished by 30 mg/L hpt because they lack the hpt resistance gene and hence perish at this level. cultivar Golden Promise using the bar gene (*Streptomyces hygroscopicus*) under the CaMV35S promoter and reported a fatal dose of 50 mg/L hpt for the selection of transformed calli. They also disclosed that 5 mg/L bialaphosin instead of hpt could produce outcomes that were comparable [127].

GM crops with altered T-DNA sequences may result from the insertion of a foreign gene into higher plants. These plants lacked T-DNA but displayed hygromycin tolerance.

Due to low hygromycin concentrations and intermittent selection, some regenerated in monocot cereal emerges from the altered therapies. Same results are obtained in the data reported in this experiment.

The rescue of shoots from kanamycin selection was using the ti plasmid found in flax seeds. Another statement which stated that the quasi cells used to create these shoots were shielded from the selection agent by the changed cells. When T-DNA is passed on to offspring through callus tissue culture, it may become volatile, lost, or altered from its steady position during crop improvement. Some

plant species can withstand varying amounts of selecting agents, whilst others are susceptible to them. To remove the potential exits, the proper selection pressure must be required for efficient transformation of selected genes.

TABLE 4.6: ANOVA of the Assessment of optimum level of ppt for transformation experiment in potato (lady Rosetta)

Source	DF	SS	MS	F	P
Replication	2	150.22	75.11		
Treatment	2	4838.22	2419.11	53.69	0.0013
Error	4	180.44	45.11		
Total	5168.89				

Grand mean = 42.889

CV 15.66

LSD = 15.226

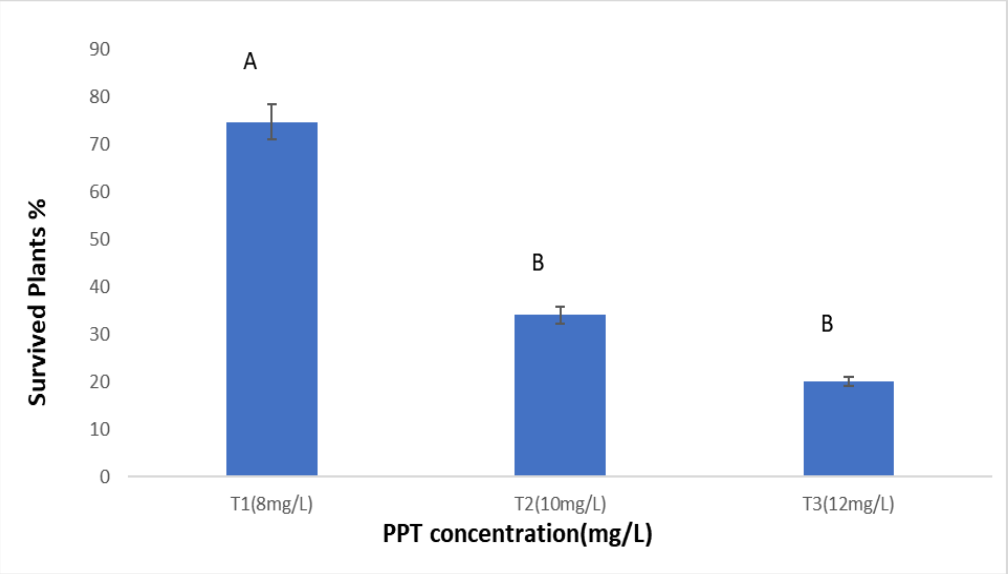


FIGURE 4.10: Effect of different ppt concentrations on Explants

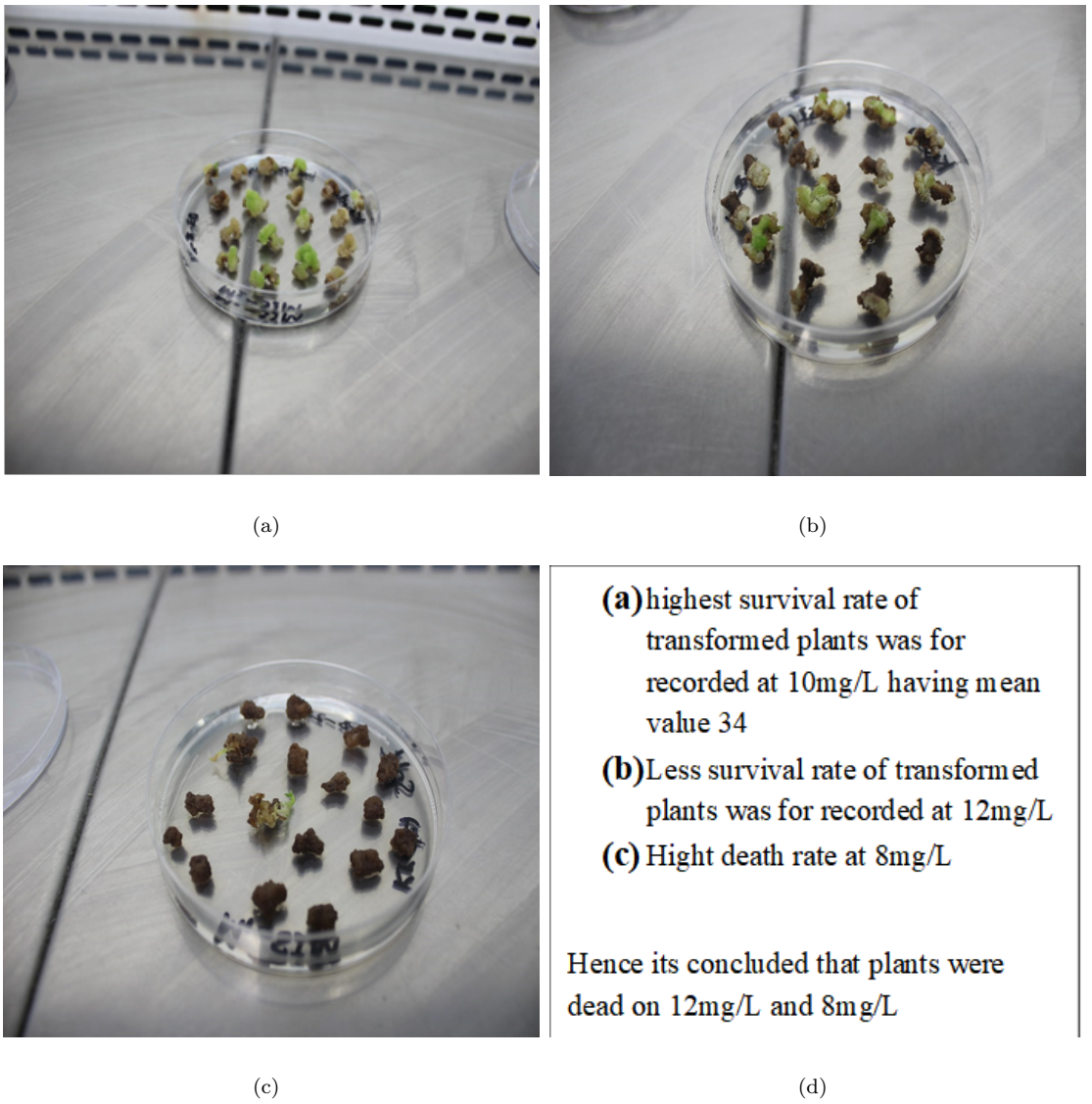


FIGURE 4.11: Effect of ppt on Explants

4.7 Regeneration

4.7.1 GA3 Concentration

Flourishing explants were shifted to regeneration media which had different concentrations of BAP (6-Benzylaminopurine) and GA3 (Gibberellic acid). In next step, different concentrations of BAP were tested. Finally, optimum concentrations of BAP were identified. Regeneration frequency was calculated from the

following formula and the best responsive treatment was sorted out for calli grown at 25^{o}C, with 16 hours light and 8 hours dark cycle.

Different concentrations of GA3 (0.1, 0.2 and 0.3mg/L) were tested to optimized the regeneration media for the transformation of potato (Lady Rosetta) as shown in table 4.7. The best regeneration of potato (Lady Rosetta) was recorded at 0.2mg/L of GA3 having mean value 41.667 which proved optimum and generated maximum transformants as shown in figure 4.13a. At this concentration combination of BAP green spots were observed. By increasing or decreasing the dose of GA3 (0.1mg/L and 0.3mg/L) false rooting was observed as shown in figure 4.13b and 4.13c.

Above findings are not supported with them during two weeks of cultivation in diet supplemented with all doses of BAP, the nodal sections demonstrated regrowth. Data presented here by another researcher which stated that BAP is necessary for propagation, as evidenced by the fact that 0.5 mg/L BAP only produced a solitary sprout and did not allow any additional propagation. More sprouts were produced as BAP content rose, peaking at 3 mg/L BAP, but as BAP content is high (5 to 7 mg/L), less shoots were produced, showed signs of death, and showed limb fasciation [128].

An improved in-vitro restoration methodology is being tested on four different wheat varieties as report indicated Murashige and Skoog medium supplemented with 0.2 milligram per liter of BAP and 4 milligrams per liter of IAA, the maximum regeneration percentage was achieved. The outcomes of this investigation are also supported the current study [127].

On the other hand, data presented in invitro regeneration of four different wheat varieties evaluated against 12 different kind of media did not support the current findings. Murashige and Skoog medium supplemented with 6 milligrams per liter of dicamba and 0.10 milligram per liter of IAA had the greatest regrowth. These results showed which may be due to genetic differences among different cultivars [128].

The best regeneration was obtained by using 5 milligrams per liter of BAP and 1 milligram per liter of 2,4-D in the solution. These researchers don't match up with the current investigation. Various plantlet origins and genotypes are to blame for the variation.

TABLE 4.7: Effect of different concentration of GA3 on Regeneration of explants of potato (Lady Rosetta)

Source	DF	SS	MS	F	P
Replication	2	73.556	36.778		
Treatment	2	417.556	208.778	63.69	0.0009
Error	4	13.111	3.278		
Total	504.222				

Grand mean= 33.556

CV 5.40

LSD= 4.125

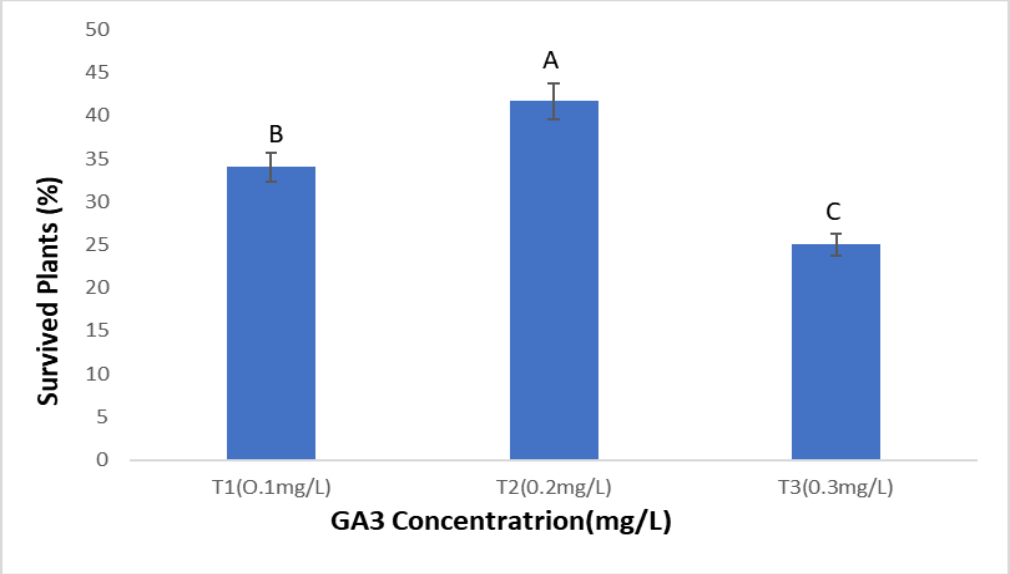
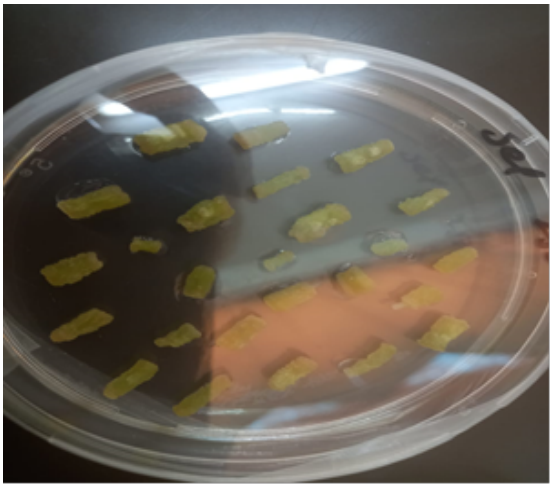


FIGURE 4.12: Effect of GA3 hormone on regenerative plants



(a)

(b)

(c)

(a) Less better regeneration rate was recoded at o.1mg/L

(b) Better regeneration rate was recorded at o.3mg/L

(c) The best regeneration of potato (Lady Rosetta) Was recorded at 0.2mg/L of GA3 having mean value 41.667.

(d)

FIGURE 4.13: Effect of GA3 Concentration on Regenerative Explants

4.7.2 Effect of GA3 in Combination with BAP on Regenerative Plant

The regeneration potato calli (Lady Rosetta) were also observed at three different concentration of BAP along with already optimized GA3 concentration both in combination and separately. When BAP alone was tested in medium no regeneration was observed for potato (Lady Rosetta).

It has been observed that at 0.2 mg/L of BAP show very good results in which maximum growth is obtained and it categorized as A as shown in figure 15a.

Any further increase or decreased of BAP dose resulted in decreased regeneration percentage and at 0.3mg/L and 0.1mg/L of BAP false rooting was recorded as shown in figure 4.15b and 4.15c.

No findings support the combined effect of BAP and GA3 as explained in the current study. The addition of a higher BAP content, based on the study, may have prevented embryogenic apical expansion, which would explain why there were less shoots. There were variations dependent on cotyledon form even though BAP and GA3 did not impact on rapid multiplication. While the addition of GA3 to a BAP-free media caused a solitary sprout to elongate, the positive control without either BAP or GA3 showed no replication or lengthening. Axillary buds grew more quickly when 1 milligram per litter BAP and 0.5 milligram per liter GA3 were utilized , but many sprouts with at least one or two reaching a height of 3 cm and containing 7 to 9 nodal sections appeared when 5 milligram per liter of BAP and 0.6 milligram per liter of GA3 were utilized [127]. The addition of GA3 caused shoot elongation, resulting in noticeable vertical sections, which may be used for further duplication during the culture, which is relevant to effective replication.

TABLE 4.8: ANOVA for the Effect of different Concentration of BAP in combination with optimized concentration of GA3 on Regeneration

Source	**DF**	**SS**	**MS**	**F**	**P**
Replication	2	58.39	29.19		
Treatment	2	2069.39	1034.69	123.75	0.0003
Error	4	33.44	8.36		
Total	8	2161.22			

Grand mean = 32.944

CV 8.78

LSD = 6.5550

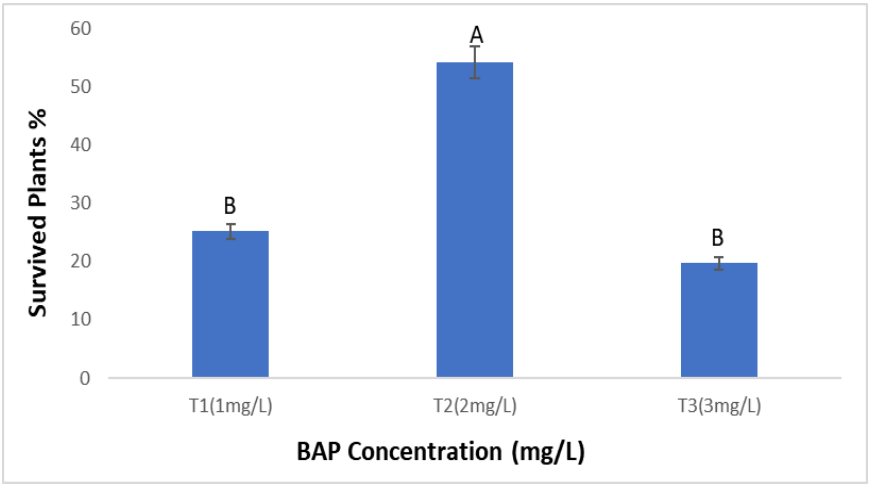
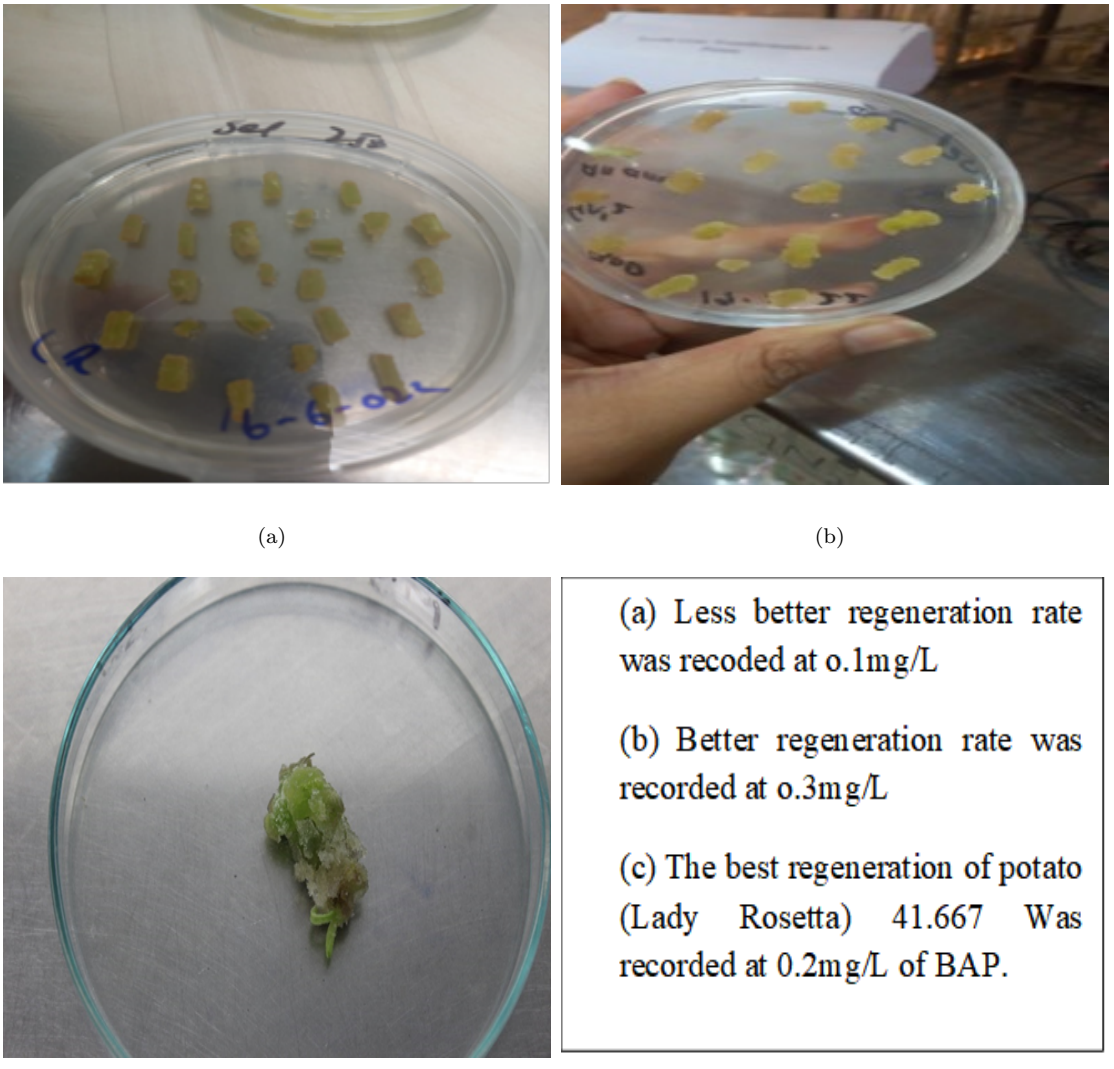


FIGURE 4.14: Effect of BAP on regenerative plants



(a) (b)

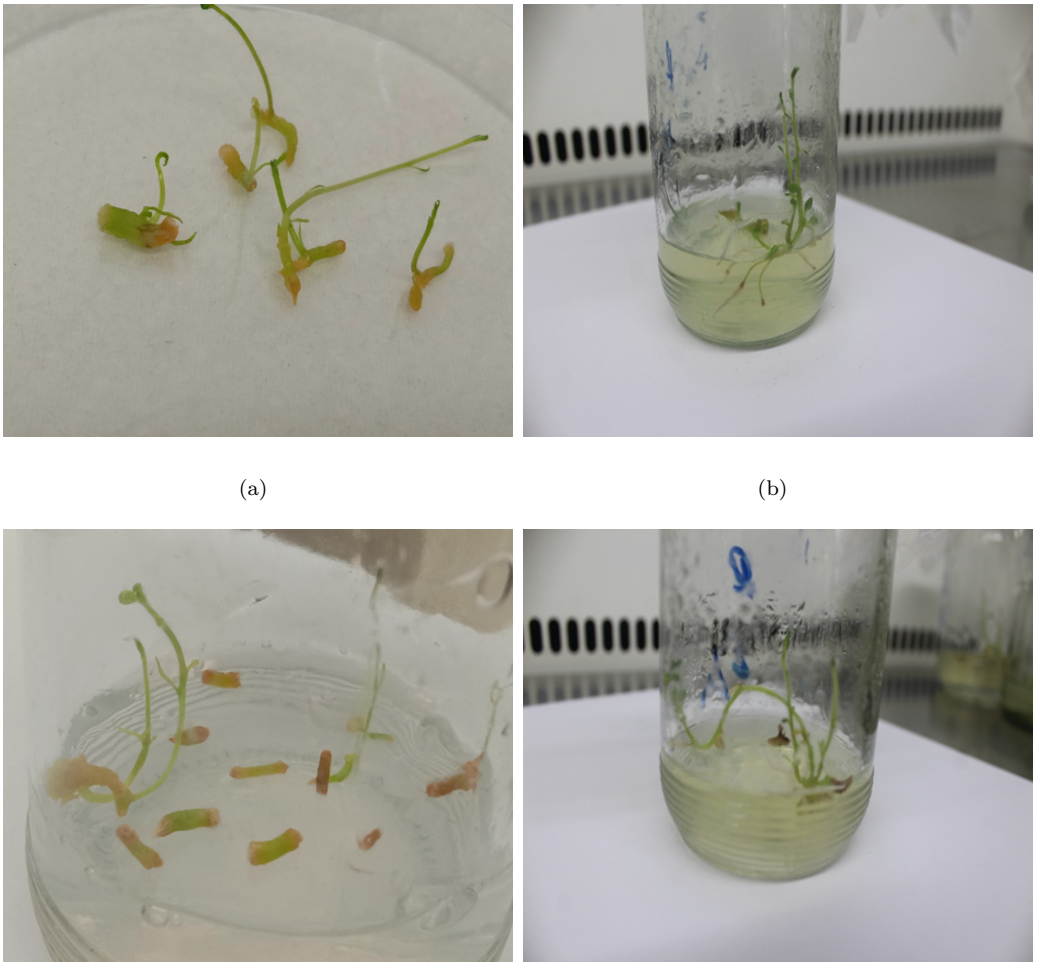
(c) (d)

FIGURE 4.15: Effect of BAP on Regenerative Explant

4.8 Development of Transgenic Plant and PCR Confirmation

4.8.1 *Agrobacterium* Mediated Lectin Gene Transformation

To develop aphid resistance in potato (Lady Rosetta), Lectin gene was transformed by using transformation protocol. Two thousand eight hundred sixty calli of Lady Rosetta were transformed with Lectin gene. The vector pCAMBIA 1201 containing lectin gene 35S promoter and PPT selectable marker was used for transformation of potato (Lady Rosetta).



(a) (b)

(c) (d)

FIGURE 4.16: Transgenic Plant of Potato (Lady Rosetta)

4.8.2 PCR Analysis

After gene transformation and were checked for lectin gene amplification by conventional PCR. Out of 7 plants 5 were found to be positive for successful transformation.

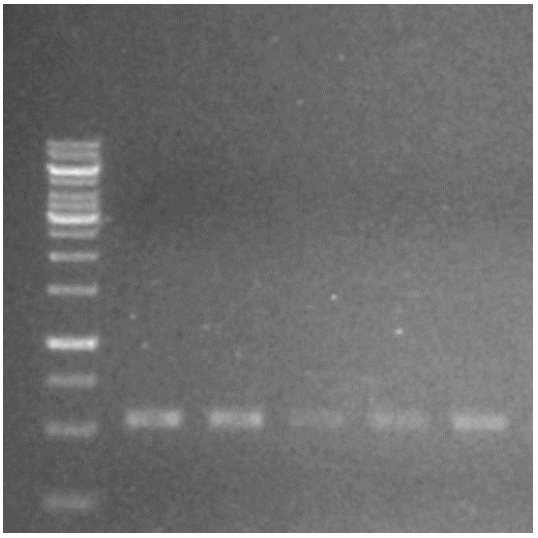


FIGURE 4.17: Confirmation of transgenic plants through PCR analysis. M, 1kb DNA ladder 1-5 amplification of 546 bp fragments of Lectin gene

TABLE 4.9

Variety	**Number of uncontaminated selected calli**	**No of Regenerated calli**	**No of transformed plants produced**	**Transformation efficiency**
Lady Rosetta	2860	1680	7	5

Chapter 5

Summary, Conclusion and Future Directions

5.1 Summary

Potato (*Solanum tuberosum*) belongs to the family of Solanaceae which arise in the South America and then spread to other countries of the world. It is considered to be important in all the regions of world after maize, wheat and rice. It singly contributes 4.0 million tons of total vegetable production in Pakistan. In the World, potato is very important crop for people used almost 140 countries cultivated potatoes.

Potato is full of starch and nutrients. It consists of 79% water, 18% starch, 2% protein and 1% vitamins. It also has fats minerals and trace elements. It is also full of carbohydrate. Potato contain many minerals such as phosphorus, potassium and magnesium and some vitamins present such as B6, B3 and B1and have pantothenic acid riboflavin and folate. Potatoes also have dietary antioxidants, which may play a part in inhibiting diseases related to fast aging, and dietary fiber.

Biotic and abiotic factors are the main problems for the losses of high and low yielding varieties of potato. Due to insects and diseases invasion the crop loss

occurs 30-40%. If insect attack in the early seedling phases it may turn even 100%.

The potato aphid (*M. euphorbiae*) originated from North America and then spread to all countries where potatoes are grown. Aphid are capable of destroying thousands of plants species. Aphid eat potato sap directly and destroy the crop by transmitting various viral infestation. Plant growth inhibit due to nonstop sucking of potato sap by these sucking insects. These aphid attack when they remove sap from potato plant and inject toxin that cause leaf deformation and poor production potato tubers. Another cause is Sooty black disease which hindered the photosynthesis process. Aphids work as vector because they transmit virus to the plant that cause diseases. Severity of diseases depend upon the nature of virus that are transmitted to the plant. Some viruses attack potato plant e.g. PLRV virus that causes tuber necrosis and leaf rolling. For example, Potato Leaf Roll Virus (PLRV) is the virus which causes leaf-rolling and also cause tuber stem necrosis in potato curling, yellowing of leaves chlorotic spotting etc. Large number of aphid population may damage heavy potato crop. Aphids are polyphagous species that help in the transmission of these viruses from one plant to another.

For the control of these diseases use of abrupt pesticides is practiced worldwide, but these pesticides create numbers of environmental and safety concerns and these problems can cause climate change. It also helps in developing resistance against pesticides in return failed to control the prevalent diseases. Insect pests are majorly affecting the potato crop and tuber quality. Due to insect pest infestation on average 16% crop are globally destroyed. If insect pest is not properly control tuber quality and potato yield can reduced from 30% to 70% and the excessive use of pesticides also caused environmental, health and safety concerns.

Although for the betterment of potato varieties it must be important to use conventional breeding techniques, but these techniques are difficult to handle and time consuming. Greater challenge is to pose the quantitative traits. The need of the hour is to control these problems with advanced molecular tools like genetic transformation techniques. In order to induce desired gene traits in the respective GM crop.

Genetic transformation techniques also help in producing the new variety with full strength against biotic and abiotic stresses. Genetic transformation technologies do not only give resistance to plants against biotic and abiotic stresses but also introduce new characters in plants which fulfill the demand and need of human.

Gene transformation with the help of *Agrobacterium* is efficient method for the transferring of foreign gene into plant and then the development of transgenic plant occurs. According to the protocol of *Agrobacterium* mediated genetic transformation, the gene of interest can be isolated from one plant and insert competently in the potato varieties which are economically very important. For virus free potato meristem culture is frequently used in a mass scale. But through in vitro methods it is not yet possible to produce fungal resistant plants. Due to this reason, different conservative breeding and biotechnological techniques are being used in diverse parts of the globe.

For this purpose, we use Lectin gene for pest control that is ecofriendly. Lectin is a hydroxyproline- rice, chitin-binding, glycoprotein which may be participated in the defense mechanism of the potato plant. Lectin gene have very two dissimilar domains. Lectin gene which are naturally present in the garlic and have defense mechanism against different types of insects including sap sucking and chewing insects' pests. Lectin gene is composed of mannose binding protein called homodimeric protein that is present in garlic. This protein is isolated from garlic and inserted into different types plants against insect pest attack. Lectin gene works by binding the antigenic structure found in the intestinal track of insect causing paralyses and ultimately die.

Sterilized the explants with 70% ethanol and 70% of Chlorox dried on filter paper. Cut the internodal area of explants, all explants are placed into LB media that consisting of Agrobacterium GV3101 strain with Lectin gene of interest for 5 min in the flask. Drained all the media and dried the seeds on filter paper for 15 min then cultivated the seeds on co-cultivation for 2 days. After two days washed seeds with autoclaved distilled water for 3 times and inoculated on selection media for regeneration. After regeneration of plant extracted the DNA and performed the

PCR by using specific primers to confirmed the production of genetically modified plants.

5.2 Conclusion

This study presents suitable hormonal composition of the regeneration media, type of explants and most suitable protocol for *Agrobacterium* mediated lectin gene transformation in potato. According to the current results, the internodal part of potato plant is inoculated with gene of interest and grow on different medias. The transformed plants having Lectin gene which have a potential resistance against aphid were produced successfully in tissue culture lab. Development of such transgenic will help the future scientists to develop insect resistance varieties to control annual potato yield losses.

5.3 Future Directions

Efficient lectin gene transformation in potato further leads to the confirmation of transgenic crop in glass house by assessing its morphological, biochemical and molecular characteristics. *Agrobacterium* mediated Lectin gene transformation can be used for the other crops to protect them from insects, bacterial and fungal diseases and enhanced crop yield. Transformation of other resistant genes in non-resistant cultivars in order to produced more resistant varieties in future. This will certainly help to overcome the challenges of yield loses by reducing the viral diseases in potato (Lady Rosetta).

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