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OPTIMIZATION OF AN EFFICIENT IN VITRO REGENERATION SYSTEM FOR AN IMPORTANT LOCALLY GROWN EGGPLANT VARIETY IN PAKISTAN

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ABSTRACT

Solanum melongena L. is an important vegetable in sub-tropical and tropical regions of the globe. It is prone to various biotic and abiotic stresses including diseases and insect pests being the most important ones. Many genes can be transferred into a plant at once because of the rapid improvement of genetic engineering tools. To apply the biotechnological approaches, first, we need to establish an efficient regeneration protocol. For tissue culture, different types of explants were tested for regeneration protocol optimization. For callus induction auxin and cytokines were used in a certain proportion. For regeneration, calli were shifted to regeneration media containing appropriate cytokines. The regenerated plantlets were shifted to rooting media containing IBA. Maximum callus fresh weight and callus induction efficiency were both achieved with 1 mg/L of 2, 4 D in combination with kinetin, BAP, and TDZ, respectively. Therefore, this research demonstrated progress in the development of regeneration and in vitro micro propagation of eggplant.

***Keywords*:** *Plant Tissue Culture, Callus, 2,4-D, IBA, Eggplant.*

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1. INTRODUCTION

Solanum melongena L. a member of Solanaceae family is generally cultivated as a fruit vegetable in sub-tropical and tropical regions of the world. It is commonly known as eggplant, guinea squash, or brinjal **(**Alam and Salimullah, 2021). Globally, eggplants are produced over an area of 1.8 million hectares. About 90% of the world's eggplant is produced in Asia. China produces more than 50% of the global eggplant crop, followed by India at 30%, and also in Egypt, Turkey, and Iran. In Pakistan, in 2019 the production of eggplants was in an area of 8566 hectares over the production of 89,724 with an average yield of 10.5 tonnes per hectare (Saini and Kaushik, 2019).

Because of the numerous biotic and abiotic challenges that eggplant is subject to, there is a constant need for cultivars that are resistant to disease and insect pests, have higher nutraceutical properties, and are climate change tolerant. In the case of vegetable crop breeding, both sexual and vegetative propagation is used to transfer the genes. The main goal is to increase genetic diversity in current plant populations. Moreover, superior plants containing desired specified features are selected and developed; this is primarily accomplished through traditional breeding procedures (Dennis et al., 2008). By using these traditional and biotechnological methods, it is possible to increase yields and so ensure food security.

The potential for improving vegetables has been greatly expanded due to the rapid growth and expansion of genetic engineering techniques. An understanding of gene structure and function enables us to make a change in a genome (Dalal et al., 2006). Genome-wide identification and characterization of Hsf and Hsp gene families and gene expression analysis under heat stress in eggplant (*Solanum melongema L.*) were carried out by (Gong et al., 2021).

With genetic transformation technologies, plant breeders now have direct access to beneficial genes that were previously inaccessible (Lusser et al., 2012). Many genes can be transferred into a plant at once because of the rapid improvement of genetic engineering tools. Through the use of genetic engineering, it is possible to integrate a desired gene into any plant while crossing the species barrier, which is not possible through traditional breeding. Through the use of genetic engineering, it is possible to integrate a desired gene into any plant while crossing the species barrier, which is not possible through traditional breeding (Lynch et al., 1999). Eggplant responds well to cell, tissue, and organ culture. Plants can be regenerated from the tissues of eggplant either through embryogenesis (Ammirato, 1983) or through organogenesis (Litz, 1992). Its capacity for regeneration has made it possible to employ biotechnology, including genetic modification, the use of somaclonal variation, haploidy, and somatic hybridization.

In Organogenesis the longitudinal leaf explant sections are transformed into shoot buds, and many meristematic zones are created. The direct regeneration potential differs from the explant type. In growth media containing

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various combinations of cytokinins and auxins, it has been discovered that different explants respond to regeneration differently. Different types of explants like hypocotyl, cotyledon, root, and leaf explants had different morphogenetic potential for the numbers of adventitious shoots on different combinations of hormones. Regeneration is also affected by 'the age' and genotype of the explant (Saini and Kaushik, 2019). An artificial procedure called somatic embryogenesis (SE) produces an embryo from a single somatic cell. Somatic embryos can be formed through direct embryogenesis and direct embryogenesis from the callus.

To make recombinant plants we need to optimize the regeneration protocol for eggplant. In Organogenesis the longitudinal leaf explant sections are transformed into shoot buds, and many meristematic zones are created. The direct regeneration potential differs from the explant type. In growth media containing various combinations of cytokinins and auxins, it has been discovered that different explants respond to regeneration differently. Different types of explants, such as hypocotyls, cotyledons, roots, and leaves, vary in their morphogenetic potential for the number of adventitious shoots on a certain hormone combination. Each kind of explant had a distinctive capacity for generating ad hoc shoots. Furthermore, elements including the genotype and age of the explant had an impact on the regeneration process.

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The first steps in the biotechnology application to eggplants entailed the creation of in vitro tissue growth and regeneration techniques. Through in vitro organogenesis and somatic embryogenesis, eggplant regeneration is possible. In these processes, cultured explants from different plant parts, such as the stem, hypocotyl, leaf, cotyledon, and root, as well as from cell suspension, anthers, isolated microspores, and protoplasts, are easily used. In order to stimulate organogenesis and somatic embryogenesis, media enriched with benzyladenine zeatin, kinetin, and thiadiazuron (TDZ) were used for explant regeneration in eggplant. From the cultivation of cotyledon explants, different Solanum species might regenerate well. The development of the leaves and cotyledons is most significantly altered by TDZ, which improves shoot organogenesis. The creation and use of several effective biotechnological methods have been made possible by improvements in laboratory methods and eggplant's extraordinary ability for tissue culture, particularly in the regeneration of cultured leaf, cotyledon, and hypocotyl segments. The management and improvement of genetic resources in the field of eggplant research have greatly benefited from these tactics. The main objectives of this work were to find a suitable medium that would aid the regeneration of these explants through optimised hormone concentrations and to accomplish effective in vitro organogenesis of eggplant shoots utilising two distinct types of explants (Taghipour et al., 2015). The plant species, genotype, explant type, culture regimen utilised throughout the in vitro process, notably the hormone content of the medium, and the frequency of subculturing are some of the elements that affect the incidence of somaclonal variation. The growth regulators may have been activated during indirect organogenesis, which would explain the variety seen in regenerated plants. It has been established that the hormone concentrations in tissue culture media are what create the somaclonal differences in eggplant. Most scientists concur that variation rates increase when growth regulator concentrations rise generally, even though the impact of particular growth regulators on variation is yet unknown.

2. MATERIALS AND METHODS

2.1. Explant Selection

Seeds of a locally grown hybrid variety HBR332C were cultured *in vitro* as a source of explants.

2.2. Sterilization of Explant

Seeds were first sterilized with double distilled water. Then the seeds were surface sterilized by using 70% ethanol 35% bleach and 0.01% triton in laminar air flow cabinet.

2.3. Callus Induction

For callus induction MS media was prepared using sucrose 30g/L, MS salts 4.33g/L, MS vitamins with varying concentrations of BAP and 2,4-D. In his media different 2,4-D concentrations were also used.

2.4. Shoot and Root Induction

Calli were transferred on MS media with cytokine-like zeatin, BAP, or kinetin for shoot induction, and for root induction calli were transferred to an MS medium containing IBA.

2.5. Acclimatization of Plants

After the roots and shoots were induced then the plantlets were subjected to hardening in plastic pots. Plantlets were transferred to pots having peat moss for acclimatization.

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3. RESULTS

A thorough analysis was carried out to determine the ideal growing conditions that may support an eggplant's callus induction, proliferation, and plantlet regeneration.

3.1. Explant Sterilization

The objective of this study was to investigate the effects of different hormone doses and combinations on the capacity of the eggplant hypocotyl and cotyledon to induce callus development and support plantlet regeneration. As a result, by using hormone-free basic MS-basal conditions, healthy explants from egg-plant seedlings were effectively produced. For seed sterilization, various mercuric chloride and bleach concentrations were used.

3.2. *In vitro* Plant Growth

After 25 days a healthy plant emerges on simple MS-basal medium. The medium plates placed on light condition. Bacteria and fungus were not aggressive. Explant with roots, leaf and stem disc was shifted into jars and cultivated in between 30 to 40 days.

3.3. Callus Induction

When living cells or tissues are exposed to the right nutritional mix, callus is formed on its own. It is believed that auxin plays a key role in determining the explant response to callus induction. Young, 1.5 to 2.0 mm thick explant discs of leaves, roots, and stems were transferred to callus induction medium and grew only in the light.

In order to assess the response of callus induction for maximal callus induction and development in the genotypes, three different 2, 4-D doses (1, 3, and 5 mg/l) were used. After a 20–30 days incubation period, callus formation began. It was discovered that callus first forms on the inner sections of immature stem discs, hypocotyl, cotyledon, and roots. When 2, 4-D concentrations are between 1 and 5 mg/l, the genotypes respond favorably.

After three to five weeks, the fresh weight of the callus was thought to vary between cultures. The mass of the calli increased as the 2, 4-D concentration increased from 1 mg/L to 3 mg/L. Callus initiation from the sides was increasing in the hypocotyl and cotyledon at 1.8 and 3 mg/L of 2, 4-D+ 9.3 mg kinetin, respectively. The calluses that came to the surface were numerous and light in hue. On the sides of the leaves, the callus began to form after 40 days. Callus growth was seen in leaves at callus induction media enhanced with 1.8 and 3 mg/L of 2, 4-D + 9 mg kinetin. At 3 mg doses, the growth was highest.

3.4. Shifting of Callus on Regeneration Media

The methods via which eggplant shoots develop are organogenesis and embryogenesis. Finally, the capacity of various eggplant seed genotypes to regenerate was tested using regeneration media containing 0.5 mg/L of BAP+13.3 mg/L TDZ. In this work, BAP-supplemented auxin-free regeneration medium was used to create five-week-old Calli discs. The calli were first cultured for a period of five weeks under low light (1400 lux day intensity) and then for an additional week under light (2000–2500 lux day power) in a growth environment at 26°C following a 16:8 hour light–dark cycle. The data was gathered and assessed after the callus had been cultured on regeneration medium for 4-5 weeks. On the callus surface, many green embryoids sprouted, and as the callus grew, they finally transformed into plantlets. After callus development, it was put into regeneration medium with a 0.5 BAP+13.3mg/L TDZ concentration. Numerous variables, including the number of shoots and shot length, was assessed after 30-35 days. The callus-producing branches that had been sown for growth were measured after 30 days.

After being developed on shooting media, the plants were then moved to MS medium to be rooted, where they were then placed in growth chambers to develop into full-fledged plant.

3.5. Acclimatization of In vitro Regenerated Eggplants

When the plants in the jars had achieved full maturity, they were put into the peat moss and covered with plastic bags. The growing chamber included these plants. After 4 to 5 weeks, when the plants are mature and huge in size, punch a hole in the polythene bags. After 6-7 weeks, the polythene bags were taken off.

4. DISCUSSION

Researcher have succeeded in developing well organized and productive plant tissue culture procedure for enhancing large scale breeding approaches over the last few years. Effectively callus induction and plant regeneration procedure are critical to the success of tissue culture technique. The physiological state of brinjal, the type of explant employed, and the nutritional component of the medium all influence callus induction and regeneration rate. The formation of an effective regeneration system is critical for improvement of vital feature.

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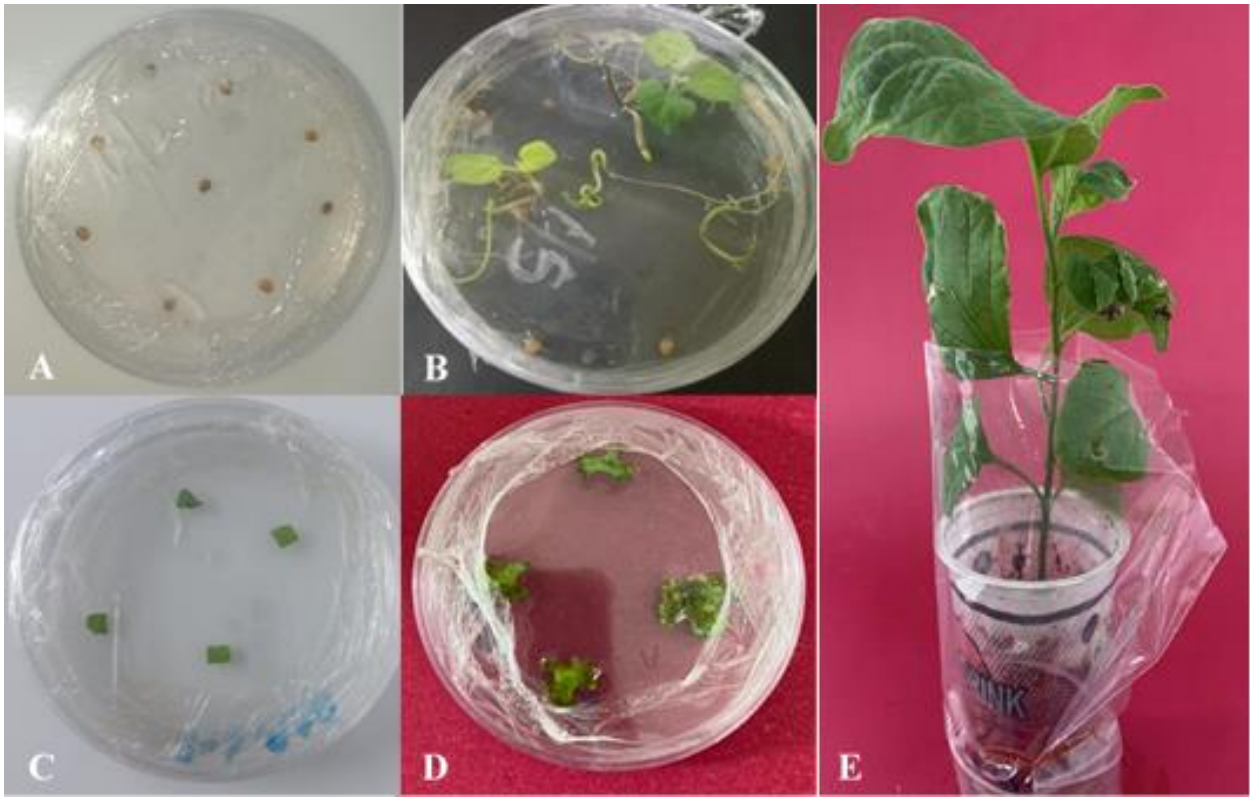

Fig. 1.0: Various steps of eggplant *in vitro* regeneration A. Germination of surface sterilized seeds B. Growth of plants for obtaining explants C. Leaf disks as explants on regeneration medium D. *In vitro* regeneration of on leaf explants E. Plant let grown from *in vitro* regenerated shoots

The selection of explant sources with the ability to regenerate into a full plant is a basic requirement for successful crop improvement using modern biotechnology approaches. Callus induction and regeneration in eggplant can be accomplished through a variety of explant source, each of which has different potential for flourishing a full plant. Eggplant seeds varieties were used in this study. After surface sterilization under aseptic condition, healthy seeds were isolated and sterilized. Seeds were sterilized and disinfected with different concentrations of bleach and Mgcl2 in laminar flow cabinet. Seeds were placed in the jars or petri plates containing MS medium and later kept those plates or jars in the growth room for the growth of plant from seeds the seeds were grown into plant after 25-30 days. Leaves and stem were used as explant which placed on callus induction medium.

The effects of several combinations and quantities of growth hormones, including2, 4 D, IAA, BAP, NAA, kinetin, and TDZ were assessed for influential eggplant tissue culture. Seeds of different varieties were successful in germinating when the MS0 medium was used without sucrose. The best concentration of 2, 4 D for callus induction in eggplant varieties is 1.8mg/L and 3mg/L. The lowest growth was observed at 3mg/L of 2, 4 D. Eggplant showed the initial sign of callus formation at 4 to 5 weeks of culture to calculate the callus induction frequency (%), data was collected for callus mass with regular interval under different concentrations of 2,4,D growth regulator. Calli were transferred at different time interval to a sterile petri dish and the weight of callus was measured. After 6 week data were recorded. The callus of eggplant was friable, globular and relatively yellowish white with slightly brown was seen. Eggplant showed 70% callus induction results. It was transferred to regeneration media with a 0.5 BAP+13.3mg TDZ concentration after callus formation. After 30-35 days, numerous metrics, including the number of shoots and roots length, was measured. After 30 days, measurements were taken of the callus-produced shoots that had been planted for growth.

(Khan et al., 2017) performed work on roots and shoot, that were grown from the eggplant seeds on MS media were used to design an important and widely used protocol for the establishment of callus and regeneration for the natural brinjal (*Solanum melongena*) The end product shown that using 2, 4-Dichlorophenoxyaetic acid 0.5 mg per L and Kinetin 0.5 mg per L on Murashige and Skoog media enabled for the best callus generation. After 4-6 weeks, callus shifted on MS media enriched with BAP 0.5 mg per L alone or BA 1.0 mg per L combined with 0.5 mg per L Kinetin regenerated into shoots. Additionally, on MS media enriched with growth-promoting hormones, the greatest rooting was observed like IAA 2 mg per L and NAA 0.5 mg per L. On simple media at 1/2 strength, calli-derived

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shoots transformed into plantlets. This enhanced approach could make it possible for developing, a useful tool for genetic studies in the future transformation of the popular vegetable crop such as eggplant which is essential for medicinal uses.

To create an effective methodology for callus formation and regeneration of shoots, evaluate the effects of explant type, media formation and growth factors has been formed (Yarmohammadi et al., 2021). At end it indicated that B5 was effective media to promote callus formation, whereas medium was the best for explant callus induction 47%, with 49% of sample plant forming callus when cultured on B5 media with 0.1 mg per L BAP and 1.0 mg per L 2,4-D. After eight weeks of culture, media plate on B5 supplemented with 1.0 mg of 2, 4-D had the highest callus induction (65.2%). The B5 medium with 1.5 mg per L of callus-proliferating growth showed the best results

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