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Plant Tissue Culture Techniques for Crop Improvement

Bilal Ahmed Awan
Institute of Horticultural Sciences, University of Agriculture Faisalabad, Pakistan

ABSTRACT
Plant tissue culture refers to a series of in vitro methods which can be used to grow and reproduce plant cells, tissues, and organs in a controlled laboratory environment. These methods have turned out to be major in contemporary farming, as it offers the means of quick multiplication, genetic enhancement, eradication of diseases and germplasm conservation. In case of plants requiring low seed viability or long reproductive cycles, the use of tissue culture enables the generation of genetically homogeneous and quality plants, which is not always possible with other traditional propagation techniques. The latest developments in plant biotechnology, such as micropropagation, somatic embryogenesis, organogenesis, and protoplast culture have played a major role in crop improvement programs, as they have provided opportunity to introduce desirable traits such as disease resistance, stress tolerance and nutritional quality enhancement. Also, genetic modification is done using tissue culture whereby plant genomes are altered with high precision and where biotic and abiotic stress-resistant genes can be inserted. This paper will examine the existing methods in plant tissue culture and how it is utilized in the enhancement of crops with reference to how this method can be used to improve food security, guarantee sustainable agriculture and add to the crop production in the world. The results highlight the necessity to include tissue culture technologies in the breeding initiatives and create the effective protocols in relation to various crop species.
Keywords:Plant tissue culture Micropropagation Somatic embryogenesis Organogenesis Genetic transformation Crop improvement Biotechnology In vitro propagation.

Corresponding Author: Bilal Ahmed Awan
Email: bilal.awan@uaf.edu.pk
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INTRODUCTION

Plant tissue culture is another paradigm shift in plant biotechnology whereby it is possible to grow plant cells, tissues or organs in sterile, nutrient-filled and controlled in vitro cultures. The methods are critical in dealing with the increasing agricultural problems such as the high yielding, disease and climate resistant crops (Pierik, 1997; Bhojwani and Razdan, 1996). As opposed to traditional forms of propagation that still depend on seed or cuttings, tissue culture allows the quick multiplication of the plants regardless of the time of the year, and parental fertility. It is especially useful with vegetatively-propagated crops, those that have recalcitrant seeds, and those that are at risk of pathogen and environmental stress (George et al., 2008; Loyola-Vargas and Ochoa-Alejo, 2016).

Among the most common techniques of tissue culture, micropropagation provides a possibility of producing genetically homogeneous and free of pathogen-free plantlets in vast amounts. It is a technique in which explants (shoot ends, nodal sections, meristematic tissues) are cultured on nutrient agar with access to plant growth regulators leading to accelerating growth and homogeneity (Debergh and Maene, 1981; Murashige and Skoog, 1962). Another important technique in tissue culture, somatic embryogenesis helps to develop embryos out of somatic cells and offers an efficient system of mass propagation and production of synthetic seeds (Feher, 2015). The formation of organs, organogenesis, which involves the formation of shoots or roots out of tissues which have been cultured, further advances the possibilities of tissue culture creating whole plants even out of undifferentiated cells or

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callus (Thorpe, 2007). All these techniques enrich the ability to propagate clones, eliminating the bottlenecks on traditional breeding, which include heterozygosity, low seed set, and poor reproductive cycles (Smith et al., 2011).

Besides propagation, the tissue culture methods are used in enhancing crops by incorporation of genetic engineering and transformation technologies. The culture of protoplast and somatic hybridization permit cells of different species or varieties to be fused to transfer the desirable traits, including disease resistance and pest tolerance, as well as stress adaptation (Davey et al., 2005; Bhojwani and Razdan, 1996). These methods give an avenue through which intentions of the accurate genetic alterations may be made which are hard to accomplish by the conventional breeding. Moreover, the in vitro selection of stress-resistant genotypes is used with the help of tissue culture, in which cells or tissues are subjected to certain stress factors including salinity, drought, or pathogen attack, and tolerant variants are selected to propagate them further (Singh et al., 2018; Xu et al., 2013). This approach hastens the production of better types of crop which can survive in unfavorable climatic conditions.

The other significant contribution of the tissue culture to the betterment of the crops is in disease eradication. Such culture has widely been applied using meristem culture together with virus indexing to obtain pathogen free plants especially in vegetatively propagated crops like potatoes, bananas and sugarcane (Karp, 1995; Jain and Gupta, 2011). Tissue culture guarantees increased harvests, less economic losses, and food security by generating healthy planting material. Besides, the genetic resources are secure due to the use of the tissue culture methods of cryopreservation of germplasm, which allows endangered or elite varieties to be preserved over a longer period of time (Engelmann, 2011; Walters et al., 2004).

The new developments in tissue culture technology have increased its application in crop enhancement further. Molecular markers, genome editing systems like CRISPR/Cas9, combined with omics systems in combination with tissue culture systems can enable the development of crops with improved nutritional values, greater yield capacity, and resistance to biotic and abiotic risks (Altpeter et al., 2016; Li et al., 2018). The secondary metabolites have also been produced in large amounts under controlled conditions in tissue culture, and have been utilized in medicinal and aromatic plants (Bhatia, 2004). Taken together, all these events have made plant tissue culture one of the pillars of current crop enhancement initiatives, occupying the gap between conventional breeding and the use of advanced biotechnological interventions.

To sum up, plant tissue culture methods present valuable and highly effective means of crop enhancement, as they allow the rapid multiplication of crops, eradication of diseases, selection of stress factors, genetic modification, and preservation of germ plasm. Not only do these methods increase productivity and quality of crops, but also, they also help in sustainable agriculture and food security. The future that lies ahead of the integration of the tissue culture to the molecular and biotechnological developments is enormous in the sense that it can come up with better varieties of crops that can satisfy the growing global food and agricultural products demands (Thorpe, 2007; Bhojwani and Razdan, 1996; George et al., 2008). It is projected that future research and development in the field of tissue culture would streamline procedures to recalcitrant crops, increase the effectiveness of genetic transformation, and discover new methods of generating biofortified as well as climate-resistant crops.

LITERATURE REVIEW

Plant tissue culture has become one of the most significant tools in contemporary plant biotechnology which offers numerous techniques of plant propagation, improvement and preservation of crop plants. Tissue culture is the aseptic culture of plant cells, tissues, organs or protoplasts in a nutrient medium under laboratory conditions (Bhojwani and Razdan, 1996). These methods have revolutionized the world of plant breeding and crop improvement by providing sure and quick ways of clonal reproduction, genetic engineering, and preservation of germ plasm in the past decades or so (Pierik,

1997; George et al., 2008).

Micropropagation is one of the major applications of tissue culture that enables the increase of plants with genetic uniformity to multiply rapidly. Micropropagation is the culture of explants, e.g. shoot tips, nodal tissue, or meristematic tissues, cultured on artificial media to add plant growth regulators (Murashige, and Skoog, 1962; Debergh and Maene, 1981). This method guarantees generation of disease free plants and it is able to combat some of the shortcomings of traditional methods of propagation especially in crops which have a slow or vegetative reproduction period (Smith et al., 2011). The versatility and effectiveness of micropropagation has been shown in a number of studies through the successful application of this method to crops like bananas, potatoes, sugarcane, orchids, and ornamental plants (Jain and Gupta, 2011; Loyola-Vargas and Ochoa-Alejo, 2016).

However, another important tissue culture strategy that has been put into focus in crop enhancement programs is known as Somatic embryogenesis. It is a process of creating the embryos out of the non-reproductive/somatic cell, which allows mass production of identical plants and creation of synthetic seeds (Feher, 2015; Thorpe, 2007). The application of somatic embryogenesis has proved successful in a number of crops such as coffee, cocoa, oil palm, and rice that have shown the ability to generate high-frequency plant regeneration and germplasm conservation (Jimenez, 2001; Xu et al., 2013). Moreover, it enables cryopreservation of embryos, which is very important in storage of genetic materials over a long period and the preservation of elite germplasm (Engelmann, 2011).

Another important tissue culture strategy that is extensively used in crop improvement involves organogenesis or the ability to grow shoots and roots out of the cultured tissues or callus. It is also applicable specifically in the regeneration of complete plants of the explant and the generation in large quantities of genetically intact plants (Thorpe, 2007; Bhojwani and Razdan, 1996). Organogenesis may be direct, in which the organ grows out of the explant, or indirect, in which the regeneration process takes place through a callus phase. Research revealed that organogenesis is very successful when applied to crops like tomato, pepper and legumes and this has served as an effective means of propagating and enhancing genetics (George et al., 2008; Bairu et al., 2009).

Not only breeding techniques such as somatic hybridization, protoplast culture, and transformation, but also propagation of crops with the help of the technique of tissue culture are applicable. Somatic hybridization is a process that entails the interfusion of protoplasts of dissimilar species or varieties to produce new genotypes that could not have been obtained through the sexual reproduction (Davey et al., 2005). This has been implemented to confer disease resistance, stress tolerance and quality into economically significant crops including potato, tobacco and citrus (Malik et al., 2010). The introduction of desired agronomic characteristics into the genomes of plants is possible with the help of protoplast culture and genetic transformation methods (Gelvin, 2003; Altpeter et al., 2016).

Another novel use of the tissue culture in crop improvement involves in vitro selection. In this technique, cells or tissues are subjected to a certain environmental stress: high salinity, drought, or pathogen attack, and only tolerant variants are propagated (Singh et al., 2018; Xu et al., 2013). Crops like wheat, rice, sugarcane and legumes have been successfully selected in vitro and have yielded to the creation of crops with enhanced tolerance to biotic and abiotic stresses (Vasil, 2008; Bhaskaran and Smith, 1990). Tissue culture combined with selection pressure helps plant breeders to develop better types of crops much faster than they would through traditional breeding methods.

Another important use of tissue culture is in the meristem culture and the elimination of viruses. A large number of crops propagated vegetatively are likely to be infected by viruses, which minimise yield and quality. With the help of thermotherapy or chemotherapy, apical meristem culture can also be used to produce planting material that is free of virus (Karp, 1995; Jain and Gupta, 2011). As an example, banana, potato and sugarcane have undergone a massive meristem culture to guarantee provision of healthy, disease free planting material that is vital in enhancing productivity and food security

(Engelmann, 2011; Walters et al., 2004).

The last developments have incorporated the use of molecular biology in conjunction with the use of tissue culture, giving greater opportunities in terms of accurate enhancement of crops. By using molecular markers, marker-assisted selection, and CRISPR/Cas9 genome editing in tissue culture systems, it is possible to generate crops with increased yield, resistance to diseases, and abiotic stresses (Li et al., 2018; Altpeter et al., 2016). In addition, transgenic plants can be assessed within a short period of time under controlled conditions using tissue culture and this would help save time and money that might be spent on breeding using conventional methods (Gelvin, 2003; Thorpe, 2007).

Other fields that Tissue culture plays a vital role in are the production of secondary metabolites in medicinal and aroma plants. Propagation of tissues and organs in vitro would be able to generate bioactive compounds in a controlled condition without being restricted by climatic or seasonal factors (Bhatia, 2004). Some of the methods used to generate alkaloids, flavonoids, and other useful plant metabolites involve the use of callus culture, cell suspension culture, and hairy root culture with Catharanthus roseus, Panax ginseng and Hypericum perforatum (Verpoorte et al., 2002; Murthy et al., 2014). Crop improvement is not the only area that is aided by this application, pharmaceutical and nutraceutical industries are fed by the application.

Although this has a lot of benefits, there are various challenges associated with tissue culture that may constrain their application in large scale. Among the significant limitation are genotype-dependent responses, somaclonal variation, contamination, and high costs of culture media (Bairu et al., 2009; Bhojwani and Razdan, 1996). To address these issues, researchers have established protocols that are optimized such as the use of plant growth regulators, antioxidants, and antibiotics to enhance regeneration performance (George et al., 2008; Loyola-Vargas and Ochoa-Alejo, 2016). Further improvements in automation, bioreceptor culture system, and molecular process are likely to increase the success and scalability of plant tissue culture in crop enhancement (Thorpe, 2007; Altpeter et al., 2016).

Conclusively, the use of plant tissue culture methods is an inseparable aspect of the contemporary concept of crop enhancement. These techniques allow the development of quality, genetically engineered plants through micropropagation, somatic embryogenesis, organogenesis, genetic transformation, in vitro selection and removation of viruses and are therefore used to create large quantity of genetically enhanced plants in a short period of time. Both molecular tools and biotechnological advancements have increased the possibilities of the tissue culture in producing stress-tolerant, high-yielding, and nutritionally fortified crops (Pierik, 1997; George et al., 2008; Bhojwani and Razdan, 1996). Further studies and optimization of tissue culture methods, as well as training and development of infrastructure, are needed to realize the full potential of these methods in sustainable agriculture and food security in the world.

METHODOLOGY

Research Design

The research design used in this study is experimental research design in order to test the efficacy of plant tissue culture techniques in the enhancement of crops. The study is directed at the evaluation of the in vitro propagation, genetic enhancement, and disease-free plant production under the supervision of the laboratory conditions. The experimental method was chosen because it provides the opportunity to control such variables as the type of explants, composition of culture media, and plant growth regulators accurately. Quantitative data involving a regeneration rate, shoot multiplication rate, rooting percentage and survival of plantlets are measured to determine the efficiency and reproducibility of the tissue culture protocols. The research is a combination of micropropagation techniques as well as organogenesis and somatic embryogenesis approaches to study their application in improving crop productivity and homogeneity.

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Selection of Plant Material

The research focuses on crop species that are economically valuable (having high agricultural value) such as potato (Solanum tuberosum), banana (Musa spp.), and tomato (Solanum lycopersicum). The choice of explants is determined by their regenerative ability such as shoot tips, nodal segments, leaf segments, and meristematic tissues. The field or the green house identifies healthy and disease-free mother plants that are used as the source of explants. Before culture initiation, the explants are subjected to extensive surface sterilization following the standard protocols of ethanol, sodium hypochlorite and sterile distilled water to reduce microbial contamination (Bhojwani and Razdan, 1996; George et al., 2008).

Culture Media and Regulators of Plant Growth

All experiments are done in Murashige and Skoog (MS) basal medium with the addition of the following; sucrose (3%) as a source of carbon and agar (0.8%) as a gelling agent (Murashige and Skoog, 1962). PGRs are incorporated in certain amounts to induce the required morphogenic effects. Cytokinins (benzylaminopurine (BAP) and kinetin) are employed in stimulating shoot growth, and auxins (indole-3-acetic acid (IAA) and naphthalene acetic acid (NAA)) are employed in rooting and callus formation (Thorpe, 2007; Smith et al., 2011). To promote the maximum amount of nutrients and growth of the plants, media pH is regulated to 5.8 and then autoclaved.

Experimental Treatments

An experimental design involving a factorial experimental design is carried out to establish the best PGR conditions, culture conditions, and explant types in order to identify the best tissue culture protocols. In the case of micropropagation, nodal and shoot tip explants are incubated on media with different cytokinin levels in an attempt to measure the rate of shoot multiplication. The effect of various ratios of auxin and cytokinin on induction of callus is determined by testing leaf and stem explants. In the case of somatic embryogenesis, immature embryos or cotyledon explants are cultured under specified conditions to cause the growth of embryos and plant regeneration (Feher, 2015; Jimenez, 2001). Every therapy is cloned at least five times and at least 10 explants per clone is used to provide statistical reliability.

Culture Conditions

The cultures of all cultures are kept in a controlled growth chamber in an aseptic environment. Temperature is kept at 25 +- 2degC with 16 hours light/8hour dark photoperiod. The intensity of the light is set to 50 mmol m-2 s-1 and cool white fluorescent lamps are used. The humidity levels are kept at close to 60-70. They are observed periodically, such as the callus formation, shoot initiation, multiplication rate, rooting percentage, and the vigor of the plantlets (George et al., 2008; Loyola-Vargas and Ochoa-Alejo, 2016).

Hardening and Acclimatization

Plantlets regenerated with well-formed roots are then carefully harvested out of cultured tubes, and washed to remove any remaining agar and then planted in pots with a mix of sterilized soil, sand and compost (1:1:1). Plantlets are kept under extreme humidity conditions at a mist chamber or covered with transparent plastic to avoid drying up. The humidity is lowered slowly, and the plantlets are acclimatized and hardened to ambient conditions (Engelmann, 2011; Walters et al., 2004). These parameters include survival rate and growth parameters, which are used to assess the success of the in vitro-to-ex vitro transition.

Data Measures and Data Collection

Different steps of the tissue culture process are measured to measure performance and efficiency by

quantitative data:

Callus Induction Rate (%) - the percentage of the explants in which the callus was inducted in a given period of time.

Shoot Multiplication Rate- mean number of shoots generated per explant.

Rooting Percentage（%) - percentage of successfully developing roots from shoots.

Plantlet Survival (%) - percentage of the amount of plantlets that survives the acclimatization period.

Morphological Measures - such as shoot length, root length and general vigor.

The measurements are performed on a weekly basis and mean values obtained on both treatments.

Statistical Analysis

The obtained data are analyzed statistically with the help of SPSS or R. Analysis of variance (ANOVA) is used to determine the impact of the treatments (explant type, PGR concentration, culture conditions) on regeneration efficiency. Post-hoc tests (such as the HSD of Tukey) are conducted to test the difference in the means and determine the significant differences between treatments. Regression analysis will be used to identify the correlation between explant type, concentration of the PGR and the response of tissue culture. Mean +-standard deviation is used to display all the results and, to determine statistical significance, $p < 0.05$ is predetermined (Bairu et al., 2009; George et al., 2008).

Ethical Considerations

Plant materials are all obtained under proper permissions after being obtained in approved germplasm banks or in experimental farms. Laboratory safety is observed during tissue culture experiments and in order to avoid contamination they are handled under sterility. None of the endangered species are utilized in research, and all the procedures adhere to institution and national biosafety measures.

Limitations

The possible constraints are the possible genotype-based variability in tissue culture reaction, the chance of somaclonal variance, contamination, and adaptation disparities throughout the acclimatization procedure. To overcome such obstacles, several explants and replicas are involved, aseptic guidelines are observed and each crop species is optimized (Bhojwani and Razdan, 1996; Thorpe, 2007).

DATA ANALYSIS AND FINDINGS

The results obtained in the experiments of in vitro propagation give the indicators of the effectiveness of plant tissue culture methods in crop improvement. Potato, banana, and tomato explants were cultured under the controlled conditions and quantitative parameters like rate of callus induction, multiplication of shoot, percentage rooting and plant survival documented. ANOVA was used to test the influence of the type of explant, concentration of the plant growth regulator and culture conditions on the regeneration efficiency.

Callus Induction

Formation of callus was evident in the leaf, stem and nodal explants of all the crop species. Potato leaf explants recorded the highest rate of callus induction 86.2 +- 2.3%, then stem segments 82.5 +- 2.7, and nodal explants had a rather low rate of 70.8 +- 3.1. Stem explants in banana had the greatest callus yield (88.5 +- 1.9%), whereas the leaf had 80.2 +- 2.5%. Tomato leaf explants exhibited a medium calibre of callus induction 75.6 +- 2.8% and a little higher with the stem segments 78.3 +- 2.4%. ANOVA statistical analysis revealed that the type of explant had a significant impact on callus induction ($p <$

0.05) and thus, it is possible to conclude that morphogenic potential is highly dependent on the type of tissue origin. These findings are in line with the previous researches which have underscored the importance of explant selection in order to achieve success in callus formation (Bhojwani & Razdan, 1996; George et al., 2008).

Shoot Multiplication

Cytokinin concentration and the type of explant played a major role in determining the rate of shoot multiplication. Nodal segments of potato grown in MS medium containing 2 mg/L BAP generated a maximum rate of shoot multiplication (9.2 +- 0.4 shoots per explant) and leaf-derived callus grew fewer shoots (4.6 +- 0.3 shoots per explant). Banana shoot tips that were cultured in presence of 1.5 mg/L BAP showed high rate of shoot proliferation with an average of 7.8 +- 0.5 shoots per explant. The best response of tomato nodal explants was seen at 1mg/L BAP with the mean shoots per explant being 6.4 +- 0.4. The explant type and PGR concentration interaction turned out to be significant ($p < 0.05$), which implies that the two parameters should be optimized in order to ensure the highest number of shoots (Thorpe, 2007; Feher, 2015).

Rooting Percentage

Root induction was accomplished through transferring regenerated shoots on MS medium with additions of auxins (IAA or naphthalene acetone). Rooting On 1 mg/L NAA resulted in an 92.5 +- 2.0 rooting percentage and efficient rooting. The optimum amount of IAA at which banana shoots could be rooted was 1.5 mg/L, with 88.7 +- 1.8 being the highest rooting of the tomato shoots under the influence of 1 mg/L IAA. The results of ANOVA proved that auxin type and concentration had significant impact on rooting efficiency ($p < 0.01$) which showed the importance of auxins in organogenesis (Smith et al., 2011; George et al., 2008). It was also noted that the root morphology was observed and the potato and banana had thick fibrous roots and tomato roots were finer and more branched and therefore could be successfully acclimatized.

Acclimatization and Survival Rate

Those plantlets that were rooted successfully were acclimatized in a soil-sand-compost mixture at controlled humidity levels. The survival rate was found to be highest in the potato plantlets (89.6 +- 1.9%), then banana (85.2 +- 2.2%) and tomato (81.4 +- 2.5%). Reduction of humidity and ambient light gradually were some of the key conditions affected the establishment of plantlets. Statistical testing revealed that the in vitro growth vigor was significantly correlated with the survival percentage ($r = 0.86$, $p < 0.01$), which was proposed to support the idea that well-grown shoots and roots increase the success rate of acclimatization (Engelmann, 2011; Walters et al., 2004).

Morphological Observations

During the procedure of tissue culture, the morphological observations were detailed. The nodal explant derived shoots were taller (6.8 +- 0.5 cm) and stronger than the leaf-derived shoots (4.3 +- 0.4 cm) in potato. There was vigorous growth as banana shoot tips generated numerous lateral shoots of average length 7.1 +- 0.4 cm. Tomato shoots were relatively shorter though they were highly branched with evenly developed leaf structure. These morphological analyses help support the quantitative data by giving the nodal segments and shoot tips the superiority in in vitro propagation.

Table 1: Tissue Culture Performance Parameters Across Crops

Crop	Explant Type	Callus Induction (%)	Shoot Multiplication (No./Explant)	Rooting (%)	Plantlet Survival (%)
Potato	Leaf	86.2 ± 2.3	4.6 ± 0.3	89.3 ± 2.0	87.5 ± 1.9
Potato	Nodal Segment	70.8 ± 3.1	9.2 ± 0.4	92.5 ± 2.0	89.6 ± 1.9

Potato	Stem	82.5 ± 2.7	6.3 ± 0.4	90.2 ± 1.8	88.4 ± 2.1
Banana	Shoot Tip	85.0 ± 2.1	7.8 ± 0.5	88.7 ± 1.8	85.2 ± 2.2
Banana	Stem Segment	88.5 ± 1.9	6.9 ± 0.4	85.5 ± 2.0	83.6 ± 2.0
Banana	Leaf	80.2 ± 2.5	5.4 ± 0.3	82.4 ± 2.1	80.1 ± 2.3
Tomato	Nodal Segment	78.3 ± 2.4	6.4 ± 0.4	85.3 ± 2.1	81.4 ± 2.5
Tomato	Leaf	75.6 ± 2.8	5.2 ± 0.3	82.1 ± 2.2	78.6 ± 2.4
Tomato	Stem Segment	77.2 ± 2.5	5.8 ± 0.4	84.2 ± 2.0	80.3 ± 2.3

Analysis of Variance (ANOVA)

The results of ANOVA indicated that the type of explant, the concentration of plant growth regulator, and crop species had a significant impact ($p < 0.05$) on the callus induction, shoot multiplication, rooting percentage, and survival of plantlets. Post-hoc analysis established that nodal segments of potato and shoot tips of banana were most effective in giving tissue culture response. The effects of interactions between the type of explant and the concentration of PGR were also important and it is necessary to optimize the two variables in each crop.

Regression Analysis

To estimate the correlation between the explant characteristics (e.g., size, type) and the tissue culture efficiency (e.g., shoot multiplication rate, rooting percentage), regression analysis was performed. The findings showed that there was a high positive correlation, ($R 2 = 0.81$, $p < 0.01$) between the explants vigor and shoot multiplication, and moderate correlation ($R 2 = 0.72$, $p < 0.05$) between root development and survival of plantlets. Such results indicate that it is imperative to choose healthy and healthy tissues as explants in order to have the best results in tissue culture (Bairu et al., 2009; Thorpe, 2007).

FINDINGS

The paper shows that plant tissue culture methods are very useful in crop improvement especially when optimized in terms of the type of explant, growth media, and plant growth regulators. Nodal segments and shoot tips presented the highest outcome regarding regeneration, multiplication and survival. Micropropagation, organogenesis and rooting regimens were effectively adopted and treatment effects were statistically found to be high. These results give solid proof that tissue culture is an effective mechanism of generating disease-free, uniform and high quality planting material, which promotes crop productivity, genetic improvement and sustainable agriculture.

DISCUSSION

The findings of this research show that plant tissue culture methods have a great potential in enhancing crop productivity and quality. The quantitative data show that explant type and concentration of plant growth regulator are the key factors which determine the in vitro regeneration efficiency which agrees with the previous research (Bhojwani and Razdan, 1996; Thorpe, 2007). The highest shoot multiplication rates were regularly obtained with nodal segments in potato and shoot tips in banana illustrating the importance of using meristematic and growing tissues in micropropagation. Although where the callus induction protocol is useful (and the induction of somatic embryos), the rate of shoot proliferation was lower in leaf explants, which validates previous studies that organogenic potential differs according to the tissue type (Feher, 2015; Jimenez, 2001).

Type and concentration of auxin had a strong effect on rooting percentage and plantlet survival with IAA and NAA showing the best result in root stimulation followed by success in acclimatization. These findings are in line with the past reports that have shown that root induction by auxin is crucial in achieving high levels of survival as plants move out of the in vitro conditions into the ex vitro

environment (Smith et al., 2011; Engelmann, 2011). The regression analysis also revealed that the vigor of the explants is a good predictor of the multiplication of shoot and rooting success and it is essential to use healthy and physiologically active explants in the tissue culture.

The induction and regeneration rate of the high level of callus which was observed in this work highlights the universal applicability of the processes of somatic embryogenesis and organogenesis in any large-scale programs of propagation and genetic enhancement. Besides high multiplication speed, tissue culture also allows the generation of free of pathogenic plantlets and this will solve a major problem with vegetatively propagated crops including potato, banana and tomato (Karp, 1995; Jain and Gupta, 2011). These are some of the findings that confirm the fact that tissue culture not only enhances crop improvement but also improves consistent and high quality planting material that is fundamental in sustainable agriculture and food security.

Altogether, the findings indicate the need to optimize tissue culture procedures in case of particular crops and explants. The interplay between the type of explants and the PGR concentration was especially notable, which showed that the species-specific modification is necessary to achieve the highest rate of regeneration. Coherent along with the complementary roles of micropropagation, organogenesis, and somatic embryogenesis in combined crop enhancement programs are also highlighted in the research, which offers flexibility in overcoming various farm-related challenges (George et al., 2008; Loyola-Vargas and Ochoa-Alejo, 2016).

CONCLUSION

To sum up, crop enhancement has potent and dynamic tools that are provided by the plant tissue culture methods. The research established that the explant type, the composition of the culture medium, and the concentration of the plant growth regulator have a significant effect on the induction of callus, the proliferation of shoots, the rooting and the survival of the plantlets. Most effective explants were nodal segments and the shoot tips that developed uniform plantlets that were disease free and of high quality in potato, banana and tomato. In addition to supporting speedy propagation and conservation of germplasm, tissue culture techniques are used as platforms to support genetic modification, in vitro selection, and generation of stress-tolerant crop varieties. The results highlight the viability of the use of tissue culture in traditional breeding and biotechnology courses to boost the productivity, uniformity of crops, and sustainable agriculture methods.

RECOMMENDATIONS

Using the findings, a number of recommendations can be made towards researchers, breeders, and practitioners that can be engaged in the use of plant tissue culture to improve crops. To begin with, the explant type and physiological condition should be properly chosen to achieve the highest level of regeneration and guarantee reproducible outcomes. The crop in which high multiplication and survival rates are required should have nodal segments and shoot tips as its priority. Secondly, it is necessary to increase the concentration and interaction of cytokinins and auxins to stimulate shoot growth, rooting, and vigor of plantlets. Third, strong aseptic methods must be employed and cultures taken regularly to reduce contamination and somaclonal variation in the research. Fourthly, the application of tissue culture in conjunction with molecular technologies including genetic transformation, marker-assisted selection, and genome editing may help to hasten the process of creating better crop varieties with increased yield, disease resistance, and stress tolerance. Lastly, investment in training, laboratory infrastructure, and automated culture will facilitate the scale of application and adoption of tissue culture methods in commercial farming, which will have a role in the food security and sustainable crop production.

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