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Micropropagation of Rose (*Rosa Hybrida*) Using Invitro Technique by Natural Hormone

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Abstract: In recent years, several Micropropagation technique has been established, to make some improved method of crop yield production the natural hormone from other plant species were selected for the beneficial result in the plant species. The Micro propagation technique is carried out in *Rosa hybrida* explant by providing the different natural shooting and rooting hormone in three different compositions with the half strength of MS medium, it is to predict the ability of which natural hormone is being utilized by the nodal explant of *Rosa hybrida*. The three different natural shooting and rooting hormone are Aloe vera gel extract and the tender coconut water with 50 ml and 100 ml and 150 ml of concentration in half strength MS media and the Cinnamon powder alone taken in the .5 g, 1g and 1.5 g of concentration in half strength MS media due to the powdery form. These were proceeded in aseptic condition of propagation as a result it has been noticed that among the three natural hormone the remarkable growth has been found in the tender coconut water provided nodal segment in all the three concentration. This article has a goal to promote the use of the natural rooting role in the plant breeding industry.

Keywords: *Rosa hybrida*, Micropropagation, natural hormone.

I. INTRODUCTION

Micro propagation is the process of cloning propagation technique which is mainly used in the plants under aseptic condition. Basically it has been done by the application of the artificial hormone such as cytokinins like benzyladenine for the propagation of shoot system, with the application of the Auxins like Indole acetic acid for the induction of multiple shoot system and the with naphthalene acetic acid for the embryo differentiation from callus. This technique has been applied to get the commercially sustainable plant species and to rid of the disease free plants species. Micro propagation has the four methods of stages for the propagation of the plants they are, selection of the explants, multiplication of the selected plant species, rooting of the plant with acclimatization and finally ex vitro preplant i.e. planting in the soil. The explant should be chosen depending upon the ability of the plant to with mature cells with the differentiating properties and time period of cell division of the mature cells so that it could produce the enormous growth in the culture medium. The explant should be determined that the plantlets will give rise to haploid or diploid set of species. In the closed container the plants were cultured with application of the media, it should mainly contain the plant growth regulators like hormone essential for the plant growth. It is commonly called as the propagation of in vitro and Ex vitro process. The Rose cultivation is propagated in this article, because it can give better propagative result when compared to other plants. It has been noticed that the rose plants are complicated give rise to the roots in normal method but by the tissue culturing technique it has been proved that it can give rise to rose plants in the large scale production and it especially requires the need of the single parent to make the next generation. The micro propagation can be carried out in the shoot multiplication and in root multiplication, it can give the remarkable growth in vitro and in vivo condition. The adventitious shoots and somatic embryos formation can give the plant cultivation by this process. This technique is widely used in the production of the commercially important plants species. It also plays a convincing role in the production of seed, conservation of germ plast and artificial seed production.

On the otherhand, it can also predict the uniformity of product and the contamination of the plant disease. Micro propagation can be carried out by the practice of the desirable phenotype cell it can yield the genetically modified plantlets, it can be further used in the process of in vivo cultivation of the shoot and root system. By the help of this technique the plants can be genetically modified with desired phenotype in the mass production of the plants and also it is cost efficient with the asexual method of propagation. The medicinally important plant species can be developed in unconditional form for the extraction of their secondary metabolites. The endangered plant species can be easily preserved by this process of propagation. The production of the plantlets in the In vitro condition can be done in the horticultural engineering and in greenhouse for the mass cultivation.

The micro propagation is the technique primarily using in the plant tissue culture by the hormones like IAA, IBA, IBB, etc are the growth promoting hormones which are produced by the stem, bud and root tips explants, they remain active under stress conditions i.e the plants can utilize the hormone such as auxins and the cytokinins which is provided in the medium when it is needed. With the application of the micropropagation steps to the plants, it has been fed under natural rooting and shooting hormone or plant growth promoting hormone naturally produced from other plant substances, in this article we have used the Aloe vera gel, cinnamon powder and coconut water, it can provide the vital strength to the plant by supporting the shoot and root development.

Among these substances, the *Rosa hybrida* plant was grown under aseptic condition with the MURASHIGE AND SKOOG medium. Due to genetic uncertainty, the widespread of this method was discouraged. This methodology of culturing, micropropagated technique and possibility of rose micropropagation by shoot and root multiplication are discussed.

II. PROPERTIES OF ROOTING AND SHOOTING HORMONE

In this technique, a rooting and shooting hormone aids in the root formation and shoot formation, it can protect the plants from the infectious organisms such as fungi and bacteria and it also promotes the better root development. During the transplantation of plants, the plant can get the transplant shock, these hormones can resist the shock. It also plays a great role in the development of the stem cuttings and in grafting. Thus, these natural rooting and shooting hormone plays a fundamental role in the plant growth development. The three major rooting and shooting hormone were taken in this process namely Aloe vera gel, Cinnamon Powder and Coconut water.

A. Aloe Vera Gel

Aloe vera contains the abundant source of nutrient such as the vitamins, minerals and amino acids which aids in the process of root development. It can induce the formation of the root development and it also acts as the natural plant growth regulator (bioregulator) it can abundantly present in the leaf of the Aloe vera gel and it also acts as a regulator in supporting the growth of cuttings in the plants as it contains the auxins, amino acid, minerals and enzymes. Aloe vera approximately contains 70 biologically active substances such as vitamin A, vitamin C, vitamin E, vitamin B2 and ions like magnesium, zinc, calcium and also constitute of lignins, choline polysaccharide and some minerals all these compounds give the moral support to the plant root development. Especially Aloe vera contains the antifungal, antibacterial and antiviral resistant properties which is produced by Acemannan polymer and it can prevent the plants from the cuttings and disease causing organisms.

B. Cinnamon

Cinnamon is the one of the rooting agent and it also known as Willow Water or Hormone rooting powder. It can provide a various significant role in promoting the stem development. The cinnamon powder can be applied directly to the plants which can give the rust free growth in the stem. It has been special characteristics of resistant property and against the fungal infection in the plants and it can induce the stem cutting and improve the root formation. The primary compound of cinnamon such as eugenol can prevent the plants from the formation of the spores and provide resistant against the fungal agents.

C. Coconut Water

The coconut water has normally consists of plant growth regulators (PGR) or phytohormone so it is used in the process of plant tissue culture. With the use of many analytical technique it has been found that the coconut water contains the auxin, gibberellins, cytokinin and abscisic acid. It has been found that it composed of other PGR such as brassinosteroids, jasmonates and strigolactones. These hormone can induce the formation of callus in the shoot and also aids in the multiplication of the cells in plants. As it contains several minerals, vitamins and hormone for the plant growth development thus it is used in the plant tissue culture technique.

III. MATERIALS REQUIRED:

A. Plants Materials

For the propagation of, the shoot explant is taken and cut with 1-2 inch, washed with the ethanol for 30s and soaked in 40% of Clorox and 5% of NaOCl. The sterilized explants were again washed for 23 times with double distilled in aseptic condition.

The fundamental media of MURASHIGE AND SKOOG medium is used which can provide the essential macro and micro molecules, it plays a vital role in plant metabolism. The half MS medium is usually provided with the composition of 1.1g of MS salts, 5g of sucrose, 4g of plant agar and combined with the natural rooting and shooting hormones in different concentration is applied to each explant. The half strength medium is used for the induction of the shoot. It is mixed with following three natural rooting and shooting hormone.

The Tender coconut water is taken in the three different concentrations of 50ml, 100ml and 150ml with media. The Aloe vera gel is taken and centrifuged at 10000 rpm and the supernatant is collected and added in different concentrations of 50ml, 100ml and 150ml with media. The Cinnamon of 3g is boiled for 15 min at low temperature in 100ml of water and then it is filtered. The extracted cinnamon powder is used in different concentration of 0.5g, 1g and 1.5g in 1000ml of medium.

B. Propagation Technique

The sterilized shoot nodal explant is taken and the process is initiated with the transfer of explant into the glass flask containing 1/2 MS medium with the Aloe vera extract in three different concentrations. The another nodal segment is propagated with Cinnamon powder of three different concentrations with 1/2 MS medium. Finally the other shoot explant is cultivated with the tender coconut water with three different concentrations with 1/2 MS medium. The propagated glass flask were incubated in sealed condition and kept in room temperature with 16h photoperiod under illumination of 20 mmolm⁻²s⁻¹ photosynthetic photon flux density provided with cool white fluorescent light. The explants were provided with half the amount of the MS medium mainly to detect the efficiency of the given natural rooting and shooting hormone in different concentrations.

IV. RESULT

The cultured explants were observed for the formation of the root development in the medium after four weeks of incubation period. Among three natural rooting and shooting hormone the better growth of plants were observed in the Tender coconut water provided with nodal explant in all the three concentrations. The moderate growth is observed in the Aloe vera gel provided explant in all the three concentrations and the poor growth is observed in the Cinnamon powder provided nodal segment.

The growth of the natural rooting and shooting hormone is calculated by the following table ;

S.NO	Test Hormone	Volume of the Hormone (ml/L) in 1/2 MS media	Growth of the plant
1.	Aloe vera gelextract	50	Well grown
2.	Aloe vera gelextract	100	Well grown
3.	Aloe vera gelextract	150	Well grown

S.NO	Test Hormone	Volume of the Hormone (g/L) in 1/2 MS media	Growth of the plant
1.	Cinnamon powder	0.5	Moderate growth
2.	Cinnamon powder	1	Moderate growth
3.	Cinnamon powder	1.5	Moderate growth

S.NO	Test Hormone	Volume of the Hormone (g/L) in 1/2 MS media	Growth of the plant
1.	Tender coconut water	50	Poor growth
2.	Tender coconut water	100	Poor growth
3.	Tender coconut water	150	Poor growth

The growth of the plants were observed frequently and it has been shown in (figure 1). The developed root system is subcultured in the another fresh MS medium with respective rooting hormone to eliminate the contamination and to observe the growth of the root system. It is mainly to identify the effective growth of the plant by which natural rooting hormone is being utilized. The root development is observed after the hormones have been utilized and it has been shown in (figure 2). In this experiment, the better growth has been successfully observed in the tender coconut water provided explant which gives the appropriate growth of the rose plant. The growth of the *Rosa hybrida* has been demonstrated in the bar graph (figure 3) and it has been mentioned after the incubation of the explants into the medium.

The growth of shoot system.



FIGURE :1

The growth of root system.



FIGURE :2

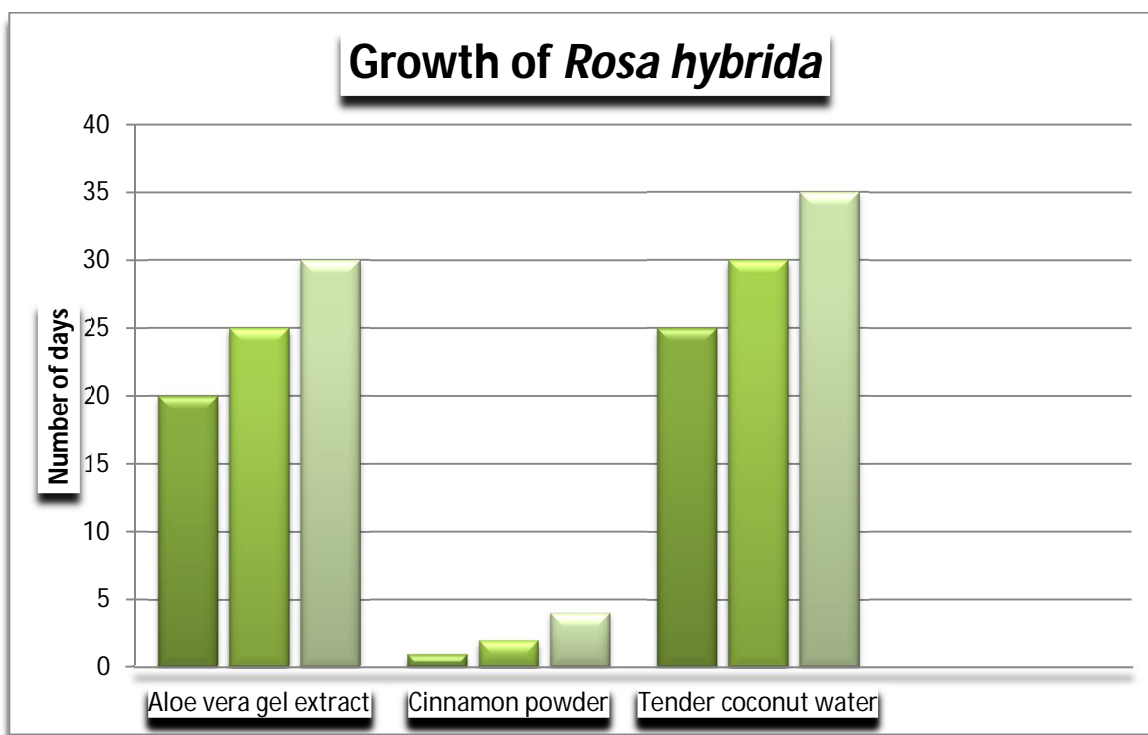


Figure :3

The growth of the *Rosa hybrida* after the incubation of explants into the medium containing the MS medium and with the natural hormone.

V. DISSUCSSION

The propagation of plants has proceeded under sterile aseptic conditions as it is cost efficient means for the mass propagation of certain plants. An important consideration of this technology is the ability to add value to plants being propagated. It can provide the large scale production of the plant cell in the provided liquid culture. The propagation using the meristem can give the shoot culture in huge number of individuals. This technique can provide the regeneration in crossing the distant related plant species. It added the great value in the field of cell biology, genetics and biochemistry. It can also eliminate the viral multiplication in plant species. The production of artificial clonal plantlets, germplasm conservation and breeding of plants in which propagation through seed is not possible can be done. By providing the improved nutritional supplements or advanced nutrient supplement like natural hormone will result in profitable development in the breeding industry.

It provides new opening opportunities for the natural rooting hormone which are more stable than artificial hormone. With the help of the plant tissue culture the high quality plant materials can yield such as the fruits, vegetables and medicinal leave parts. However, with the implementation of the secondary metabolites like plant growth promoting hormone will give the more beneficial role in this technique. This can be achieved by the selection of specific cell producing high amount of desired compounds and development of a suitable medium. The in vitro propagation of the plants can give the enormous biomass energy in tissue culture technique. The chromosomal disorder of the plant species can be rectified by the employment of the tissue culture. Micropropagation can improve the crop production commercially as it doesn't depend on any climatic condition.

Moreover, it can be cultivated in large scale with the short period of time in the limited surface. This technique also generate the disease free plant species. This technique will enhance the growth of the plant by cell elongation, cell division, dormancy, adventitious root formation, Parthenocary, control of premature fruit formation and delay leaf abscission, initiation of flowering and eradication of weeds. It can also be used in the genetic dwarfism plants to eliminate such growth and in counteraction of the apical dominance. In some plants it can be used to promote the chloroplast growth and pigments and in morphogenesis. It can inhibit the leaf senescence and for the stimulation of the fruit ripening. The plant stress can be reduced by the method of propagation and it can able to reduce the breeding period of the plants. The rare varieties of the plants can be easily able to propagate and it can be preserved for long time for some particular uses in the fields like medicine, forestry, horticulture and also for the development of the secondary metabolites.

VI. CONCLUSION

Micropropagation is an in vitro technique, it can produce a lot more progeny plants from the tissue culture method. The role of the natural hormone has been discussed. The common growth factor is artificial hormones like Auxin, cytokinin, gibberellin etc., it gives a predominant growth for root and shoot development. In this study, the focus is given to the natural hormones throughout the micropropagation, this natural hormones have a growth factors similar to Auxin and cytokinin and also it given a well defined growth effects under in vitro condition as well as in aseptic conditions. We have used Tender coconut water, Aloe vera gel extract and cinnamon powder with the moderate compositions along with half MS medium. In contrast, the natural hormone gains a more effective growth than the artificial hormone. The expected growth has been obtained from the tender coconut water provided explant and it has made a significant growth in the *Rosa hybrida*, then the considerable growth has been obtained from the Aloe vera gel extract and the cinnamon powder provided explants. Moreover, the intake of the natural hormone from the tender coconut water, Aloe vera gel and cinnamon powder has been proved that it can make a remarkable use in the plant tissue culture. This application will provide the further use of the natural hormone from the plant substances and it definitely yield desirable quantity of the plant breeding and it also can also be used for various sectors like, to preserve the seeds and plant, genetical breeding of crops, Gene bank etc.

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