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Propagation of Rose (*Rosa Hybrida* L.) Under Tissue Culture Technique

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Abstract
The rose is the most popular ornamental plant in the world, as well as the most important cut flower. Throughout history no other plant has such wide appeal and been the center of so much attention than the Rose. Roses are one of the world's most important ornamentals for a long time and are most often used for ornamental, medicinal and aromatic purposes. The experiment was conducted at Tissue Culture Laboratory; Plant Breeding and Genetics Division, Tando Jam during 2014. The aim of present investigation was to determine appropriate basal medium and growth regulators for *in vitro* propagation of *Rosa hybrida* from nodal meristem explants. The basal medium of Murashige and Skoog (1962) containing with different concentrations of MS + 30 g L^{-1} sugar, MS + BAP 0.5 mg L^{-1} + 30 g L^{-1} sugar, MS + IBA 0.1 mg L^{-1} + BAP 5 mg L^{-1} + 30 g L^{-1} sugar, MS + NAA 0.5 mg L^{-1} + BAP 0.5 mg L^{-1} + 30 g L^{-1} sugar, MS + NAA 0.1 mg L^{-1} + BAP 2 mg L^{-1} + 30 g L^{-1} sugar for shoot induction and MS½ + 30 g L^{-1} sugar, MS½ + NAA 1 mg L^{-1} + 30 g L^{-1} sugar, MS½ + NAA 2 mg L^{-1} + 30 g L^{-1} sugar, MS½ + IBA 1 mg L^{-1} + 30 g L^{-1} sugar, MS½ + IBA 2 mg L^{-1} + 30 g L^{-1} sugar for root induction were used in this study. The statistical analysis of variance showed that days to initiation, number of shoots, shoot length, number of leaves, number of roots and root length were highly significant at 5% probability level. The results showed that early days to initiation was recorded, maximum number of shoots $bottle^{-1}$, shoot length $bottle^{-1}$ and number of leaves $bottle^{-1}$ were recorded under the concentration of MS + NAA 0.1 mg L^{-1} + BAP 2 mg L^{-1} + 30 g L^{-1} sugar, followed by number of shoots $bottle^{-1}$, shoot length $bottle^{-1}$ and number of leaves $bottle^{-1}$ were obtained under the concentration of MS + NAA 0.5 mg L^{-1} + BAP 0.5 mg L^{-1} + 30 g L^{-1} sugar and minimum number of shoots $bottle^{-1}$, shoot length $bottle^{-1}$ and number of leaves $bottle^{-1}$ were recorded under the concentration of MS + 30 g L^{-1} sugar. The results indicated that maximum number of roots and root length were achieved under MS½ + IBA 2 mg L^{-1} + 30 g L^{-1} sugar and minimum number of roots and root length were recorded under MS½ + 30 g sugar L^{-1}. IBA is an auxin plant growth regulator used to promote and accelerate root formation of plant. It was concluded from this study that MS + NAA 0.1 mg L^{-1} + BAP 2 mg L^{-1} + 30 g L^{-1} sugar for shoot induction and MS½ + IBA 2 mg L^{-1} + 30 g L^{-1} sugar proved best for root induction in rose.

Keywords: Rose, Tissue Culture Technique

Introduction

Rose (*Rosa hybrida* L.) is the most popular of the flowers because of its beauty and fragrance that is why it is rightly called the queen of flowers. The genus Rosa contains more than 1400 cultivars and 150 species, which are grown for rootstocks, curiosity value and striking floral display. Apart from its ornamental value, it is also used for the production of essential oil and vitamin C and is rightly called the queen of flowers Jafar *et al*, (2005) [12]. Roses are best known as ornamental plants grown for their flowers in the garden and sometimes indoors. Rose is one of the most important commercial flower crop used in the floriculture and cut flower industry throughout the world (Rajeshbabu *et al*, 2014) [22]. The rose is one of the leading cut flowers in the global floriculture trade and is used at almost every event in both local and international markets. The major rose producing countries of the world include Netherlands, Colombia, Kenya, Israel, Italy, United States, and Japan (Evans, 2009) [7]. Rose has always been the favorite flower in Pakistan and has a special place in our culture as there is hardly any event where roses are not displayed. Rose production has great potential in Pakistan because it has an agricultural economy with diverse climatic conditions (Khan, 2005) [15].

There are more than 20,000 commercial cultivars, which collectively are based on only 8 wild species (Kim *et al*., 2003) [16]. They belong to the *Rosaceae* and are grown worldwide as cut flowers and potted plants and in home gardens. The flowers vary greatly in size, shape and color. They serve as rootstocks, onto which other species or cultivars are grafted to increase their rate of propagation; they supply the cut-flower market used in the extraction of attar as rose oil (Hameed *et al.*, 2006) [9]. Conventionally, it is propagated asexually through cuttings, budding or grafting scion cultivars on specific rootstocks in the particular seasons. These methods are laborious and time taking with very low percentage of success. It has also been observed that plants raised from these methods are infected with different diseases that affect flower production and quality, and ultimately their

market value is decreased (Norton and Boe, 1998). In general cuttings of hybrid roses are difficult to root. Tissue culture methods have been developed as a potential tool for rapid and mass propagation in number of plant species. The central concept of tissue culture is totipotency i.e., every living cell has the genetic information needed to develop into complete organism (Khan and Shaw, 1988) [14].

Micropropagation of plants through tissue culture has been considered as an important and very popular method to produce plants which are very difficult to propagate conventionally by seeds and other natural means. The great benefit of *in vitro* propagation technique is the enormous multiplicative capacity to produce disease free plants in a relative short period of time with independent of seasonal factor in a cost effective manner. The plantlets developed through tissue culture reduce input costs, increase effective management and enable market pricing because of contamination and disease free products. Although vegetative propagative method like cutting, layering, budding and grafting is a predominant technique in roses, yet it does not ensure healthy and disease free plants (Dhawan and Bhojwani, 1986) [6]. Various tissue culture techniques, such as propagation, may decrease propagation time and could virtually eliminate the need for grafting onto root stocks; propagation has been shown to be a highly effective method of rapidly propagating disease free, uniform rose plants (Wang *et al.*, 2002) [24].

Plant tissue culture is a propagation technique widely used in modern agriculture that allows a complete plant to be grown from a single plant cell. Tissue culture is considered an asexual propagation technique since it only involves the cells from a single parent plant. Asexual propagation techniques produce plants that are genetically identical to the parent plant and to each other (Kane, 1991) [13].

Eventually, most of the new growth in the plant becomes restricted to specific areas at the tips of the stems and roots called meristems. The cells that compose the meristems (meristematic cells) are relatively unspecialized and retain the ability to become any of the specialized cells in the plant. Meristematic cells are usually the preferred cell to initiate new plants since they begin to develop into stems and leaves very quickly. The use meristematic cells from a part of the plant called an axillary bud (Hasegawa, 1980) [10].

All of the different cells in a plant must develop and work together in a coordinated manner in order to carry out the various processes necessary for the plant to live. During normal development, the specialized cells within the plant are produced at the proper times in response to growth stimulating and regulating chemicals called hormones. During tissue culture, the hormones must be supplied artificially to the plant at the proper time. Two important classes of hormones used in tissue culture are cytokinins and auxins. These hormones promote division and specialization of cells, and later development of stems, leaves, and roots. It is often necessary to treat the developing plant with different hormones at different times because a hormone that promotes stem and leaf development may inhibit root formation (Hyndman et al, 1982) [11]. Tissue culture techniques should minimize the time necessary for the introduction of new cultivars into the commercial market and thus increase the availability of plants with improved horticultural characteristics (Previati *et al.*, 2008) [21]. To establish an *in vitro* flowering research system, it is necessary to develop a reliable and rapid shoot organogenesis protocol. In the present study planned an efficient tissue culture technique to yield large number of shoots from nodal explants of rose in controlled condition segmented with different hormonal effects on *in vitro* propagation in rose (*Rosa hybrida* L.).

Materials And Methods

The experiment was conducted in Tissue Culture Laboratory, Plant Breeding and Genetics Division at Nuclear Institute of Agriculture, (NIA), Tando Jam. Fresh plant materials (lateral buds) were collect from the rose plant grown in garden at Nuclear Institute of Agriculture (NIA), Tando Jam. The excised young and mature shoot tips were washed in running water for ten minutes. The Murashige and Skoog (1962) [17] medium containing with different concentrations of MS + 30 g L^{-1} sugar, MS + BAP 0.5 mg L^{-1} + 30 g L^{-1} sugar, MS + IBA 0.1 mg L^{-1} + BAP 5 mg L^{-1} + 30 g L^{-1} sugar, MS + NAA 0.5 mg L^{-1} + BAP 0.5 mg L^{-1} + 30 g L^{-1} sugar, MS + NAA 0.1 mg L^{-1} + BAP 2 mg L^{-1} + 30 g L^{-1} sugar for shoot induction, MS½ + 30 g L^{-1} sugar, MS½ + NAA 1 mg L^{-1} + 30 g L^{-1} sugar, MS½ + NAA 2 mg L^{-1} + 30 g L^{-1} sugar, MS½ + IBA 1 mg L^{-1} + 30 g L^{-1} sugar, MS½ + IBA 2 mg L^{-1} + 30 g L^{-1} sugar were used for root induction. Rose explants excrete phenolic compounds, which caused browning of media and mortality of explants. Therefore, activated charcoal (0.5 mg L^{-1}) was added to MS media to control browning. Nodal explants containing lateral buds of actively field grown rose were used for multiplication in the experiment. They were cut in 3-4 cm length segments and surface disinfested using 70% ethanol for 30 seconds and then immersed in 10 % sodium hypochlorite solution of commercial laundry bleach (5.25% NaOCl) containing 2 drops of Tween-20 emulsifier to aid wetting for 20 minutes. The pH of medium was adjusted at 5.7-5.8 before autoclaving and media was autoclaved at 121 °C and 1.05 kg^{cm2} (15-20 psi) for 20 minutes. Uniform culture conditions were maintained as 16-hour photoperiod at 25±2 °C for growth temperature. The experiments were laid out in completely randomized design (CRD) with three replications. The days to initiation, number of shoots $bottle^{-1}$, shoot length (cm) $bottle^{-1}$, number of leaves $bottle^{-1}$, number of roots $bottle^{-1}$, number of root length $bottle^{-1}$ were recorded. The experimental data were recorded and subjected to factorial design of analysis of variance (ANOVA) under linear models of statistics to observe statistical differences among different traits of wheat using computer program, Student Edition of Statistix (SWX), Version 8.1 (Copyright, 2005, Analytical Softwear-USA). Further least significant difference (LSD) test was also applied to test the level of significance among different combination means (Gomez and Gomez, 1984) [8].

Results And Discussions

Shoot induction

The statistical analysis of variance showed that days to initiation, number of shoots, shoot length and number of leaves were highly significant at 5% probability level and data are presented in Appendix-I, Table 1. The results showed that early days to initiation was recorded 10.00 days, while, maximum number of shoots $bottle^{-1}$ (7.00), shoot length (6.79 cm) $bottle^{-1}$ and number of leaves $bottle^{-1}$ (11.00) were recorded under the concentration of MS + NAA 0.1 mg L^{-1} +

BAP 2 mg L^{-1} + 30 g L^{-1} sugar, followed by number of shoots bottle^{-1} (5.00), shoot length (5.57 cm) bottle^{-1} and number of leaves bottle^{-1} (9.66) were recorded under the concentration of MS + NAA 0.5 mg L^{-1} + BAP 0.5 mg L^{-1} + 30 g L^{-1} sugar and minimum number of shoots bottle^{-1} (1.66), shoot length (3.05 cm) bottle^{-1} and number of leaves bottle^{-1} (2.66) were recorded under the concentration of MS + 30 g L^{-1} sugar.

Yan *et al.*, (1996) [25]; Ara *et al*., (1997) [3] reported that the most important technique of micropropagation. The meristem proliferation in which apical buds or nodal segments having an axillary bud are cultured to regenerate multiple shoots without any intervening callus phase achieved that in spite of many improvements for some cultivars of roses and observed that the influence and interaction of growth regulators (BA, NAA) and carbohydrates (sucrose, glucose) in multiplication of rose cultivars. The proliferation with BAP and NAA significantly increased number of new green leaves and axillary shoots and leaves. The results supported by Skirvin *et al*, (1990) [23] Pati *et al* (2001) [20], Allahverdi *et al*, (2010) [1]. The results fully support by Asad *et al.* (2010) [4] that the treatment containing BAP was found to be the best one for shoot regeneration from nodal segments. The treatment with NAA in combination with BAP was found to be suitable treatments for production from leaf explants for micropropagation.

Table 1: Days to initiation, number of shoots bottle^{-1}, shoot length (cm) bottle^{-1}, and number of leaves bottle^{-1} as affected under different concentrations of phytohormones in rose (*Rosa hybrida* L.)

MS + Concentrations	Days to initiation	Number of shoots bottle^{-1}	Shoot length (cm) bottle^{-1}	Number of leaves bottle^{-1}
MS + 30 g L^{-1} sugar	18.00 a	1.66 d	3.05 c	2.66 c
MS + BAP 0.5 mg L^{-1} + 30 g L^{-1} sugar	15.00 b	2.66 c	3.61 c	4.66 b
MS + IBA 0.1 mg L^{-1} + BAP 5 mg L^{-1} + 30 g L^{-1} sugar	12.00 c	4.33 b	4.97 b	5.66 b
MS + NAA 0.5 mg L^{-1} + BAP 0.5 mg L^{-1} + 30 g L^{-1} sugar	12.66 c	5.00 b	5.57 b	9.66 a
MS + NAA 0.1 mg L^{-1} + BAP 2 mg L^{-1} + 30 g L^{-1} sugar	10.00 d	7.00 a	6.79 a	11.00 a

Days to initiation SE (0.7601) LSD (5%) (1.7528)
Number of shoots bottle^{-1} SE (0.3944) LSD (5%) (0.9095)
Shoot length (cm) bottle^{-1} SE (0.46951) LSD (5%) (1.0827)
Number of leaves bottle^{-1} SE (0.7601) LSD (5%) (1.7528)

Root induction

The statistical analysis of variance showed that number of roots and root length were highly significant at 5% probability level and data are presented in Appendix I, Table 2. The results showed that maximum number of roots and root length were obtained (3.00 and 3.75 cm) under MS½ + IBA 2 mg L^{-1} + 30 g L^{-1} sugar, followed by (2.00 and 2.55 cm) with concentration of MS½ + IBA 1 mg L^{-1} + 30 g L^{-1} sugar, while minimum number of roots and root length were obtained (0.33 and 0.90 cm) under MS½ + 30 g L^{-1} sugar. IBA is an auxin plant growth regulator used to promote and accelerate root formation of plant. IBA is also used on ornamental turf to promote growth development of flowers and fruit to increase crop yields. It is the most effective and widely used for rooting of plantlets. IBA is an auxin plant growth regulator to promote and accelerate root formation of plant. IBA is also to improve growth and development for rooting in flowers. It is the most effective and widely used for rooting of plantlets and these results supported by Ozel and Arsalan (2006) [19] and Chakrabarty *et al*., (2000) [5]. The results agreed with Asad *et al.* (2010) [4] that medium containing MS½ prepared with 1.0 mg L^{-1} IBA proved best root induction in rose.

Table 2: Number of roots bottle^{-1}, number of Root length (cm) bottle^{-1} as affected under different of phytohormones in rose (*Rosa hybrida* L.)

MS + Concentrations	Number of roots bottle^{-1}	Root length (cm) bottle^{-1}
MS½ + 30 g L^{-1} sugar	0.33 c	0.90 c
MS½ + NAA 1 mg L^{-1} + 30 g L^{-1} sugar	0.66 c	1.66 bc
MS½ + NAA 2 mg L^{-1} + 30 g L^{-1} sugar	1.00 bc	2.53 b
MS½ + IBA 1 mg L^{-1} + 30 g L^{-1} sugar	2.00 ab	2.55 b
MS½ + IBA 2 mg L^{-1} + 30 g L^{-1} sugar	3.00 a	3.75 a

Number of roots bottle^{-1} SE (0.558) LSD (5%) (1.2862)
Root length (cm) bottle^{-1} SE (0.4447) LSD (5%) (1.0255)

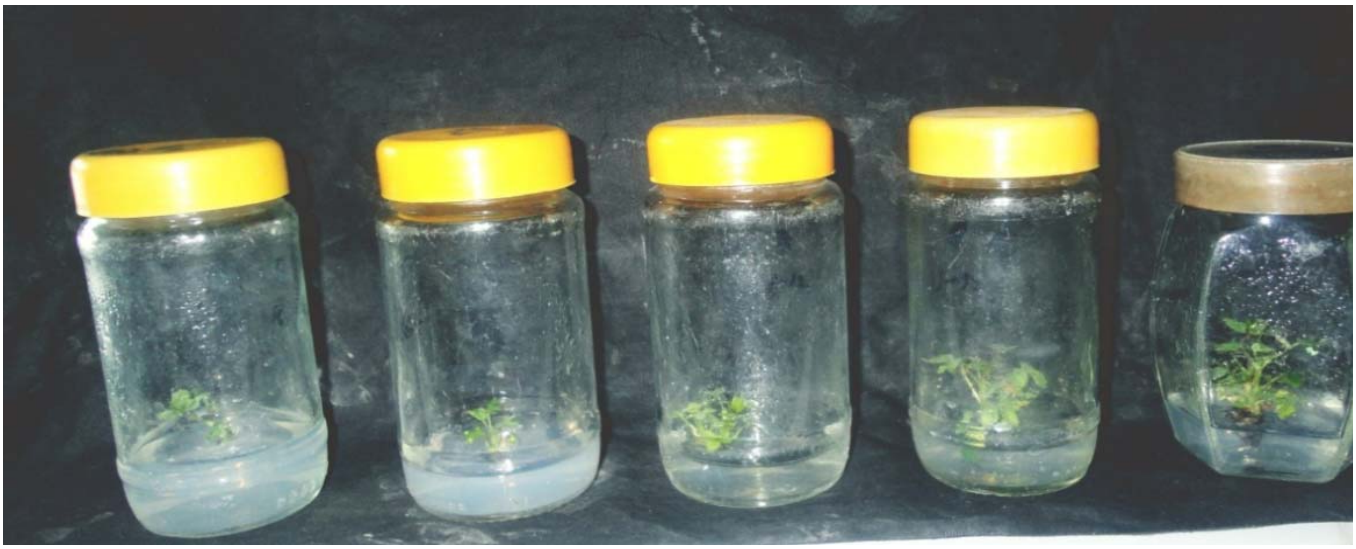

Fig 1: Effect of different concentrations of growth hormones on rose (*Rosa hybrida* L.)

MS + 30 g L^{-1} sugar	MS + BAP 0.5 mg L^{-1} + 30 g L^{-1} sugar	MS + IBA 0.1 mg L^{-1} + BAP 5 mg L^{-1} + 30 g L^{-1} sugar	MS + NAA 0.5 mg L^{-1} + BAP 0.5 mg L^{-1} + 30 g L^{-1} sugar	MS + NAA 0.1 mg L^{-1} + BAP 2 mg L^{-1} + 30 g L^{-1} sugar

Conclusion

The results of the experiment for the conclusion that the concentration of MS + NAA 0.1 mg L^{-1} + BAP 2 mg L^{-1} + 30 g sugar L^{-1} performed very well for shoot induction, while MS½ + IBA 2 mg L^{-1} + 30 g L^{-1} sugar proved best for root induction under *in vitro* condition in rose.

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