An International Journal of Floriculture Science & Landscaping

THE JOURNAL OF THE GREENS AND GARDENS

Volume 01, No. 01 July 2018

Publication Division

ROY'S GREENS & GARDENS FOUNDATION

1/342, Vijayant Khand, Gomti Nagar, Lucknow - 226 010
Uttar Pradesh, India

The Journal of the Greens and Gardens
An international Journal of Floriculture Science & Landscaping
Volume 01, No. 01, July, 2018

Profile:
Received on – April, 2018
Accepted on – May, 2018

Propagation of Rose: status, progress and future

Tejaswini, Shivakumar, Vinutha, M.R. and Saidulu Yeluguri
Indian Institute of Horticultural Research, Bangalore, INDIA
E. Mail: tejaswini@iihr.res.in

Rose, the 'Queen of flowers' is aesthetically appreciated by each and every one. No garden is complete without a rose plant and no event is complete without a rose flower. *Rosa* is an economically important genus of ornamental crops. Rose belongs to the family *Rosaceae*. The genus *Rosa* consists of approximately 200 species and more than 20,000 varieties. Cultivated roses are of complex hybrid origin. Hence, it is referred as either *Rosa hybrida* or simply as *Rosa spp*.

Geographically rose cultivation is distributed both in temperate and tropical countries. Being a favorite ornamental plant, the flowers are cherished as exclusive rose gardens, as well as an integral part of any garden, be it small or large. Area of cut rose flower production worldwide particularly in developing countries is expanding with a remarkable progression. Flowers with short and long stalk, ranging in size and colour are traded across the globe. In the world trade of cut flowers, the rose is by far the most important crop both in terms of quantity and the amount involved in trade. The demand for new rose varieties having varying size and colour along with biotic and abiotic resistance is constantly on demand. Continued creation and supply of new varieties is dependent on rose breeding programs. Production of large number of progenies with different gene combination is basic necessity of any breeding program and that is constrained in rose by the inherent problems of seed dormancy and seed germination. A lot of research has been done for addressing the problem to break the seed germination barrier and to make advances in seed propagation (Banuprakash *et al.*, 2004, Hosafci *et al.*, 2005, Anderson and Byrne, 2007, Nadeem *et al.*, 2013,). Biotechnological approaches in rose improvement have necessitated the research in tissue culture and micro propagation studies (Canli and Kazaz, 2009, Alsemaan, 2013, Vinutha, 2017).

Besides growing for their ornamental beauty as a garden plant for its flowers, rose cultivation is doe for varying utilities in pharmaceutical and cosmetic Industry. Every part of the plant that includes root, stem, leaves, flowers and fruits are reported to be useful for varying purpose (Tejaswini and Prakash, 2005). Roses are cultivated for extraction of aromatic compounds for perfume industry to traditional use in incense stick and attar. *R.damascena*, *Rosa bourboneana*, *R. gallica* and *R. centifolia* are some of the major fragrant rose species used in aromatic industry (Amjad *et al.*, 2016). Most of these species are propagated by cuttings for commercial cultivation.

Rose cultivation ranges wide from simple open field cultivation to growing within naturally ventilated polyhouses and in green houses with automised control of all weather parameters. In order to address the varying needs of cultivated area in terms of soil and water quality, rose propagation methods have changed over the years, from the simple own-root multiplication progressing to the budded hybrids, grafting, stenting and micro propagation.

There is a growing demand of rose plants for commercial flower production as well as for garden and pot purposes. In this paper, we have made an attempt to consolidate the status of sexual and asexual propagation methods, the progress that has been made over the years and the research required in future.

Asexual Propagation Methods

Budding is the most widely practiced asexual method of propagation in rose. Grafting, stenting, cutting and layering are the other methods being followed depending upon the rose variety multiplied. Cutting is the easiest and least expensive method of propagation, and is a prerequisite for propagation of rootstocks required for all other methods.

Propagation by cutting - Not all rose varieties can survive on their own root. Particularly, commercially cultivated varieties of *R. hybrida* meant for flower production and garden beautification are unable to survive on their own root. On the contrary, commercially cultivated fragrant rose varieties of *R. damascena*, *R. borboniana* and landscape varieties of *R. rugosa*, as well as varieties of *R. canina* cultivated for its hips are generally grown on their own root. Most of the climbing and rambler roses, as well as miniature roses are good to be grown on their own root. Varieties that can grow well on their own root are multiplied by cuttings. *R. multiflora*, *R. canina*, *R. indica* and *Rosa odorata* are the major species used as rootstocks and propagated by cutting.

Generally, the hard wood cuttings are better in rooting than soft wood cuttings. Preferably cuttings should be taken after flowering is over. Cuttings of 6-8 inch with 4-6 good leaves are ideal for propagation. The basal portions of the stem made up of less than five-leaflets and from those which have open or swollen auxiliary buds are eliminated. When the leaves are too small it means that insufficient photosynthesis has occurred for the auxiliary buds that should shoot. Original leaf area of stem cutting impacts upon number and dry weight of roots as well as length and dry weight of the primary shoot formed (Costa and Challa, 2002). Rose cuttings are normally planted without leaves, in exceptional cases of certain varieties, leaves retained on upper part of the cuttings leads to better rooting.

Cuttings can be directly rooted in beds, in covers or in containers filled with media. Sand is the most preferred media for quick rooting. However, cuttings to be used as rootstock for budding need to be better rooted on fine soil having high clay content so that the root system is not disturbed and remains intact while budding. The rooting performance of rose appeared to be less affected by the poor aeration and more correlated with oxygen diffusion rate than with air content. (Hans 1983). Callus formation is a pre-requisite for root formation in rose cuttings. In case of bourbon roses and in *R.damascena*, to get better callus formation, farmers of Uttar Pradesh, India follow an unique practice where in, they dig up pits in soil, place the rose cuttings and completely cover them with soil for 15 days after which the cuttings with callus planted out in beds are expected to give better rooting. Not much scientific studies have done on this farmers practice to understand and improvise it further. Scientific studies have concentrated more in propagation chambers with controlled environment than with field situations. For instance, multiplication of cuttings in propagation chambers with enhanced ambient carbon dioxide concentration (350 ppm) has been reported to hasten the formation of callus, callus proliferation and enhancing the number of roots formed (Costa *et al.*, 2007).

Rooting hormones were reported to be beneficial to get better rooting of cuttings but is not essential in many of the species being used as rootstock. Research studies regarding use of hormones for rooting have reported varying results with wide range of auxin concentrations to be used for efficient rooting. Using 3000-3500 ppm. of rooting hormones either NAA or IBA was reported to encourage number of roots, average root length as well as fresh and dry root weight per cutting (Hajian and Khosh Khui *et al.*, 2000). On the contrary in R. *canina*, highest rooting percentage with maximum root length was reported in cuttings treated with 25 ppm IBA for 20 min and the largest number of roots per cutting was obtained with 100 ppm IBA for 30 minutes (Hoşafçı *et al.*, 2005). Concentration of hormone required varies between genotypes and species. There is a need for scientific investigations to figure out the essentiality of hormone requirement and associated factors to be considered besides the genotypes.

Single node cuttings - There are few cut flower rose varieties, that are multiplied by cuttings with single nodes. The selected floral stem is usually divided into 3-5 small cuttings of 6-8 cm each with a five-leaflet leaf at the extremity. These cuttings are rooted in high humidity growth chambers after pretreatment with rooting hormones.

Layering - Layering is particularly used when a single plant of rare species or a rare plant, have to be multiplied into number of plants. It is practiced mainly in case of climber and rambler varieties where the stem can be easily bent.

Budding, grafting and stenting - Propagation by these techniques has the advantage of adaptability across wide range of soil condition and reduced vegetative phase. All these methods involve root stock and scion combinations. All most all the rose varieties can be multiplied on suitable root stock and most of the rose varieties perform better when budded or grafted on roots stock compared to the performance on their own root. Fresh weight and diameter of flowering stem, fresh and dry weight of flower, flower diameter and length, petal number, leaf chlorophyll content and quality index were higher in grafted plants compared to those propagated by cuttings (Farzad Nazari *et al.*, 2009).

Root stock - Rootstock can be prepared in polybags or in beds. Various species and varieties are being used as rootstocks across the globe. Use of rootstocks varies for different soil and environmental conditions. *Rosa banksiae* Ait., R. *canina* L., R. *chinensis* Jacq., R. *multiflora* Thunb, R. *indica*, and R. *manetti* are some of the most widely used species as rootstocks. 'Indica major'; 'Natual Brier'; 'Inermis'. 'Dr. Huey', 'Masquerade', are some of the popular varieties of rootstock used worldwide. In northern India, R. *indica* is widely used while in southern India it is R. *multiflora* and R. *canina*. 'Nishkant' bred by ICAR-Indian Institute of Horticultural Research, Bengaluru is a rootstock variety without prickles making it convenient for budding. This variety having resistance for both black spot and powdery mildew and with high bud uptake percentage makes it a better choice particularly when there is a need to save the rare varieties.

Number of cut flower, stem and flower diameter, leaf fresh and dry weights, flower stem fresh and dry weights and leaf chlorophyll index were affected by the type of rootstock used. R. *canina* was reported to be the best rootstock for producing the highest number of flowers (Khosh-Khui and Zargarian 2010). These rootstock-imposed alterations varied between the varieties and in response to the rootstocks used.

Like breeding of rose varieties, root stock breeding is also done to develop ideal root stocks. Apart from conventional breeding program, non conventional approach of genetic transformation is also being attempted for development of the ideal rootstock. For developing rootstock with improvement in the root characteristics, the role A, B and C genes from *Agrobacterium rhizogenes* were used for transformation. Transformant expressing rol C resulted in good growth. Grafting experiments on this transformed rootstock resulted in altered plant architecture with more basal shoots producing more flowers (Van Der Salm *et al.*, 1998).

Scion - The scion variety is that whose flowers are desired to be produced. The right stage of scion should have the flower just opened with full-grown leaves. Axillary buds are used in either grafting or budding. In case of budding, individual bud is scooped out of the stem whereas in grafting and stenting, stem with the node and bud is used. There is a need of research to understand the axillary bud quality and its interaction with resulting plant quality. Scientific understanding of each and every axillary bud on flowering stem and its regeneration ability will facilitate propagation and pruning techniques to be followed in rose.

Budding – It is by far the most widely used method of producing rose plants. Budding is a special method of grafting in which a single vegetative bud taken from scion is budded to root stock. Budding is done either in polybag or in bed. Common budding methods are T budding, chip budding and patch budding. All the three methods with minor variation are being followed depending upon the expertise of budders. T budding is the most common budding method used in rose. The name comes after the shape of the cut made on root stock to insert the bud. 'T' budding can be done only when the root stock is in active growth and when bark can be easily separated from the wood. Best bud can be selected from the centre portion of the flowering shoot. Age of the bud was thought to be the influencing factor in the bud growth. However a systematic study demonstrated that the age of the bud was not a major factor in determining the rate of bud growth (Bris et al.,1998). In case of 'T' budding, any cambium wood portion attached to it has to be removed exposing the bud inside the bark. On the contrary, in chip budding, the bud is sliced out with very thin layer of bark so that no effort is further made to remove the cambium layer inside the bark. The method is also called as shield budding. Chip or shield budding results in stronger bushes with a considerably greater fresh mass and a greater number of shoots than the "T" budding. Number of flowers and the length of shoots were also better with chip budding (Pudelska, 2001).

To perform the budding, rootstocks should be planted in beds with a distance of 2ft. These would have stronger rooting system and can wait in bed for considerably longer time for uprooting and transfer to the place of planting. In case of bed planted root stocks, they are maintained in such a way as to have a single main shoot removing all the side branches. Normally a single bud is budded into a rootstock. Two to three buds can also be budded into the same rootstock, giving a better chance for the bud and to the rootstock so as to ensure any one of the bud to sprout. Multiple budding is done at various heights of the same shoot or on different shoots of the same plant. In such case more than one shoot is left on the rootstock to facilitate budding of more than one bud.

Enhancement in area and spread of rose cultivation necessitated long distance transportation of budded plants from specialised rose nurseries and that resulted in polybag method of propagation. In case of polybag method, rootstocks are raised in small covers of 3"x4" size and budding of desired varieties is taken up on these.

Grafting - Several different methods are used to join the scion variety to the rootstock. Different types of grafting are practiced in rose. Most common methods are - Cleft grafting, Whip and tongue grafting. The most common form of grafting is cleft grafting. This is useful for joining a thin scion to a thicker branch or stock. The root stock should be split to form a cleft and the end of the scion is cut to a long shallow wedge. Union of scion cleft into wedge of root stock result into new plant. Whip-and-tongue grafting is another form that requires more skill to make the cuts so that the scion and the stock fix up neatly.

Stenting - Van de Pol and Van der Vliet (1979) introduced a modified technique wherein cutting and grafting is performed in one action and called it as "stenting". The procedure is similar to grafting except the size of stock and scion, and the unrooted stock. In stenting, the graft union must be formed before root initiation. After leaves formation on the scion there must be a free transport flow of carbohydrates and natural hormones from the leaf to the base of the rootstock for new root initiation. When a suitable rootstock is used the root formation after stenting can be better than those produced by conventional rooting. This technique is successful in humid chambers. Root stocks are cut into sections of 3-4 inches and the stems of scion to be used as a scion are cut into sections having 2 or 3 leaves on each section. Wedge grafting is done with the sections and is kept for rooting. If kept sufficiently moist and warm, this grafted cutting will root within two or three weeks. This method was further improved by grafting a scion on rootstock with just one internode without bud (Van de Pol and Breukelaar, 1982). In this method, an internode length of one inch is sufficient to be used as rootstock for stenting. This improvement lowered the problem of wild suckering. Application of rooting hormones to the graft site, resulted in more success of graft union (Cummins and James 1997). A minor modification of stenting was also attempted where in instead of grafting, budding was done (Chien-Young Chu, 1990).

In most of the budding and grafting procedure, the grafted bud or scion is over powered by the activity of the rootstock resulting in reduction of success percentage. Stenting procedure address this problem as the formation of the graft union and of adventitious roots occur simultaneously. Advances in stenting techniques needs further improvement in mechanisation of the procedure making the industry more economical and efficient. Success of a graft union depends on the establishment of a callus bridge between the cut surfaces of scion and stock, and the subsequent establishment of a functioning vascular cylinder connecting scion and stock. Histo chemical investigation of the stem above and below the graft union indicated starch content of the scion and stock varying throughout the establishment period of stenting, indicating the photosynthetic activity responsible for rooting and establishment (Van de Pol *et al.*,1998). Scientific research of microscopic and histo chemical studies will help to understand the cellular level reasoning and for further advancements.

Seed Propagation

In general rose is multiplied by vegetative propagation and rose seeds are not used for commercial propagation. Botanically, rose fruits are known as hips and seeds are known as achenes. For easy understanding and for convenience, terminology of fruits and seeds is retained in most of the research works reported. Seed germination is essentially required in rose hybridization program to develop new varieties and rootstocks. Most of the commercial rose varieties do not set seed. Seed setting ability varies among genotypes and attempts to enhance seed set starts from selection of parents to method of crossing (Tejaswini and Dhananjay, 2006). Rose seeds do not germinate easily due to inherent seed dormancy problem. Reasons responsible for seed dormancy varies from anatomical features to biochemical besides hormonal constituents. In addition to hard seed coat (Bekendam, 1973, Gudin *et. al.*,1990, Jin Bo *et. al.*,1993 and 1995, He *et. al.*,2001), anatomical features of achene (He *et. al.*,2001), seed constituents (Zeng *et al.*,2000) and localisation of ABA in the pericarp and testa (Jin-Bo *et al*,1995, He *et al*,2001) are known to be responsible for dormancy in rose.

Attempts to enhance seed germination percentage needs to address all the inherent problems associated with dormancy. Starting with simple methods of manipulation in stage of seed harvest (Lamont, 1985, Dadlani *et al.*, 1989, Jin Bo *et al.*, 1993 and 1995, He et *al.*, 2001) several methods have been tried to eliminate the problem of seed dormancy and to enhance seed germination. Being kept for two years after its collection, seeds of *R. canina* show a high level of germination without any additional process (Alp *et al.*, 2010). In an attempt to decrease the duration for germination and to enhance the percentage of germination various treatments of light (Yambe *et al.*,1995, Younis et al., 2007), stratification (Voyiatzi *et al.*, 1999, Benetka, 1998), scarification (Jin Bo *et al.*,1993, Bhanuprakash *et al.*, 2004, Younis et al., 2007), pretreatment with enzymes (Yambe and Takeno, 1992) and harmones (Bhanuprakash *et al.*, 2004) were tried. Role of varying combinations of warm plus cold stratification was found to be effective in breaking seed dormancy (Zhou *et al.*, 2008, Haouala *et al.* *2013*, Nadeem *et al.2013*, Zhou and Bao 2011). Research gaps in terms of germination percentage and period for germination needs to be addressed for long term benefit of the rose industry.

Biotechnology and Rose Propagation

Micropropagation - It is widely accepted propagation method for mass multiplication of disease free plants in short time. Virus-free shoot culture lines can be obtained by thermotherapy (Previati *et al.*, 2008) and shoot tip culture (Golino, 2007). On an annual basis, around four lakh plants can be multiplied from a single rose plant by micropropagation (Martin, 1985). *In vitro* plant material can be tested by molecular methods like ds-RNA and RT-PCR for viruses and viroids to ensure pathogen-free and genetically uniform rose micro-plants (Minas 2007). *Micropropagation* of roses are one of the most exciting supporting procedures of producing new varieties by rapid multiplication and speeding up breeding programs. This also plays a significant role in preserving high-quality shoot cultures.

Shoot regeneration and multiple shoots can be obtained from nodal explants cultured on MS basal medium (Murashige and Skoog,1962) supplemented with 3% (w/v) sucrose, containing various concentrations of N6-benzyladenine, kinetin separately or in combination depending upon the cultivar and species (Senapati and Rout, 2008 and Nak Udom *et al.*, 2009). Though MS medium is the most commonly used and best suited media for regeneration (Nizamani *et al.*, 2016, Vinuth 2017), there is report of Van Der Salm medium giving good shoot induction (Pahnekolayi *et al.*, 2015). Axillary buds or nodal meristerms are the preferred explants used for shoot regeneration (Pahnekolayi *et al.*, 2015, Nizamani *et al.*, 2016). However, leaf segments can also be used for shoot regeneration **(Moallem *et al.*, 2012).** Shoot elongation can be achieved with BAP and GA_3 of various concentrations and combinations (Hamama *et al.* 2015). Inclusion of **GA_3,** IAA, NAA ,TDZ, and Phloroglucinol were also reported in various species and varieties of rose to enhance frequency of shoot proliferation (**Zohreh Jabbarzadeh and Morteza Khosh-Khui , 2005,** Ozel and Arslan, 2006, Khosh-Khui *et al.*,2010, **Attio *et al.* 2012, Moallem *et al.*, 2012,** Salekjalali, 2012, Alsemaan, 2013). Requirement of growth hormones depends on endogenous concentration that varies across species and varieties. Miniature roses have low endogenous concentrations of auxin and cytokinins and demand high concentration of auxin for callus induction and of BAP for shoot regeneration (Zakizadeh *et al.*, 2010). The frequency of shoot multiplication increased up to the 6th-7th subculture, and then declined thereafter (Senapati, and Rout, 2008).

Regenerated shoots readily rooted on 1/4 MS medium devoid of growth regulators (Nak Udom *et al.*, 2009). Rooting could also be obtained upon transferring the microshoots into half-strength MS medium (Ruchika Bharadwaj *et al.* 2006, Senapati, and Rout, 2008, Salekjalali, 2012, Alsemaan, 2013 Kumari *et al.*, 2013, Pahnekolayi *et al.*, 2015). Various concentrations and combinations of NAA or IAA, IBA, 2,4-dichlorophenoxyacetic acid (2,4-D) were reported to impact on percentage of rooting (**Zohreh Jabbarzadeh and Morteza Khosh-Khui, 2005,** Ruchika Bharadwaj *et al.*, 2006, **Attio *et al.* 2012, Moallem *et al.*, 2012 and** Nizamani *et al.*, 2016) and needs standardization with varieties and species to be used (Vinutha, 2017). The regenerated plantlets were best acclimatized in peat in combination with equal proportions of either soilrite (Ruchika Bharadwaj *et al.*, 2006), or sand **(Zohreh Jabbarzadeh and Morteza Khosh-Khui, 2005),** or perlite (Khosh-Khui *et al.*, 2010).

Synthetic seeds - Regeneration of plants through the techniques of plant tissue culture and their subsequent acclimatization and delivery to the field poses many problems. Synthetic seeds concept has emerged to address these problems. Synthetic seeds are basically defined as encapsulated somatic embryos which functionally mimic seeds and can develop into seedlings under sterile conditions. In a broader sense, it would also refer to encapsulated buds or any other form of meristems which can develop into plants. The direct delivery of encapsulated material will save many subcultures to obtain plants and also eliminate the difficult stage of acclimatization of *in vitro* plants. In rose, synthetic seeds were produced by hardening 3% Na-alginate beads, containing apical buds, in 100 mM $CaCl_2.2H_2O$ solution for 30 minutes. All the synthetic seeds were then able to germinate in 10-11 days, independently from the presence of sucrose in the artificial endosperm of the bead (Previati et. al.,2008). Research to standardise the production of synthetic seed will also go a long way in adopting this strategy for conservation of valuable genetic material.

Seed / Planting Materials Standards

There are no specific standards fixed for planting material in rose. In case of rose, wide range of planting material are available such as budded, grafted, bare-root, own-root, and containerized plants of various growth stage as well as in various size of polybags and pots. Normally for ease of transportation as well as for large scale cultivation for commercial production of flowers, budded plants in small polybags of one month old are used. However, plants in bigger polybags of 6-10 months old are stronger and percentage of establishment is higher. For establishment of gardens, bare root plants are preferred that are stronger and can establish well. Care should be taken to ensure the production of planting material free from pests, pathogens, viruses and root knot nematode (*Meloidogyne hapla*). *Establishment of region specific rootstocks and production specific planting material standards will be of significant contribution for the rose industry.*

Future Needs of Rose Propagation

Techniques of propagation and ideal root stock development are two lines of research required for advancing the production of quality planting material. Root stock breeding is an important area to be considered to develop roots stock that can impart vigour and is resistant to pest and disease. With the area expanding under cultivation of roses, it is essential to find out suitable rootstocks that are well adaptable to marginal land as well as problematic soil conditions of saline and alkaline. Environmental issues such as soil and water quality along with chemical residues pose great challenges in the future which can be addressed by research in the development of ideal rootstock. The need of the hour is root stocks that can efficiently absorb and assimilate toxic metals to impart vigorous growth of scion are.

There is an emerging market of pot roses. Potted plants are expected to have different growth habit, with dwarf stature and early flowering with multiple flower stalks. Genetic potential of a variety can be expressed by suitable propagation technique to alter structure of plant. In contrast to small pot roses, tree roses are another extreme form in demand. Right propagation technique of pot as well as tree rose plant production needs to be evolved. In order to fulfill the constant demands of novelty in flower, it is but imperative to make advance in seed propagation technique. There is a need of scientific understanding and applicable research in the area of shortening stratification period prerequisite to seed germination and to enhance the seed germination percentage. Advance in synthetic seed production as well as micro propagation techniques would also provide opportunities to enter into unexplored areas of International trading of plants.

Micropropagated Rose Plants

References

Alp S, Ipek A and N Arslan (2010) The effect of gibberellic acid on germination of rosehip seeds (*Rosa canina* L.). *Acta Hort.* 885:33-37.

Alsemaan T (2013) Micro-propagation of Damask rose (*Rosa damascena* Mill.) cv.Almarah, *Internation journal of agricultural research* 8(4): 172-177.

Amjad F, Lee S, Muhammad N, Asif N, Gulzar A and JB Shahid (2016) Cross compatibility in various scented rosa species breeding. *Pak. J. Sci* 53(4): 863-869.

Anderson N and DH Byrne (2007) Methods for *Rosa* germination. *Acta Hort* 503-507.

Anuradha M and GR Rout (2005) Study of embryo rescue in floribunda rose. *Plant cell tiss orgen cul* 81: 113-117.

Attio AO, Dessoky ELS and AE EL-tarras (2012) *In vitro* propagation of *Rosa hybrida* (L.) cv. Al-Taif Rose plant. *African. J. Biotechnol* 11(48): 10888-10893.

Bekendam J (1973) Germination capacity of *Rosa canina* and *Rosa inermis*. *Bedrijfsontwikkeling*, 4(12): 1143-1151.

Benetka V (1998) Effect of warm stratification on seed viability of the rootstock rose 'Pávův červený (Pollmeriana). *Acta Pruhoniciana* 66:37-41.

Bhanuprakash K, Tejaswini, Yogeesha HS and L Naik (2004) Effect of scarification and gibberellic acid on breaking dormancy of rose seeds. *Seed Res* 32 (1):105-107.

Bris M LE, Champeroux A, Bearez P and M T LE Page-degivry (1998) Basipetal Gradient of Axillary Bud Inhibition along a Rose (Rosa hybrida L.) Stem: Growth Potential of Primary Buds and their Two Most Basal Secondary Buds as Affected by Position and Age. Annals *of Botany* 81: 301-309.

Canli FA and S Kazaz (2009) Biotechnology of rose: progress and future prospects. suleyman Demirel Universitesi Orman Fakultesi Dergisi.163-183.

Chien-Young Chu (1990) Budded cuttings for propagating roses. *Scientia Horticulturae* 43 (1-2): 163-168.

Costa JM and H Challa (2002)The effect of the original leaf area on growth of softwood cuttings and planting material of rose. Scientia Hort. 95(1-2): 111-121.

Costa JM, Heuvelink E, Van de Pol PA and HMC Put (2007) Anatomy and morphology of rooting in leafy rose stem cuttings and starch dynamics following severance. *Acta Hort.* 751:495-502.

Cummins and NY James (1997) State Agricultural Experiment Station, Geneva, NY 14456. Pomona, Spring 2:34

Dadlani NK, Venkataramana KT, Mathew RR and Brijendra (1989) Seed germination in roses. *Seed Res.* 17(2): 193-196.

Das P (2010) Mass cloning of Rose and Mussaenda, popular garden plants, via somatic embryogenesis. *Horticultural Science* 37(2): 70-78.

Farzad Nazari, Morteza, Khosh-Khui and Hassan Salehi (2009) Growth and flower quality of four *Rosa hybrida* L. cultivars in response to propagation by stenting or cutting in soilless culture. *Scientia Hort.* 119 (3):302-305.

Golino DA, Sim ST, Lee JA and A Rowhani (2007) Elimination of rose mosaic viruses using microshoot tip tissue culture. *Acta Hort.* 751:237-239.

Gudin S, Arene L, Chavagnat A and C Bulard (1990) Influence of endocarp thickness on rose achene germination: genetic an environmental factors. *Hort. Sci.*, 25:786-788.

Hajian S and M Khosh-khui (2000) Sexual and asexual treatments in Damask rose (Rosa damascena Mill.) *Iran Agricultural Research*, 19(1): 1-16.

Hamama L, Cesbron D, Voisine L, Lecerf M, Jareno C, Dorion N, Foucher F, Sakr S and HS Oyant (2015) Effects of Carbohydrate Sources and BAP Concentrations on *In Vitro* Morphogenesis of Four Rose Genotypes. *Acta Hort.* 3: 1083

Hans R (1983) Gislerod Physical Conditions of Propagation Media and their Influence on the Rooting of Cuttings. *Plant and Soil* 75 (1): 1-14.

Haouala Nissaf H, Zohra B and A Cheikh (2013) Enhancing seed germination in Rose (*Rosa rubiginosa* L.). *Med. Aro. Pl.* 2:613-142.

He H, Ueda Y, Kurosawa T, Ogawa S and E Nishino (2001) Morphological character and germination in achenes of *rosa persica* michx. *Acta Hort.* **547**: 129-140.

Hosafci H, Arslan N and EO Sarihan (2005) Propagation of dog roses (*Rosa canina* L.) by softwood cuttings. *Acta Hort.* **690**:139-142.

Jin BO, Dong HR and XH Yang (1993) Studies on the cause of dormancy of rose achenes. *Acta Hort.* 20(1): 86-90.

Jin BO, Dong HR and XH Yang (1995) Shortening hybridization cycle of rose-A study on mechanisms controlling achenes dormancy. In: *Acta horticulturae*, Zhu Dewei (eds.).International symposium on cultivar improvement of horticultural crops. ISHS publishers. **404**, pp.40-47.

Khosh-Khui M and M Zargarian (2010) Effects of four rootstocks on growth and development of three rose scion cultivars. *Acta Hort.* **870**:207-212.

Khosh-Khui M, Honarvar M and K Javidnia (2010) The first report on in vitro culture of musk rose. *Acta Hort.* **870**:213-218.

Kumari S, Singh KP, Janakiram T and DVS Raju (2013) Standardization of *in vitro* mass multiplication protocol for hybrid tea rose cv. Grand Gala. *Indian J. Hort.*, **70(4)**: 560-565.

Lamont GP (1985) Native rose - 2. Propagation Australian Plants. 13(105): 210-212.

Martin C (1985) Plant breeding *in vitro.* Endeavour 9:81–86.

Minas GJ (2007) a rapid protocol for in vitro micropropagation of three types of gardenia (*gardenia* spp. ellis) for quality produce flowering pot-plants. *Acta Hort.* **755**:81-86.

Moallem S, Behbahani M, Mousavi E and N Karimi (2012) Direct regeneration of rosa canina through tissue culture. *Trakia J. Sci.* **10(3)**: 23-25.

Murashige T and F Skoog (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiologia Plantarum* **15**:473-497.

Nadeem M, Atrif R, Adnan Y, Akond M, Amjad F and T Usman (2013) Improved technique for treating seed dormancy to enhance germination in Rosa x hybrid. *Turkish . J. Bot.* **37**(3): 521-529.

Nak-Udom N, Kanchanapoom K and K Kanchanapoom (2009) Micropropagation from cultured nodal explants of rose (*Rosa hybrida* L. cv. 'Perfume Delight'). *J. Science and Technology* **31**(6): 583-586.

Nizamani F, Nizamani GS, Nizamani MR, Ahmed S and N Ahmed (2016) Propagation of Rose (*Rosa hybrida* L.) Under Tissue Culture Technique. *Int. J. Bio. Res. 1*: 23-27.

Ozel CA and O Arslan (2006)Efficient micropropagation of English shrub rose "Heritage" under *in vitro* conditions. *International J. Agriculture and Biology* 8(5) 626-629.

Pahnekolayi MD, Samiei L, Tehranifar A and M Shoor (2015) The effect of medium and plant growth regulators on micropropagation of Dog rose (*Rosa canina* L.). *J. Plant Mol. Br* **3**: 61-71.

Previati A. Benelli C., Re F, da Ozudogru A and M Lambardi (2008). Micropropagation and *in vitro* conservation of virus-free rose germplasm. *Propagation of Ornamental Plants*, **8(2)**: 93-98.

Pudelska K (2001)The influence of budding techni- que on the quality of rose plants. *Acta Hort.* **547**:353-356.

Ruchika Bharadwaj, Singh SK, Surinder Paland and Surendra Kumar (2006) An improved protocol for micro-propagation of miniature rose (*Rosa chinensis* Jacq. var. *minima*) cultivars. *J. Orna.l Hort.* **9**(4): 238-242.

Salekjalali M (2012) Phloroglucinol, BAP and NAA Enhance Axillary Shoot Proliferation and other Growth Indicators In vitro Culture of Damask Rose (Rosa damascena Mill.), American-Eurasian. *J. Agric. Environ. Sci.* **12(7)**: 960-966.

Senapati SK and GR Rout (2008) Study of culture conditions for improved micropropagation of hybrid rose. *Hort Sci.* **35**:22-26.

Tammy Estabrooks, Robin Browne and Zhongmin Dong (2007) 2,4,5-Trichlorophenoxyacetic Acid Promotes Somatic Embryogenesis in the Rose Cultivar 'Livin' Easy' (*Rosa* sp.) *Plant Cell Reports* **26**(2):153-160.

Tejaswini and MS Prakash (2005) Utilization of wild rose species in India. *Acta Hort.* **690**:91-96.

Van de pol PA and A Breukelaar (1982) Stenting of roses; a method for quick propagation by simultaneously cutting and grafting. *Scientia Hort.* **17**: 187-196.

Van de Pol PA, and G Van der vliet (1979) Rozen stekken en enten in ~n handeling. *Vakbl. Bloemisterij* **26**: 40-41.

Van Der Salm T, Bouwer R, Van Dijk AJ, Keizer LCP, Hänisch Ten Cate CH and LHW Van Der Plas Jim Dons (1998) Stimulation of Scion Bud Release by *rol* Gene Transformed Rootstocks of *Rosa hybrida* L. *J. Experimental Botany* **49** (322) 847-852.

Vinutha MR (2017) Varietal response in rose for micropropagation. M.Sc thesis University of Horticultural sciences, Bagalkot.

Voyiatzi CI, Koutsika MS and E Vavdinoudi (1999) Pre-sowing treatments affect seed germination and plantlet vigour of some climbing rose varieties. *Plant Varieties & Seeds* **12**(1):65-72.

Yambe Y and K Takeno (1992) Improvement of rose achene germination by treating with macerating enzymes. *Hort. Sci.* **27** (9): 1018-1020.

Yambe Y, Takeno K and T Saito (1995) Light and phytochrome involvement in Rosa multiflora seed germination. *J American Society for Hort. Sci.* **120** (6) 953-955.

Younis A, Riaz A, Ahmed R and A Raza (2007) Effect of hot water, sulphuric acid and nitric acid on the germination of rose seeds. *Acta Hort.* **755**:105-107.

Zakizadeh H, Debener T, Sriskandarajah S, Frello S and M Serek M (2010) Somatic embryogenesis in miniature potted rose (*Rosa hybrida* L.). *Acta Hort.* **870**:227-232.

Zhou Z and Bao (2011) Levels of physiological dormancy and methods for improving seed germination of four rose species. *Scientia Hort.* **129**:818-824.

Zhou ZQ, Wu NB and PF Qiu (2008) Post-dispersal factors regulating dormancy and germination of *Rosa soulieana* seeds. *Belgian J. Botany* **141**(1):103-111.

Zohreh Jabbarzadeh, Morteza Khosh-Khui (2005) Factors affecting tissue culture of damask rose (*Rosa damascena* mill.). *Scientia Hort.* 105(4): 475-482