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Auxin concentration and sampling time affect rooting of *Chrysanthemum morifolium* L. and *Rosmarinus officinalis* L.

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ABSTRACT

Vegetative propagation is the most commonly used method for the multiplication of ornamental, medicinal and aromatic plants mainly due to the low seed germination percentage and rate as well as the prolonged time needed for the plant growth and development. *Chrysanthemum morifolium* and *Rosmarinus officinalis* are two major ornamental medicinal plants routinely used in landscape and with pharmaceutical and food industries. Owing to the constant needs for these two species, the mass production of the plants in a given short time is more demanding. For the study of the effects of PGRs; NAA and IBA (0,1000, 2000 and 3000 mgl^{-1}) and different sampling times (July, August and September) on cuttings rooting and the subsequent root growth a factorial experiment based on RCBD with three factors (auxin type, auxin concentration and sampling time) with three replications was conducted. The results revealed that the highest rooting percentage (with three sampling time) and survival rate for *Chrysanthemum morifolium* (in August and September) was attained with 3000 mgl^{-1} NAA. The greatest roots number in September and, root weight in August and September in *Chrysanthemum morifolium* again were belonged to 3000mgl^{-1} NAA. Auxin concentration had significant effect on root number, root fresh weight and survival rate of rosemary. For both IBA and NAA, 3000 mgl^{-1} had positive effects on root fresh weight and survival rate. In total, 3000 mgl^{-1} auxin and September were defined as the time of choice for rosemary cutting preparation and multiplication.

INTRODUCTION

Propagation via stem cutting is a predominant procedure for the mass clonal production of many plant species. Roots organogenesis from the cuttings is controlled by internal (nutrition and hormonal balance) and external (temperature, light and humidity) factors (Kollarova 2005). RGRs such as NAA and IBA commercially have been tried for the promoting of cuttings rooting potential and for to increase rooting percentage. Adventitious roots emergence at the bottom of cuttings is an important developmental phenomenon in the growth and survival of the young plants. The main stages in the adventitious roots formation in plants are induction, initiation and the roots growth. The timings for the mentioned stages are quite different with diverse plant taxonomy ranging from some hours (in mung beans) up to several weeks in Camelia (Kollarova 2005). Adventitious root formation is stimulated by auxins and the response to auxin and its role in controlling the roots formation and their length and number are really important (Yan et al. 2014). Auxin role in root induction is much more highlighted than its initiation (Yan et al. 2014). In general, roots formation in plants is happening by two phases; the first phase is sensitive to auxin content but, the second phase is auxin insensitive (Yan et al. 2014). Roots primordia formation in cuttings is dependent upon internal auxin content and some synergistic components such as diphenyls. These compounds stimulate the related RNAs biosynthesis and hence improve the roots

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primordia initiation (Henrique et al. 2006). Besides hormones and RGRs, sucrose is another main criterion affects rooting phenomenon.

Along with being energy source, sucrose is a structural monomer for the biosynthesis of many other skeletal compounds. Sugars promote the growth and development in plants. It seems that oligosaccharides deposited in the cell wall have positive effect on root induction and growth. Galactoglucomannan oligosaccharides (GGMOs), commonly available in the primary and secondary cell walls and the related compounds hold preventative effect on rooting even with suitable availability of 2.4.D and IAA (Kollarova 2005). Galactoglucomannan oligosaccarides stimulate the rooting in the absence of auxins, but, its effect on rooting is much lower than auxin in Mung bean (Kollarova 2005). With auxins, GGMOs prevent the rooting. The preventative potential is dependent upon GGMOs concentration and auxin type. An experiment by Abu-Zahra et al. (2013) revealed that 3000 mgl^{-1}NAA had positive effect on rosemary rooting.

Rosemary (*Rosmarinus officinalis*; Fam:Lamiaceae) is a perennial woody evergreen hard rooting plant (Abu-zahir et al. 2013). Seed propagation is quite time consuming. Apart from being used in landscape, rosemary essential oil has widespread applications in food and pharmaceutical industries.

Chrysanthemum morifolium L. from Asteraceae family is a pot and landscape plant (Oladipupo et al. 2014). In *Chrysanthemum morifolium* propagation the traits of interest are the flower color, shape and size (Waseem et al. 2009). Limited gene pools and the great difference in ploidy levels impose large limitations in homogenous growth and flowering and propagation of *Chrysanthemum morifolium* plants. The major propagation methods for those plants are seeding, cutting and *in vitro* culture (Waseem et al. 2009).

Owing to the multidisciplinary application of this species, mass economical propagation of these species has been a focus of research and extension section for a long time. The present experiment was conducted to evaluate the effects of some auxin and sampling time on rooting potential of these two species.

MATERIALS AND METHODS

This experiment was conducted at the Research Greenhouse of Azerbaijan Shahid Madani University during 2014-2015. Experiment was factorial based on RCBD with three factors of auxin type (NAA[2], IBA[3]), concentration and sampling time with three replications. Auxins concentrations were 0,1000, 2000 and 3000 mgl^{-1}. Sampling times were July, August and September.

Mother plants were acquired from a commercial greenhouse and were incubated in greenhouse under 25:18 degrees centigrade temperature regime under 16:8 hrs photoperiod. Cutting were taken from the shoots distal ends (10-12 cm length, having 2-4 leaves for *Chrysanthemum morifolium* and 10-14 leaves for rosemary). The growing condition for cuttings was the same as mother plants. The cuttings were pretreated with a fungicide (Benomyl) (0.5%) for 30 seconds; later, cuttings basal ends were dipped in PGRs solution for 30 seconds. Medium–sized perlite was the growing bed employed. Sampling was around in one month intervals during July, August and September. 40 days after the treatment, rooted cuttings were assayed for the rooting percentage, roots number, roots fresh weight, longest root and survival rate.

To assay the survival rate, the rooted cuttings were transferred to the pots containing 1:1:1 sand, soil and green manner. Survival rate was traced 40 days later. The data were analyzed by SPSS and MSTATC.

RESULTS AND DISCUSSION

Results was showing that in *Chrysanthemum morifolium* the interaction effects of auxin type, concentration and sampling time were significant on all the traits measured except root length (Table1). The highest rooting percentage and survival rate was achieved at 3000 mgl^{-1} of PGRs (Table 1). There was no significant difference between sampling times and 3000 mgl^{-1} PGRs. (Table 1). The highest root number and roots fresh weight was obtained during September at 3000 mgl^{-1} PGRs. Auxin type and sampling date (August and September) had no significant effects on rooting percentage (Table 2). The greatest root number was obtained with IBA at sampling September (Table 2). The highest data for roots fresh weight was belonged to NAA during August and September considering survival rate (Table 2). The plants from both auxin treatments hold the highest rate during September sampling time (Table 3). August and September were defined the best times for obtaining the greatest rooting percentage and roots fresh weight. Sampling time had no meaningful effect on roots length (Table 3). For roots number and survival rate, September was the optimum sampling time (Table 3).

Immediate roots formation and growth are the main factors influencing survival of cuttings. Time course needed for roots induction and initiation as well as later development of roots greatly affect the emerged roots quality and quantity. PGRs greatly

2 - Naphthaleneacetic acid (NAA)
3 -Indole-3-butyric acid (IBA)

Table1. Combined effects of auxin concentration and sampling time on rooting traits of *Chrysanthemum morifolium*.

Auxin level (mgl^{-1})	Sampling time	Rooting (%)	Root number	Root length (cm)	Root fresh weight (g)	Survival rate (%)
0	July	0.05^{h}	0.40^{f}	0.416^{d}	0.02^{f}	0.017^{g}
0	August	0.13^{g}	0.35^{f}	0.31^{d}	0.400^{f}	0.141^{f}
0	September	0.00^{h}	0.00^{f}	0.00^{d}	0.00^{f}	0.00^{g}
1000	July	0.67^{ef}	1.86^{de}	1.60^{c}	2.86^{e}	0.368^{e}
1000	August	0.70^{de}	1.44^{e}	2.03^{abc}	3.95^{d}	0.52^{d}
1000	September	0.81^{c}	1.98^{cd}	2.85^{a}	4.45^{c}	0.57^{d}
2000	July	0.62^{f}	2.38^{c}	1.89^{bc}	3.75^{d}	0.55^{d}
2000	August	0.76^{cd}	2.06^{cd}	2.43^{ab}	4.75^{c}	0.55^{d}
2000	September	0.92^{b}	2.36^{c}	2.55^{ab}	4.51^{c}	0.81^{c}
3000	July	0.93^{ab}	3.46^{b}	2.88^{a}	5.71^{b}	0.89^{b}
3000	August	0.99^{a}	3.10^{b}	2.86^{a}	5.75^{b}	0.95^{ab}
3000	September	1.00^{a}	7.78^{a}	2.66^{ab}	6.38^{a}	1.00^{a}
LSD 1%		0.069	0.444	0.769	0.430	0.069

Similar letters in the columns are non-significant based on LSD test.

Table 2. Combined effect of auxin type and sampling date on rooting traits of *Chrysanthemum morifolium.*

Auxin type	Sampling date	Rooting percentage	Root number	Root length (cm)	Root fresh weight (g)	Survival rate (%)
IBA	July	0.55^{b}	1.67^{d}	1.48^{c}	2.54^{d}	0.384^{d}
IBA	August	0.65^{a}	1.36^{d}	1.75^{bc}	2.65^{c}	0.51^{c}
IBA	September	0.66^{a}	3.05^{a}	1.67^{bc}	2.68^{c}	0.57^{ab}
NAA	July	0.57^{b}	2.38^{bc}	1.90^{abc}	4.12^{b}	0.53^{bc}
NAA	August	0.64^{a}	2.11^{c}	2.10^{ab}	4.79^{a}	0.56^{b}
NAA	September	0.70^{a}	2.65^{b}	2.45^{a}	4.99^{a}	0.62^{a}
LSD 1%		0.049	0.314	0.544	0.304	0.049

Similar letters in the columns are non-significant based on LSD test.

influence the rooting potential and roots quality of cuttings.

Roots initiation in plants is greatly dependent on internal hormones and diphenyl compound availability. These compounds induce the RNA biosynthesis and hence promote the roots primordial initiation (Ullah et al. 2013).

Seemingly, the high rates of root formation with *Chrysanthemum morifolium* and subsequent survival rate is due to high content of internal hormones during the sampling times. Wiegel et al. (2006) reported that the *Chrysanthemum morifolium* cutting contained the high amounts of both free and ester–bound IAA during the summer sampling and hence increased rooting potential. There was positive correlation between IAA content of mother plants at the time of sampling and the initiated roots from cutting about 20 days after sampling time.

Table 4 depicts that with both auxins, the highest rooting percentage and roots number was belonged to 3000 mgl^{-1} auxin. However, the highest root fresh weight and survival rate just belonged to 3000 mgl^{-1} of NAA.

Cuquel et al. (1992) reported that rooting in *Chrysanthemum morifolium* plants occurs even in the absence of IBA. However, any increase in the hormone concentration and time exposure led to increased rooting potential of plants. Considering survival rate, root fresh weight and root length and number, NAA was superior to IBA (Table 5). 3000 mgl^{-1} IBA was the concentration of choice for all the before mentioned traits (Table 4).

Samanda et al. (2015) reported that in chrysanthemum, the application of Ethrel (2-chloroethyl-phosphoric acid) and IBA positively influenced the rooting behavior, so that, IBA increased the roots number initiated along with ethrel which increased root length. Moreover, they reported that, root initiation and later development went to reduce the carbohydrates pool accumulated at the cutting basal end (Samanda et al. 2015)

Table 3. Mean comparison for the effect of different sampling time on rooting traits of *Chrysanthemum morifolium*.

Sampling time	Rooting percentage	Root fresh weight (g)	Root number	Survival rate (%)
July	0.55^{b}	3.13^{b}	2.52^{b}	0.45^{c}
August	0.65^{a}	3.72^{a}	1.74^{c}	0.54^{b}
September	0.66^{a}	3.83^{a}	2.85^{a}	0.59^{a}
LSD 1%	0.58^{b}	0.215	0.221	0.034

Similar letters in the columns are non-significant based on LSD test.

Table 4. Combined effects of auxin type and concentration on rooting traits of *Chrysanthemum morifolium*.

Auxin type	Auxin concentration (mgl^{-1})	Rooting (%)	Root number	Root length (cm)	Root fresh weight (g)	Survival rate (%)
IBA	0	0.11^{d}	0.46^{e}	0.389^{d}	0.322^{f}	0.10^{f}
IBA	1000	0.68^{c}	1.37^{d}	1.62^{c}	2.56^{e}	0.43^{e}
IBA	2000	0.71^{c}	1.60^{d}	2.05^{bc}	2.87^{e}	0.52^{d}
IBA	3000	0.98^{a}	4.70^{a}	2.45^{b}	4.20^{d}	0.91^{b}
NAA	0	0.011^{e}	0.00^{f}	0.10^{d}	0.07^{f}	0.00^{g}
NAA	1000	0.78^{b}	2.15^{c}	2.70^{ab}	4.96^{c}	0.54^{d}
NAA	2000	0.81^{b}	2.94^{b}	2.53^{ab}	5.80^{b}	0.76^{c}
NAA	3000	0.96^{a}	4.50^{a}	3.15^{a}	7.70^{a}	0.98^{a}
LSD 1%		0.056	0.362	0.628	0.072	0.056

Similar letters in the columns are non-significant based on LSD test.

Table 5. Mean comparison for the effects of two auxins tested on the rooting traits of *Chrysanthemum morifolium*.

Auxin type	Root number	Root length (cm)	Root fresh weight (g)	Survival rate (%)
IBA	2.53^{b}	1.63^{b}	2.49^{b}	0.49^{b}
NAA	2.39^{a}	2.12^{a}	4.64^{a}	0.57^{a}
LSD 1%	0.05	0.08	0.05	0.01

Similar letters in the columns are non-significant based on LSD test.

Rosemary

Auxin concentration had significant effects on rooting percentage of rosemary (Table 6). NAA increased the roots number and length (Table 6).

Table 6. Mean comparison for the effects of auxin type on rooting traits of rosemary.

Auxin type	Root number	Root length (cm)
IBA	1.420^{b}	1.810^{b}
NAA	2.580^{a}	2.16^{a}
LSD 1%	0.07	0.06

Similar letters in the columns are non-significant based on LSD test.

Mean comparisons revealed that there was no meaningful differences in rooting percentage considering auxins levels (Table 7). Auxin concentration had statistical effects on roots number, roots fresh weight and rooted cutting survival rate. The highest survival rate was obtained by 3000 mgl^{-1}auxins (Table 7). Table 8 revealed that, 3000 mgl^{-1} of both auxins had positive effects on root fresh weight and survival rate of potted cuttings. Mean comparisons for auxins levels and sampling times depicted that 3000 mgl^{-1} auxins had promotion effects on root fresh weight, roots number, survival rate as well as on root length at the September sampling time (Table 9). Elhaak et al. (2014) reported that with rosemary plants, optimal root number was achieved by soaking cuttings for three hours in 60 ppm IBA. Sampling time had no meaningful effect on rooting percentage in rosemary, however, sampling time effectively influenced the roots number, root length, roots fresh weigh and survival rate and the best selected time for the traits mentioned was

Table 7. Mean comparison for the effects of auxin concentration on rooting traits of rosemary.

Auxin concentration (mgl^{-1})	Root number	Root length (cm)	Root fresh weight (g)	Survival rate (%)
0	0.11^{d}	0.10^{d}	0.016^{d}	0.005^{d}
1000	1.86^{c}	2.05^{c}	4.31^{c}	0.48^{c}
2000	2.26^{b}	2.52^{b}	5.40^{b}	0.59^{b}
3000	3.40^{a}	3.23^{a}	6.85^{a}	0.80^{a}
LSD 1%	0.35	0.341	0.615	0.069

Similar letters in the columns are non-significant based on LSD test.

Table 8. Mean comparison for the effects of auxin type and concentration on rooting traits of rosemary

Auxin type	Auxin concentration (mgl^{-1})	Root number	Root length (cm)	Root fresh weight (g)	Survival rate (%)
IBA	0	0.52^{f}	0.22^{e}	0.03^{e}	0.011^{d}
IBA	1000	1.00^{e}	1.56^{d}	3.63^{d}	0.41^{c}
IBA	2000	1.66^{d}	2.24^{c}	5.42^{bc}	0.59^{b}
IBA	3000	2.36^{c}	3.17^{a}	7.49^{a}	0.80^{a}
NAA	0	0.00^{f}	0.00^{e}	0.00^{e}	0.00^{d}
NAA	1000	3.11^{c}	2.53^{bc}	4.98^{c}	0.59^{b}
NAA	2000	3.47^{b}	2.80^{ab}	5.38^{bc}	0.59^{b}
NAA	3000	4.14^{a}	3.29^{a}	6.21^{a}	0.81^{a}
LSD 1%		0.505	0.482	0.87	0.74

Similar letters in the columns are non-significant based on LSD test.

Table 9. Mean comparison for the effects of auxin concentration and sampling date on rooting traits of rosemary

Auxin level (mgl^{-1})	Sampling time	Root number	Root length (cm)	Root fresh weight (g)	Survival rate (%)
0	July	0.33^{g}	0.33^{e}	0.05^{e}	0.016^{f}
0	August	0.00^{g}	0.00^{e}	0.00^{e}	0.00^{f}
0	September	0.00^{g}	0.00^{e}	0.00^{e}	0.00^{f}
1000	July	0.99^{f}	1.26^{d}	2.70^{d}	0.24^{e}
1000	August	1.77^{e}	2.22^{bc}	4.31^{c}	0.53^{d}
1000	September	2.81^{bc}	2.66^{b}	5.91^{b}	0.68^{bc}
2000	July	2.13^{de}	1.97^{c}	4.58^{c}	0.52^{d}
2000	August	2.41^{cde}	2.73^{b}	5.71^{b}	0.56^{cd}
2000	September	3.31^{b}	2.86^{ab}	5.93^{b}	0.69^{b}
3000	July	2.51^{cd}	2.80^{b}	6.15b	0.72^{b}
3000	August	2.92bc	2.71^{b}	6.45^{b}	0.71^{b}
3000	September	4.80^{a}	4.13^{a}	7.95^{a}	0.98^{a}
LSD 1%		0.118	0.590	1.066	0.120

Similar letters in the columns are non-significant based on LSD test.

Table 10. Mean comparison for the effects of sampling time on rooting traits of rosemary

Sampling time	Root fresh weight (g)	Root number	Root length (cm)	Survival rate (%)
July	3.37^{c}	1.49^{b}	1.61^{c}	0.377^{c}
August	4.11^{b}	1.77^{c}	1.91^{b}	0.453^{b}
September	4.95^{a}	2.73^{a}	2.41^{a}	0.59^{a}
LSD 1%	0.532	0.309	0.295	0.06

Similar letters in the columns are non-significant based on LSD test.

Table 11. Mean comparison for the effects of auxin type and sampling date on rooting traits of rosemary

Auxin type	Sampling date	Root number	Root length (cm)	Root fresh weight (g)	Survival rate (%)
IBA	July	1.19^{d}	1.69^{c}	3.06^{cd}	0.379^{c}
IBA	August	1.26^{d}	1.65^{dc}	4.24^{bc}	0.45^{bc}
IBA	September	1.85^{c}	2.05^{bc}	4.53^{b}	0.53^{b}
NAA	July	1.79^{c}	1.52^{d}	3.08^{d}	0.37^{c}
NAA	August	2.33^{b}	2.16^{b}	3.99^{bc}	0.45^{bc}
NAA	September	3.61^{a}	2.77^{a}	5.37^{a}	0.64^{a}
LSD 1%		0.437	0.417	0.753	0.084

Similar letters in the columns are non-significant based on LSD test.

September (Table 10). NAA at the September sampling, imposed positive effects on survival rate, root weight, roots length and root number in rosemary (Table 11). The main possible reason could be the inevitable impact of NAA on cell division, enlargement, and further cell growth and development.

CONCLUSION

Owing to the great horticultural value of these two plants, the easy and on- time propagation of these plants is of crucial importance for the greenhouse producer and extension sections. Just standing on classical propagation methods is not satisfactory enough for the huge needs for the plants, so, the use of updated methods and some chemicals along with knowledge of the right phenological stages of the plants are inevitable to reach the suitable propagation rates in these species. The present experiment clearly revealed that the application of the right auxin type plus the correct sampling time provide us with a pool of rooted cuttings ready to use as propagation material. Overall, it seems that with some careful selection of the propagules and choosing the right PGRs, these two species have the potential of mass propagation to fulfill the demands of greenhouses and the related producers and industries.

REFERENCES

Abu-Zahra T. Al-Shadaideh R. Abubaker A N. Qrunfleh S.M. (2013) Influence of auxin concentration on different ornamental plant rooting. International Journal of Botany, 9: 96-99.

Adekola O.F. Akpan I.G. (2012) Effects of growth hormones on sprouting and rooting of *Jatropha Curcas* stem cuttings. Journal of Applied Sciences and Environmental Management, 16: 165 –168.

Cuquel F.L. Granja N.P. Minam K. (1992) Rooting evaluation in *Chrysanthemum morifolium* cv. white Reagan 606 stem cuttings as affected by indole butyric acid (IBA). Scientia Agricola, 49: 15-22.

Elhaak M. Matter M.Z. Zayed M.A. Gad D.A. (2015) Propagation Principles in Using Indole-

3-butyric acid for rooting Rosemary stem cuttings, 2:121. doi:10.4172/2376-0354.1000121.

Henrique A. NogueiraCampinhos E. OrikaOno E. Zambello de Pinho S. (2006) Effect of plant growth regulators in the rooting of Pinus cuttings. Brazilian Archives of Biology and Technology, 49:2. On-line version ISSN 1678-4324

Kollarova K. Henselova M. Liskova D. (2005) Effect of auxins and plant oligosaccharides on root formation and elongation growth of Mung bean hypocotyls. Plant Growth Regulation, 46: 1-9.

Oladipupo A. Lawal Isiaka A. Ogunwande Olusola F. Opoku A.R. (2014) The essential oils of *Chrysanthemum morifolium* Ramat. from Nigeria. American Journal of Essential Oils and Natural Products, 2: 63-66.

Samananda N.D. Ormrod P. Adedipe N.O. (2015) Rooting of chrysanthemum stem cuttings as affected by (2-chloroethyl) phosphonic acid and Indole butyric acid. Annals of Botany, 36: 961-965.

Ullah Z. Abbas S.J. Naeem N. Lutfullah G. Malik T. Ullah Khan M.A. Khan I. (2013) Effect of indolebutyric acid (IBA) and naphthaleneacetic acid (NAA) plant growth regulators on Marigold (*Tagetes erecta* L.). African journal of agricultural research, 8: 4015-4019.

Waseem M. Jilani S. Khan M.S. (2009) Rapid plant regeneration of chrysanthemum (*Chrysanthemum morifolium* l.) through shoot tip culture. African journal of biotechnology, 8:1871-1877.

Weigel U. Horn W. Hock B. (2006) Endogenous auxin levels in terminal stem cuttings of *Chrysanthemum morifolium*. Physiologia plantarum, 61:422 - 428.

Yan h. Li J. Zhang X. Yang W. Wan Y. Mei Ma Y. Huang L. (2014) Effect of Naphthalene acetic acid on adventitious root development and associated physiological changes in stem cutting of *Hemarthria compressa*. PLoS One. 9(3): e90700.Published online (2014) Mar 4. doi: 10.1371/journal.pone.0090700

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