

USING A MULTIFACTOR APPROACH FOR IMPROVING STAGE II RESPONSES OF *HELLEBORUS* HYBRIDS IN MICROPROPAGATION

Lindsay Caesar and Jeffrey Adelberg*

Department of Plant and Environmental Science, Clemson University, Clemson, SC, USA 29634,
*Fax: + 1-864-656-4960, *E-mail: jadlbrg@clemson.edu

Abstract

Plant quality and multiplication ratios of hellebores (*Helleborus* spp.) *in vitro* were observed with sucrose (1-3%), phosphate (1.25-6.25 mM), nitrate (39.8-69.8 mM), ammonium (5-35 mM), plant density, micronutrient dilution (from Woody Plant Medium), and thidiazuron (1-9 μ M) as treatment factors. Treatment combinations were chosen using D-optimal criteria and tested in liquid media for two hybrid hellebore genotypes (*Helleborus* \times *nigercors* and *Helleborus* \times *ballardiae* 'Raulston Remembered'). Analysis of variance indicated that sucrose, nitrate, and the sucrose \times phosphate interaction had the largest positive effects on plant multiplication in liquid media. Multiplication was highest at maximum concentrations of phosphate and sucrose, indicating that ideal concentrations may be greater than the range tested. A 3 \times 3 factorial study extending the range of sucrose and phosphate levels was conducted in gelled media. Optimal phosphate concentration for multiplication was 7.25 mM and optimal sucrose levels were 3% or less. The D-optimal response surface approach was successful for defining factors of interest to improve micropropagation of these hellebore hybrids. Plant nutrition appeared to be more important than the concentration of growth regulator present in the culture medium.

Key words: liquid culture, mineral nutrition, optimality criterion, response surface design, Thidiazuron

LOCATION IN CANOPY OF THE STOCK PLANT AFFECTS ROOTING AND SUBSEQUENT GROWTH IN CUTTING PROPAGATION OF ROSES

Chul Hwan Hwnag¹, Dal Jin Sim¹, Ji Eun Park¹, Yoo Gyeong Park², and Byoung Ryong Jeong^{1,2,3*}

¹Department of Horticulture, Division of Applied Life Science (BK21 Plus), Graduate School of Gyeongsang National University, 660-701 Jinju, Korea

²Institute of Agriculture and Life Science, Gyeongsang National University, 660-701 Jinju, Korea

³Research Institute of Life Science, Gyeongsang National University, 660-701 Jinju, Korea

*Fax: + 82-55-757-7542, *E-mail: brjeong@gmail.com

Abstract

This study was conducted to investigate rooting and subsequent growth as affected by location in the canopy of the stock plant for cutting propagation of cut roses (*Rosa hybrida* Hort.) 'Pink Mimi' and 'Free Sun'. Cuttings were prepared as single node cuttings, each with a five-leaflet leaf and were stuck in rockwool cubes. Effect of location in the canopy of the flowering stem on cutting propagation revealed that rooting value was equal or higher than 78.4% in both cultivars regardless of location in the canopy of the flowering stem. In 'Pink Mimi', there were little differences in growth as affected by location in the canopy of the flowering stem. In 'Free Sun', length of the shoot and the longest root, and fresh and dry weights of the shoot increased, while chlorophyll content, and fresh and dry weights of the stem decreased, in the cuttings harvested from the central area of the canopy in comparison with those from the marginal area of the canopy. These results suggest that shoot growth of rooted cuttings was significantly affected by location in the canopy of the stock plant, and additional research is needed to reveal the effect of location in the canopy of the stock plant in more cultivars.

Key words: cut rose, light intensity, photosynthesis, physiological status of the stock plant, root formation

MICROPROPAGATION OF *SORBUS COMMIXTA* HEDL.

Byoung Ryong Jeong¹ and Iyyakkannu Sivanesan^{1,2*}

¹Institute of Agriculture and Life Science, Department of Horticulture, Division of Applied Life Science (BK21 Plus), Gyeongsang National University, Jinju 660-701, Republic of Korea

²Department of Molecular Biotechnology, Konkuk University, 1 Hwayang-dong, Gwangjin-gu, Seoul 143-701, Republic of Korea *Fax: + 8224503310 *E-mail: isivanesan@yahoo.com

Abstract

Nodal explants obtained from greenhouse-grown plants of *Sorbus commixta* were cultured on Murashige and Skoog (MS) medium supplemented with 0, 0.5, 1.0, 2.0 or 4.0 mg l⁻¹ N⁶-benzyladenine (BA) or thidiazuron (TDZ), and 1.0 or 2.0 mg l⁻¹ BA in combination with 0.5 and 1.0 mg l⁻¹ indole-3-acetic acid (IAA) or α -naphthaleneacetic acid (NAA) for axillary shoot multiplication. The maximum frequency of shoot formation (78.1%) with a mean of 6.6 shoots was obtained on MS medium supplemented with 2.0 mg l⁻¹ BA. Addition of IAA or NAA to BA-containing medium improved further the shoot induction response. The highest frequency of shoot induction (96%) with a mean of 16.2 shoots obtained when the nodal explants were cultured on MS medium supplemented with 2.0 mg l⁻¹ BA and 0.5 mg l⁻¹ NAA. The regenerated shoots were cultured on half-strength MS medium supplemented with 0, 0.5, 1.0, 2.0 or 4.0 mg l⁻¹ IAA, indole-3-butyric acid (IBA) or NAA for root induction. Out of the three auxins studied, IBA was found to be the most effective in promoting root induction in *S. commixta* followed by NAA and IAA. The regenerated shoots were rooted best on half-strength MS medium supplemented with 2.0 mg l⁻¹ IBA after 30 days of culture.

Key words: auxin, BA, nodal explants, Rosaceae, shoot multiplication, thidiazuron

THE EFFECT OF THE AUXIN APPLICATION METHODS ON ROOTING OF *PHYSOCARPUS OPULIFOLIUS* MAXIM. CUTTINGS

Andrzej Pacholczak

Department of Ornamental Plants, Faculty of Horticulture, Biotechnology and Landscape Architecture,
Warsaw University of Life Sciences (SGGW),
166 Nowoursynowska str., 02-787 Warszawa, Poland,
*Fax: + 48 (22) 5932268, *E-mail: pacholczak@poczta.onet

Abstract

In nursery production of ornamental woody plants, rooting is routinely stimulated by application of auxin-containing rooting powders. This study compares various application methods of the indole-3-butyric acid (IBA) and their effects on rhizogenesis and investigates the mechanism of its action in stem cuttings of two cultivars of ninebark *Physocarpus opulifolius* Maxim. The cuttings were sprayed with water solution of IBA (50 mg l⁻³), or their bases were treated with the commercial rooting powder Rhizopon AA (1% IBA), soaked 24 h in water solution after dissolving a Rhizopon AA tablet (50 mg l⁻³) or immersed for 5 s in 150 mg l⁻³ IBA (Rhizopon AA tablets – the „quick dip” method); untreated cuttings serving as controls were sprayed with water. Almost all treatments increased the rooting percentage in both cultivars tested. The effects of foliar IBA application and soaking the cuttings bases in the IBA solution (50 mg l⁻³) were comparable to those of the commercial rooting powder. Auxin applications resulted in changes in the contents of several organic compounds in both tested cultivars: the levels of the chlorophyll content (a + b), total soluble sugars, free amino acids, and the indole-3-acetic acid were increased by some treatments while the levels of polyphenolic acids were reduced. The results of biochemical analyses indicate that the auxin preparations not only stimulate rhizogenesis but also affect the metabolism in cuttings, in a different way, depending on the application method and the cultivar.

Key words: growth regulators, IBA, ninebark, organic compounds, shrubs

**EFFECT OF NITROGEN, APPENDAGE REMOVAL, LOCALITY,
AND YEAR ON SEED GERMINATION OF THE ENDANGERED DRY GRASSLAND
SPECIES *PULSATILLA PRATENSIS* (L.) MILL.**

Martina Bochenková^{1*}, Petr Karlík¹, and Michal Hejman²

¹Department of Forest Ecology, Faculty of Forestry and Wood Sciences,
Czech University of Life Sciences Prague, 1176 Kamýčká str., 165 21 Prague 6 – Suchdol, Czech,

*Fax: + 420 234 383 778, *E-mail: bochenkova@fzp.czu.cz

²Department of Ecology, Faculty of Environmental Sciences, Czech University of Life Sciences Prague,
1176 Kamýčká str., 165 21 Prague 6 – Suchdol, Czech

Abstract

Pulsatilla pratensis (L.) Mill. is a plant species widely used for ornamental purposes in gardens, but in nature it grows in dry grasslands and is considered an endangered species whose seeds can be directly affected by higher nitrogen (N) deposition. Here we investigate how the germination of *Pulsatilla pratensis* is affected by concentrations of dissolved nitrogen (NH_4^+ and NO_3^- ions), appendage removal, the locality of seed origin, and the year of seed collection. Seeds from different years and two localities, one on clay shale and the other on limestone, were germinated under laboratory conditions in solutions of NH_4NO_3 ranging from 0 to 4239 mg l^{-1} N. Mean germination of seeds from the clay shale locality in 2009 and 2011 and from the limestone locality in 2011 across treatments with low concentrations of N (0-170 mg l^{-1} N) ranged between 35 - 49%. Seeds were able to germinate under a wide range of N concentrations, and a substantial decrease in germination rate was recorded only if N concentration exceeded 848 mg l^{-1} . Seeds germinated in cases where they were totally covered by fungal mycelium, which indicates their high tolerance to fungal infection. There were almost no differences in germination between seeds with and without appendages. Lower germination of seeds collected from the limestone locality could be caused by calcareous bedrock connected with very low P and high Ca availability in the soil, producing lower P and higher Ca concentrations in seeds than at the clay shale locality. Seeds from the clay shale locality also showed differences from year-to-year. We conclude that common N concentrations in rainwater, which range from 10 to 13 mg l^{-1} N in Prague, and seed appendage removal have no direct effect, but locality, weather conditions during pollination and seed development play important roles in the establishment of *Pulsatilla pratensis* seeds.

Key words: deposition, establishment of pasqueflower, fungal infection, nutritional maternal effect