REFERENCES

1. Salmerón-Manzano E, Garrido-Cardenas JA, Manzano-Agugliaro F. Worldwide Research Trends on Medicinal Plants. Int J Environ Res Public Health. 2020;12,17(10):3376. https://doi.org/10.3390/ijerph17103376
2. Singh R, Geetanjali. Chemotaxonomy of medicinal plants: possibilities and limitations. Natural products and drug discovery. 2018;119-136. https://doi.org/10.1016/B978-0-08-102081-4.00006-X
3. Salmerón-Manzano E, Manzano-Agugliaro F. Worldwide Research on Low-Cost Technologies through Bibliometric Analysis. *Inventions.* 2020; 5:9. doi: https://doi.org/10.3390/inventions5010009
4. Christenhusz M, Byng, J. The number of known plant species in the world and its annual increase. Phytotaxa. 2016; 261. 201-217. DOI: https://doi.org/10.11646/phytotaxa.261.3.1
5. Malik MA, Bhat SA, Fatima B, Ahmad SB, Sidiqui S, Shrivastava P. *Rheum emodi* as valuable medicinal plant. *International Journal of General Medicine and Pharmacy (IJGMP).* 2016; 5, 4: 35-44
6. Singh R, Tiwari T, Chaturvedi P. *Rheum emodi* Wall ex. meissn (Indian Rhubarb): Highly endangered medicinal herb. Journal of Medicinal Plants Studies. 2017; 5(4):13-16
7. Jabeen N, Shawl AS, Dar GH and Sultan P. Micropropagation of *Inula racemosa Hook.f.* A valuable medicinal plant. International Journal of Botany. 2007; 3(3): 296-301
8. Bhat TA, Wani AA, Gulfishan M. Medicinal Plants Sector in Northern India: An Ethno-Medicinal Appraisal. The Journal of Ethnobiology and Traditional Medicine. Photon. 2015; 124, 978-992
9. Bilal S, Bhat SA, Ahanger AA, Hussain I, Ahmad SP, Mir MR. Healing potential of *Rheum emodi* (rhubarb) root powder on excision wounds in rabbit. *World J Pharm Pharm Sci.* 2014; 3(3):1317-1323.

10. Upadhyay S, Uniyal A, Kumar V, Gupta S. Occurrence, Distribution, Conservation, Ethanobotanical and Pharmacological aspects of endangered medicinal herb belonging to Genus *Rheum*, Research Journal of Chemistry and Environment. 2022; 26(7): 168-186.
11. Aslam M, Dayal R, Javed K, Fahamiya N, Mohd Mujeeb, Husain A. Pharmacognostical and Phytochemical Evaluation of *Rheum emodi* Wall. *Journal of Current Pharma Research*. 2012; 2(2):471-479.
12. Clapa D, Borsai O, Harta M, Bonta V, Szabo K, Coman V, Bobis O. Micropropagation, genetic fidelity and phenolic compound production of *Rheum rhabarbarum L. Plants.* 2020; 9(5):656. https://doi.org/10.3390/plants9050656
13. Rolta R, Sharma A, Kumar V, Sourirajan A, Baumler DJ, Dev K. Methanolic extracts of the rhizome of *R. emodi* act as bioenhancer of antibiotics against bacteria and fungi and antioxidant potential. *Med Plant Res.* 2018; 8:74–85. doi:10.5376/mpr.2018.08.0009.
14. Rolta R, Kumar V, Sourirajan A, Upadhyay NK, Dev K. Bioassay guided fractionation of rhizome extract of *Rheum emodi* Wall as bio-availability enhancer of antibiotics against bacterial and fungal pathogens. *J Ethnopharmacol.* 2020; 257: 112867. doi: https://doi.org/10.1016/j.jep.2020.112867.
15. Neyrinck AM, Etxeberria U, Tamminiau B, Daube G, Hul MV, Everard A, Cani PD, Bindels LB, Delzenne NM. Rhubarb extract prevents hepatic inflammation induced by acute alcohol intake, an effect related to the modulation of the gut microbiota. *Mol Nutr Food Res.* 2017; 61(1), 1500899.
16. Cohen JI, Williams JT, Plucknett DL, Shands H. Ex situ conservation of plant genetic resources: global development and environmental concerns. *Science.* 1991; 253 (5022), 866-872. DOI: 10.1126/science.253.5022.866
17. Pence VC. The application of biotechnology for the conservation of endangered plants. Plant conservation biotechnology. 1999; 15, 227-241.
18. Rao NK. Plant genetic resources: Advancing conservation and use through biotechnology. *Afr. J. Biotechnol.* 2004; 3 (2), 136-145. DOI: 10.5897/AJB2004.000-2025

19. Bridgen M, Van HW, Eeckhaut T. Plant Tissue Culture Techniques for Breeding. In *Ornamental Crops, Handbook of Plant Breeding*; Van Huylenbroeck, J., Ed.; Springer International Publishing AG: Cham, Switzerland. 2018; 127–144.
20. Abdalla N, El-Ramady H, Seliem MK, El-Mahrouk ME, Taha N, Bayoumi Y, Shalaby TA, Dobránszki J. An Academic and Technical Overview on Plant Micropropagation Challenges. *Horticulturae*. 2022; *8*, 677. https://doi.org/10.3390/horticulturae8080677
21. Gantait S, Kundu S, Ali NM, Sahu NC. Synthetic seed production of medicinal plants: a review on influence of explants, encapsulation agent and matrix. Acta Physiol Plant. 2015; 37:98
22. Cangahuala-Inocente GC, Dal Vesco LL, Steinmacher D, Torrees AC, Guerra MP. Improvements in somatic embryogenesis protocol in Feijoa (Acca sellowiana (berg) biuret). Induction, conversion and synthetic seeds. Sci Hortic 2007; 111:228–234. https://doi.org/10.1016/j.scienta.2006.10.030
23. Verma SK, Rai MK, Asthana P, Jaiswal VS, Jaiswal U. In vitro plantlets from alginateencapsulated shoot tips of Solanum nigrum L. Sci Hortic. 2010. 124:517–521
24. Sundararaj SG, Agrawal A, Tyagi RK. Encapsulation for in vitro short-term storage and exchange of ginger (Zingiber officinale Rosc.) germplasm. Sci Hortic. 2010; 125:761–766
25. Rai MK, Asthana P, Singh SK, Jaiswal VS, Jaiswal U. The encapsulation technology in fruit plants-a review. Biotechnol Adv. 2009; 27:671–679
26. Asrar AA. Seed germination induction of Hommaidh (Rumex vesicarius L.) by gibberellic acid and temperature applications. Eurasian J Agricult Environ Sci 2011; 10:310–317
27. Mandal J, Pattnaik S, & Chand PK. Alginate encapsulation of axillary buds of *Ocimum americanum* L. (hoary basil), *O. Basilicum* L. (sweet basil), *O. Gratissimum* L. (shrubby basil), and *O. Sanctum*. L. (sacred basil). *In Vitro Cell.Dev.Biol.-Plant.* 2000; 36, 287–292. https://doi.org/10.1007/s11627-000-0052-0

28. Anand Y, Bansal YB. Synthetic seed: a novel approach of in vitro plantlet formation in Vasaka (Adhatoda vasica Nees). Plant Biotechnol. 2002; 19:159–162.
29. Bekheet SA. A synthetic seed method through encapsulation of in vitro proliferated bulblets of garlic (Allium sativum L). Arab J Biotechnol. 2006; 9:415–426
30. Chandrasekhar T, Hussain TM, Gopal GR, & Rao JV. Somatic Embryogenesis of Tylophora indica (Burm.f.) Merril., an Important Medicinal Plant. *International Journal of Applied Science and Engineering*. 2006; *4*, 33-40.
31. Latif Z, Nasir IA, Riazuddin S. Indigenous production of synthetic seeds in Daucus carota. Pak J Bot. 2007; 39:849–855
32. Baskaran P, Kumari A, & Van Staden J. In vitro propagation via organogenesis and synthetic seeds of *Urginea altissima* (L.f.) Baker: a threatened medicinal plant. *3 Biotech.* 2018; **8**, 18. https://doi.org/10.1007/s13205-017-1028-7
33. Baro T and Das S. Invitro propagation and somaclonal variation study of *Phlogacanthus thyrsiformis nees* an ethnic medicinal plant. Plant science today 2022; 9. (sp2), 18-23. https://doi.org/10.14719/pst.1703
34. Sultana KW, Das S, Chandra I, Roy A. Efficient micropropagation of Thunbergia coccinea wall. and genetic homogeneity assessment through RAPD and ISSR markers. Scientific reports. 2022; 12(1): 1-11. https://doi.org/10.1038/s41598-022-05787-7
35. Bargali H, Kumar A, Singh P. Plant studies in Uttarakhand, Western Himalaya–A comprehensive review. Trees, Forests and People. 2022; 8, 100203. https://doi.org/10.1016/j.tfp.2022.100203
36. Lakshman CD. Bio-diversity and conservation of medicinal and aromatic plants. *Adv Plants Agric Res.* 2016; 5(4): 561-566. 10.15406/apar.2016.05.00186

37. Bhattacharyya P, Kumaria S, Bose B, Paul P, Tondon P. Evaluation of genetic stability and analysis of phytomedicinal potential in micropropagated plants of Rumex nepalensis – A medicinally important source of pharmaceutical biomolecules. Journal of Applied Research on Medicinal and Aromatic Plants. 2017; 6: 80-91. https://doi.org/10.1016/j.jarmap.2017.02.003
38. Liang HX, Dai HQ, Fu HA, Dong XP, Adebayo AH, Zhang LX and Cheng YX. Bioactive Compounds from Rumex Plants. Phytochemistry Letters. 2010; 3, 181-184. https://doi.org/10.1016/j.phytol.2010.05.005
39. Foust & Clifford M. Rhubarb: The wondrous drug. Princeton, New Jersey: Princeton University Press. 1992
40. Newby LJ. The Empire and the Khanate. Leiden, The Netherlands: Koninklijke Brill NV. 2005
41. Agarwal SK, Singh SS, Verma S and Kumar S. Antifungal activity of anthraquinone derivatives from *Rheum emodi*. Journal of Ethnopharmacology. 2000; 72:43-46. https://doi.org/10.1016/S0378-8741(00)00195-1
42. Agarwal SK, Singh SS, Lakshmi V, Verma S and Kumar S. Chemistry and pharmacology of Rhubarb (*Rheum* species) - A Review. Journal of Scientific and Industrial Research, 60: 1-9. 2001; http://nopr.niscpr.res.in/handle/123456789/26456
43. Tayade A, Dhar P, Ballabh B, Kumar R, Chaurasia OP, Bhatt RP, & Srivastava RB. *Rheum webbianum royle*: A potential medicinal plant from trans-himalayan cold deserts of Ladakh, India. *Plant Archives* 2012; *12*(2), 603-606.
44. Clementi EM, Misiti F. Bioactive foods in promoting health, fruits and vegetables. Cambridge, MA, USA: Academic Press. 2010
45. Kirtikar KR & Basu BD. Indian Medicinal Plants – with illustrations, 2nd ed., Oriental Enterprises, Dehra Doon, India. 200; 652.
46. Kandari LS, Rao KS, Payal KC, Maikhuri RK, Chandra A, Van Staden J. Conservation of aromatic medicinal plant *Rheum emodi* through improved seed germination. Seed Science & Technology. 2012; 40:95-101. DOI:10.15258/sst.2012.40.1.10

47. Badoni A, Bisht C, Chauhan JS. Seed Age Effect on Germinability in Seeds of *Rheum emodi* Wall. ex Meissn: An Endangered Medicinal Plant of Garhwal Himalaya. New York Science Journal. 2009; 2(4):81-84.
48. Chopra RN, Chopra IC, Handa KL, Kapur LD. Chopras's indigenous drugs of India. 2nd edn. UN Dhur & Sons, Pvt. Ltd., Calcutta. 1958; 233-236.
49. Haq SM, Khoja AA, Lone FA, Waheed M, Bussmann RW, Casini R, Mahmoud EA, Elansary HO. Keeping Healthy in Your Skin—Plants and Fungi Used by Indigenous Himalayan Communities to Treat Dermatological Ailments. *Plants*. 2023; 12(7):1575. https://doi.org/10.3390/plants12071575
50. Rashid S, Kaloo ZA, Singh S, Basher I. Medicinal importance of Genus *Rheum* -A review. *Int. J. of Adv. Res.* 2014; **2, 7: 261-267.**
51. Mishra SK, Tiwari S, Shrivastava A. *et al.* Antidyslipidemic effect and antioxidant activity of anthraquinone derivatives from *Rheum emodi* rhizomes in dyslipidemic rats. *J Nat Med*. 2014; 68, 363–371. https://doi.org/10.1007/s11418-013-0810-z
52. Radhika R, Kumari DK, Sudarsanam D. Antidiabetic activity of *Rheum emodi* in Alloxan induced diabetic rats. *Int. J. Pharm. Sci. Res.* 2010; 1:296–300.
53. Ibrahim M, Khan AA, Tiwari SK, Habeeb MA, Khaja M, Habibullah C. Antimicrobial activity of Sapindus mukorossi and Rheum emodi extracts against H pylori: In vitro and in vivo studies. *World J Gastroenterol*. 2006; 12(44): 7136-7142. DOI: 10.3748/wjg.v12.i44.7136
54. Rajkumar V, Guha G, Kumar RA. Antioxidant and Anti-Cancer Potentials of *Rheum emodi* Rhizome Extract. *Evid. -Based Complementary Altern. Med.* 2011; 1-9. https://doi.org/10.1093/ecam/neq048
55. Ibrahim M, Khaja NM, Aara A, Khan AA, Habeeb MA, Devi PY, Narasu ML, Habibullah M. Hepatoprotective activity of *Sapindus mukorossi* and *Rheum emodi* extracts. *World J Gastroenterol*. 2008; 14(16): 2566–2571. doi: 10.3748/wjg.14.2566
56. Akhtar MS, Amin M, Ahmad M, Alamgeer. Hepatoprotective Effect of *Rheum emodi* Roots (Revandchini) and Akseer-e-Jigar Against Paracetamol-induced Hepatotoxicity in Rats. *Ethnobot. Leaflets*. 2009; 13: 310-315. https://opensiuc.lib.siu.edu/ebl/vol2009/iss2/3

57. Alam MM, Javed K, Jafri MA. Effect of *Rheum emodi* (Revand Hindi) on renal fuctions in rats. *J Ethnopharmacol.* 2005; 96(1-2) :121-5. DOI: 10.1016/j.jep.2004.08.028
58. Park SK, Lee YK. Antioxidant Activity in *Rheum emodi* Wall (Himalayan Rhubarb). Molecules. 2021; 27,26(9):2555. DOI: 10.3390/molecules26092555
59. Sussex IM. The scientific roots of modern plant biotechnology. The Plant Cell. 2008; 20(5):1189-98. https://doi.org/10.1105/tpc.108.058735
60. Daltoso R, Melandri F. Sustainable sourcing of natural food ingredients by plant cell cultures. Agro Food Ind. Hi Tech. 2011; 22, 30–32
61. Lepse L. Comparison of in vitro and traditional propagation methods of rhubarb (Rheum rhabarbarum) according to morphological features and yield. Acta Hortic. 2009; 812, 265–270. https://doi.org/10.17660/ActaHortic.2009.812.35
62. Sudheer WN, Thiruvengadam M & Nagella P. A comprehensive review on tissue culture studies and secondary metabolite production in *Bacopa monnieri* L. Pennell: a nootropic plant, Critical Reviews in Biotechnology. 2022; 43:6, 956-970. https://doi.org/10.1080/07388551.2022.2085544
63. Arora K, Rai MK & Sharma AK. Tissue culture mediated biotechnological interventions in medicinal trees: recent progress. *Plant Cell Tiss Organ Cult.* 2022; 150, 267–287. https://doi.org/10.1007/s11240-022-02298-1
64. Kolewe ME, Gaurav V, Roberts SC. Pharmaceutically active natural product synthesis and supply via plant cell culture technology. Mol. Pharm. 2008; 5, 243–256. https://doi.org/10.1021/mp7001494
65. Ali H, Musa IF, Anu Bakar NA, Karsani SA, Yaacob JS. In vitro regeneration and ISSR-Based genetic fidelity analysis of Orthosiphon stamineus Benth. Agronomy. 2019; 9, 778. https://doi.org/10.3390/agronomy9120778
66. Kozak D, Salata A. Effect of cytokinins on in vitro multiplication of rhubarb (Rheum rhaponticum L.) ‘Karpow lipskiego’ shoots and ex vitro acclimatization and growth. Acta Sci. Pol. Hortorum Cultus. 2011; 10, 75–87.
67. Zhao Y, Grout BWW, Crisp C. Inadvertent selection for unwanted morphological forms during micropropagation adversely affects field performance of European Rhubarb (Rheum rhaponticum). Acta Hortic. 2003; 616, 301–308. DOI:10.17660/ActaHortic.2003.616.42

68. Padma-Mallaya N, Ravishankar GA. In vitro propagation and genetic fidelity study of plant regenerated from inverted hypocotyl explants of eggplant (*Solanum melongena* L.) cv. Arka Shirish. *3 Biotech.* 2013; 3, 45–52. https://doi.org/10.1007/s13205-012-0068-2
69. Nešković M, Ćulafić L, Vujičić R. Somatic Embryogenesis in Buckwheat (*Fagopyrum* Mill.) and Sorrel (*Rumex* L.), Polygonaceae. In: Bajaj, Y.P.S. (eds) Somatic Embryogenesis and Synthetic Seed II. Biotechnology in Agriculture and Forestry, vol 31. Springer, Berlin, Heidelberg. 1995. https://doi.org/10.1007/978-3-642-78643-3_33
70. Kakarla L, Rama C, Botlagunta M, Krishna MSR, & Mathi PS. Somatic embryogenesis and plant regeneration from leaf explants of Rumex vesicarius L. African Journal of Biotechnology. 2014; 13(45), 4268-4274. https://doi.org/10.5897/AJB2014.14058
71. Fei Y, Wang LX, Fang ZW, Liu ZX. Somatic Embryogenesis and Plant Regeneration from Cotyledon and Hypocotyl Explants of *Fagopyrum esculentum* Moench *lpls* Mutant. *Agronomy*. 2019; *9*, 768. https://doi.org/10.3390/agronomy9110768
72. Nurwahyuni I, Situmorang M, Sinaga R. "Plant Regeneration through Callus Cultures from Leaf Explant of Sumatra Benzoin (*Styrax benzoin*)", *International Journal of Forestry Research*. 2020; 7. https://doi.org/10.1155/2020/8860178
73. Singh R, Chaturvedi P. *In vitro* propagation and establishment of adventitious root cultures of *Rheum emodi* Wall. ex Meisn for quantification of emodin production. *In Vitro Cell. Dev. Biol.-Plant*. 2022; 58, 80–92. https://doi.org/10.1007/s11627-021-10220-1
74. Tuncer B. Development of an efficient in vitro callus proliferation protocol for edible wild rhubarb (Rheum ribes L.). *Acta Scientiarum Polonorum Hortorum Cultus*. 2021; *20*(5), 119–126. https://doi.org/10.24326/asphc.2021.5.11
75. Tuncer B. Germination and Emergence of Wild Rhubarb (Rheum ribes L.) Seeds In Vitro Culture Conditions. Journal of Agricultural Sciences. 2019; 29 (3), 425-432. https://doi.org/10.29133/yyutbd.569671

76. Mun SC & Mun GS. Development of an efficient callus proliferation system for *Rheum coreanum Nakai*, a rare medicinal plant growing in Democratic People's Republic of Korea. *Saudi Journal of Biological Sciences*. 2016; *23*(4): 488–494. https://doi.org/10.1016/j.sjbs.2015.05.017
77. Malik S, Kumar R, Vats SK, Bhushan S, Sharma M, Ahuja PS. Regeneration in Rheum emodi wall.: A step towards conservation of an endangered medicinal plant species. Eng. Life Sci. 2009; 9(2): 130-134. https://doi.org/10.1002/elsc.200800071
78. Sahai A, Shahzad A. *In Vitro* Clonal Propogation of *Coleus forskohlii* via Direct Shoot Organogenesis from Selected Leaf Explants. *J. Plant Biochem. Biotechnol.* 2010; 19: 223–228. https://doi.org/10.1007/BF03263344
79. Sahai A, Shahzad A. High frequency in vitro regeneration system for conservation of *Coleus forskohlii*: a threatened medicinal herb. *Acta Physiol Plant.* 2013; 35, 473–481. https://doi.org/10.1007/s11738-012-1090-z
80. Tabin S, Kamili AN, Gupta RC. *et al.* In Vitro Culture of *Rheum emodi* Wall: An Endangered Medicinal Plant of Northwestern Himalaya. *Proc. Natl. Acad. Sci., India, Sect. B Biol. Sci.* 2018; 88, 995–1006. https://doi.org/10.1007/s40011-016-0835-7
81. Manoj P, Kumar SS & Giridhar P. In vitro shoot multiplication of *Rumex vesicarius* L., and quantification of ascorbic acid and major phenolics from its leaf derived callus. *3 Biotech.* 2019; 9, 353. https://doi.org/10.1007/s13205-019-1877-3
82. Khan MI, Shahzad A, Ganie IB, Pandith SA. Organogenesis, direct somatic embryogenesis, and shoot proliferation of *Rheum spiciforme* Royle: an endemic and vulnerable medicinal herb from Indian Trans Himalayas. *In Vitro Cell.Dev.Biol.-Plant.* 2022; 58, 35–50. https://doi.org/10.1007/s11627-021-10211-2
83. Kikowska MA, & Thiem B. Alginate-encapsulated shoot tips and nodal segments in micropropagation of medicinal plants. A review. *Herba Polonica.* 2011; *57*.

84. Murashige T. Plant Cell and Organ Cultures as Horticultural Practices. Symposium on Tissue Culture for Horticultural Purposes. 1977; 78, 17-30. http://dx.doi.org/10.17660/actahortic.1977.78.1
85. Ara H, Jaiswal U, Jaiswal VS. Synthetic seed: prospects and limitations. *Current science*. 2000; 1438-1444.
86. Maqsood M, Khusrau M, Mujib A, Kaloo ZA. Synthetic seed technology in some ornamental and medicinal plants: An overview. Propagation and genetic manipulation of plants. 2021; 19-31. https://doi.org/10.1007/978-981-15-7736-9
87. Micheli M, Hafiz IA, & Standardi A. Encapsulation of in vitro-derived explants of olive (Olea europaea L. cv. Moraiolo): II. Effects of storage on capsule and derived shoots performance. *Scientia Horticulturae*. 2007; *113*: 286-292. https://doi.org/10.1016/j.scienta.2007.04.001
88. Hu G, Dong N, Zhou Y. *et al.* In vitro regeneration protocol for synthetic seed production in upland cotton (*Gossypium hirsutum* L.). *Plant Cell Tiss Organ Cult.* 2015; 123: 673–679. https://doi.org/10.1007/s11240-015-0858-4
89. Reddy MC, Murthy KS, Pullaiah T. Synthetic seeds: A review in agriculture and forestry. African Journal of Biotechnology. 2012; 11(78): 14254-14275. DOI: 10.5897/AJB12.770
90. Sharma N, Gowthami R, Pandey R. Synthetic Seeds: A Valuable Adjunct for Conservation of Medicinal Plants. In: Faisal, M., Alatar, A. (eds) Synthetic Seeds. Springer, Cham. 2019. https://doi.org/10.1007/978-3-030-24631-0_7
91. Sharma S, Shahzad A, Teixeira da Silva JA. Syn seed technology-a complete synthesis. Biotechnol Adv. 2013; 31(2): 186-207. https://doi.org/10.1016/j.biotechadv.2012.09.007
92. Redenbaugh K. Synseeds: applications of synthetic seeds to crop improvement. CRC Press Inc. 1993.
93. Saiprasad GVS. Artificial seeds and their applications. *Reson.* 2001; 6; 39–47. https://doi.org/10.1007/BF02839082

94. Swamy MK, Balasubramanya S, and Anuradha M. Germplasm conservation of patchouli (*Pogostemon cablin Benth.*) by encapsulation of in vitro derived nodal segments. *International Journal of Biodiversity and Conservation.* 2009; *1*, 224-230.

95. Cheruvathur MK, Najeeb N. & Thomas TD. In vitro propagation and conservation of Indian sarsaparilla, *Hemidesmus indicus* L. R. Br. through somatic embryogenesis and synthetic seed production. *Acta Physiol Plant.* 2013; 35, 771–779. https://doi.org/10.1007/s11738-012-1117-5

96. M Faisal and M Anis. Regeneration of plants from alginate-encapsulated shoots of *Tylophora indica* (Burm. f.) Merrill, an endangered medicinal plant, The Journal of Horticultural Science and Biotechnology. 2007; 82,3: 351-354. DOI: https://doi.org/10.1080/14620316.2007.11512241

97. Murthy K, Reddy M, & Kondamudi R. Synthetic seeds - A novel approach for the conservation of endangered C. spiralis wt. and C. pusilla. *Bangladesh Journal of Scientific and Industrial Research.* 2013; *48*(1): 39–42. https://doi.org/10.3329/bjsir.v48i1.15412

98. Onishi N, Sakamoto Y. & Hirosawa T. Synthetic seeds as an application of mass production of somatic embryos. *Plant Cell Tiss Organ Cult.* 1994; 39, 137–145. https://doi.org/10.1007/BF00033921

99. Ipekci Z, Gozukirmizi N. Direct somatic embryogenesis and synthetic seed production from *Paulownia elongata*. *Plant Cell Rep.* 2003; 22: 16–24. https://doi.org/10.1007/s00299-003-0650-5

100. Manjkhola S, Dhar U. & Joshi M. Organogenesis, embryogenesis, and synthetic seed production in *Arnebia euchroma*—A critically endangered medicinal plant of the Himalaya. *In Vitro Cell.Dev.Biol.-Plant.* 2005; 41, 244–248. https://doi.org/10.1079/IVP2004612

101. Cheruvathur MK, Kumar GK & Thomas TD. Somatic embryogenesis and synthetic seed production in *Rhinacanthus nasutus* (L.) Kurz. *Plant Cell Tiss Organ Cult.* 2013; 113: 63–71. https://doi.org/10.1007/s11240-012-0251-5

102. Priyadharshini SD, Manokari M, & Shekhawat MS. In vitro conservation strategies for the critically endangered Malabar River lily (Crinum malabaricum Lekhak & Yadav) using somatic embryogenesis and synthetic seed production. *South African Journal of Botany*. 2020; *135*: 172-180. https://doi.org/10.1016/j.sajb.2020.08.030
103. Gangopadhyay G, Bandyopadhyay T, Poddar R, Gangopadhyay SB, & Mukherjee KK. Encapsulation of pineapple micro shoots in alginate beads for temporary storage. *Current Science*. 2005; *88*(6): 972–977. http://www.jstor.org/stable/24110394
104. Singh AK, Varshney R, Sharma M, Agarwal SS, Bansal KC. Regeneration of plants from alginate-encapsulated shoot tips of Withania somnifera (L.) Dunal, a medicinally important plant species. J Plant Physiol. 2006 Feb;163(2):220-3. https://doi.org/10.1016/j.jplph.2005.06.013
105. Naik SK and Chand P. Nutrient-alginate encapsulation of in vitro nodal segments of pomegranate (Punica granatum L.) for germplasm distribution and exchange. Scientia Horticulturae. 2006; 108. 247-252. https://doi.org/10.1016/j.scienta.2006.01.030
106. Ray A & Bhattacharya S. Storage and plant regeneration from encapsulated shoot tips of Rauvolfia serpentina--An effective way of conservation and mass propagation. South African Journal of Botany. 2008; 74: 776-779. https://doi.org/10.1016/j.sajb.2008.06.002
107. Srivastava V, Khan SA, Banerjee S. An evaluation of genetic fidelity of encapsulated microshoots of the medicinal plant: *Cineraria maritima* following six months of storage. *Plant Cell Tiss Organ Cult*. 2009; 99, 193–198. https://doi.org/10.1007/s11240-009-9593-z
108. Sharma S, Shahzad A, Jan N, & Sahai A. In vitro studies on shoot regeneration through various explants and alginate-encapsulated nodal segments of Spilanthes mauritiana DC., an endangered medicinal herb. *Int J Plant Dev Biol*. 2009; *3*(1): 56-61.

109. Kumar S, Rai MK, Singh N, & Mangal M. Alginate-encapsulation of shoot tips of jojoba [Simmondsia chinensis (Link) Schneider] for germplasm exchange and distribution. *Physiology and Molecular Biology of Plants*. 2010; *16*: 379-382. https://doi.org/10.1007/s12298-010-0043-5
110. Tabassum B, Nasir IA, Farooq AM, Rehman Z, Latif Z & Husnain T. Viability assessment of in vitro produced synthetic seeds of cucumber. *African Journal of Biotechnology*. 2010; *9*(42): 7026-7032.
111. Kumar K.G and Thomas TD. High frequency somatic embryogenesis and synthetic seed production in *Clitoria ternatea* Linn. *Plant Cell Tiss Organ Cult.* 2012; 110, 141–151. https://doi.org/10.1007/s11240-012-0138-5
112. Islam S and Bari MA. In vitro regeneration protocol for artificial seed production in an important medicinal plant Mentha arvensis L. Journal of Bio-Science. 2012; 20: 99-108. https://doi.org/10.3329/jbs.v20i0.17722
113. Maqsood M, Mujib A, & Siddiqui ZH. Synthetic seed development and conversion to plantlet in Catharanthus roseus (L.) G. Don. *Biotechnology (faisalabad)*. 2012; *11*: 37-43. DOI: 10.3923/biotech.2012.37.43
114. Mohanty P, Nongkling P, Das MC, Kumaria S & Tandon P. Short-term storage of alginate-encapsulated protocorm-like bodies of Dendrobium nobile Lindl.: an endangered medicinal orchid from North-east India. *3 Biotech*. 2013; *3*: 235-239. https://doi.org/10.1007/s13205-012-0090-4
115. Faisal M, Alatar AA and Hegazy AK. Molecular and Biochemical Characterization in *Rauvolfia tetraphylla* Plantlets Grown from Synthetic Seeds Following In Vitro Cold Storage. *Appl Biochem Biotechnol.* 2013; 169, 408–417. https://doi.org/10.1007/s12010-012-9977-0
116. Gupta AK, Harish Rai MK, Phulwaria M, Agarwal T, & Shekhawat NS. In vitro propagation, encapsulation, and genetic fidelity analysis of Terminalia arjuna: a cardioprotective medicinal tree. *Applied biochemistry and biotechnology*. 2014; *173*: 1481-1494. https://doi.org/10.1007/s12010-014-0920-4

117. Devi PS, Satyanarayana B, Arundhati A & Rao TR. Plant regeneration from encapsulated nodal segments of Sterculia urens Roxb., an endangered gum-yielding tree. *Journal of Crop Science and Biotechnology*. 2014; *17*: 21-25. https://doi.org/10.1007/s12892-013-0097-3
118. Sharma S, Shahzad A, Kumar J, Anis M. In vitro propagation and synseed production of scarlet salvia (Salvia splendens). Rendiconti Lincei. Scienze Fisiche e Naturali. 2014; 25: 359-368. https://doi.org/10.1007/s12210-014-0308-y
119. Baskaran P, Kumari A & Van Staden J. Embryogenesis and synthetic seed production in Mondia whitei. *Plant Cell, Tissue and Organ Culture (PCTOC)*. 2015; *121*: 205-214. https://doi.org/10.1007/s11240-014-0695-x
120. Shaheen A and Shahzad A. Nutrient encapsulation of nodal segments of an endangered white cedar for studies of regrowth, short term conservation and ethylene inhibitors influenced ex vitro rooting. *Industrial Crops and Products*. 2015; *69*, 204-211. https://doi.org/10.1016/j.indcrop.2015.02.032
121. Rathore MS & Kheni J. Alginate encapsulation and in vitro plantlet regeneration in critically endangered medicinal plant, Withania coagulans (Stocks) Dunal. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*. 2017; *87*: 129-134. https://doi.org/10.1007/s40011-015-0577-y
122. Naz R, Anis M, Alatar AA, Ahmad A, & Naaz A. Nutrient alginate encapsulation of nodal segments of Althaea officinalis L., for short-term conservation and germplasm exchange. *Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology*. 2018; *152*(6): 1256-1262. https://doi.org/10.1080/11263504.2018.1436610
123. Nandini BP, Sudarshana MS & Rajashekar N. Plant regeneration through somatic embryogenesis and synthetic seed production in Rumex vesicarius L. - A Potent medicinal herb. *IOSR Journal of Pharmacy and Biological Sciences.* 2014; *9*, 129-136. DOI 10.9790/3008-0952129136

124. Biswas P, Nandy S, Dey A, Tikendra L, Nongdam P. Molecular Markers in Assessing Genetic Clonal Fidelity for in Vitro Propagated Endangered Medicinal Plants. In: Kumar, A., Choudhury, B., Dayanandan, S., Khan, M.L. (eds) Molecular Genetics and Genomics Tools in Biodiversity Conservation. Springer, Singapore. 2022. https://doi.org/10.1007/978-981-16-6005-4_6
125. Larkin PJ and Scowcroft W. Somaclonal Variation—A Novel Source of Variability from Cell Cultures for Plant Improvement. Theoretical and Applied Genetics. 1981; 60: 197-214.http://dx.doi.org/10.1007/BF02342540
126. Upadhyay R, Kashyap SP, Tiwari KN. *et al.* Micropropagation of *Phyllanthus fraternus* Webster (Euphorbiaceae) from field-derived shoot tip explant and assessment of its genetic fidelity. *Braz. J. Bot.* 2015; 38, 517–525. https://doi.org/10.1007/s40415-015-0171-9
127. Ahmad N, Javed SB, Khan MI *et al.* Rapid plant regeneration and analysis of genetic fidelity in micropropagated plants of *Vitex trifolia*: an important medicinal plant. *Acta Physiol Plant.* 2013; 35, 2493–2500. https://doi.org/10.1007/s11738-013-1285-y
128. Faisal M, Alatar AA, Ahmad N. *et al.* An Efficient and Reproducible Method for in vitro Clonal Multiplication of *Rauvolfia tetraphylla* L. and Evaluation of Genetic Stability using DNA-Based Markers. *Appl Biochem Biotechnol.* 2012; 168, 1739–1752. https://doi.org/10.1007/s12010-012-9893-3
129. Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers, *Nucleic Acids Research*. 1990; 18, 22: 6531–6535. https://doi.org/10.1093/nar/18.22.6531
130. Rani V, Raina SN. Genetic fidelity of organized meristem-derived micropropagated plants: A critical reappraisal. *In Vitro Cell. Dev. Biol.-Plant.* 2000; 36, 319–330. https://doi.org/10.1007/s11627-000-0059-6
131. Varshney A, Lakshmikumaran M, Srivastava PS. *et al.* Establishment of genetic fidelity of *In vitro*-raised *Lilium* bulblets through rapd markers. *In Vitro Cell. Dev. Biol.-Plant.* 2001; 37, 227–231. https://doi.org/10.1007/s11627-001-0040-z

132. Devarumath R, Nandy S, Rani V. *et al.* RAPD, ISSR and RFLP fingerprints as useful markers to evaluate genetic integrity of micropropagated plants of three diploid and triploid elite tea clones representing *Camellia sinensis* (China type) and *C. assamica* ssp. *assamica* (Assam-India type). *Plant Cell Rep.* 2002; 21, 166–173. https://doi.org/10.1007/s00299-002-0496-2
133. Bhattacharyya P, Kumaria S, Job N & Tandon P. Phyto-molecular profiling and assessment of antioxidant activity within micropropagated plants of *Dendrobium thyrsiflorum*: a threatened, medicinal orchid. *Plant Cell, Tissue and Organ Culture (PCTOC).* 2015; *122*, 535-550. https://doi.org/10.1007/s11240-015-0783-6
134. Bhattacharyya P, Kumaria S, Job N, Tandon P. En-masse production of elite clones of Dendrobium crepidatum: A threatened, medicinal orchid used in Traditional Chinese Medicine (TCM), Journal of Applied Research on Medicinal and Aromatic Plants. 2016; 3,4: 168-176. https://doi.org/10.1016/j.jarmap.2016.04.001
135. Rout G and Das G. An Assessment of Genetic Integrity of Micropropagated Plants of *Plumbago Zeylanica* by RAPD Markers. *Biologia Plantarum.* 2002; 45, 27–32. https://doi.org/10.1023/A:1015186709691
136. Pradhan S, Paudel YP, Qin W, Pant B. Genetic fidelity assessment of wild and tissue cultured regenerants of a threatened orchid, Cymbidium aloifolium using molecular markers. Plant Gene. 2023; 34, 100418. https://doi.org/10.1016/j.plgene.2023.100418
137. Kumar S, Mangal M, Dhawan AK. *et al.* Assessment of genetic fidelity of micropropagated plants of *Simmondsia chinensis* (Link) Schneider using RAPD and ISSR markers. *Acta Physiol Plant.* 2011; 33: 2541–2545. https://doi.org/10.1007/s11738-011-0767-z
138. Seth S and Panigrahi J. In vitro organogenesis of Abutilon indicum (L.) Sweet from leaf derived callus and assessment of genetic fidelity using ISSR markers, The Journal of Horticultural Science and Biotechnology. 2019; 94:1, 70-79. https://doi.org/10.1080/14620316.2018.1447314

139. Leroy X, Leon K, Charles G. *et al.* Cauliflower somatic embryogenesis and analysis of regenerant stability by ISSRs. *Plant Cell Reports.* 2000; 19, 1102–1107. https://doi.org/10.1007/s002990000252
140. Martins M, Sarmento D & Oliveira MM. Genetic stability of micropropagated almond plantlets, as assessed by RAPD and ISSR markers. *Plant Cell Rep.* 2004; 23, 492–496. https://doi.org/10.1007/s00299-004-0870-3
141. Joshi P and Dhawan V. Assessment of genetic fidelity of micropropagated *Swertia chirayita* plantlets by ISSR marker assay. *Biol Plant.* 2007; 51, 22–26. https://doi.org/10.1007/s10535-007-0005-0
142. Narula A, Kumar S & Srivastava PS. Genetic fidelity of in vitro regenerants, encapsulation of shoot tips and high diosgenin content in *Dioscorea bulbifera* L., a potential alternative source of diosgenin. *Biotechnol Lett.* 2007; 29, 623–629. https://doi.org/10.1007/s10529-006-9276-3
143. Venkatachalam L, Sreedhar RV & Bhagyalakshmi N. Micropropagation in banana using high levels of cytokinins does not involve any genetic changes as revealed by RAPD and ISSR markers. *Plant Growth Regul.* 2007; 51, 193–205. https://doi.org/10.1007/s10725-006-9154-y
144. Tawar PN, Sawant RA, Dalvi SG. *et al.* An assessment of somaclonal variation in micropropagated plants of sugarcane by RAPD markers. *Sugar Tech.* 2008; 10, 124–127. https://doi.org/10.1007/s12355-008-0021-1
145. Yuan XF, Dai ZH, Wang XD. *et al.* Assessment of genetic stability in tissue-cultured products and seedlings of *Saussurea involucrata* by RAPD and ISSR markers. *Biotechnol Lett.* 2009; 31, 1279–1287. https://doi.org/10.1007/s10529-009-9984-6
146. Sharma M, Rai SK, Purshottam DK. *et al.* In vitro clonal propagation of *Clerodendrum serratum* (Linn.) Moon (barangi): a rare and threatened medicinal plant. *Acta Physiol Plant.* 2009; 31: 379–383. https://doi.org/10.1007/s11738-008-0245-4
147. Agnihotri RK, Mishra J & Nandi SK. Improved in vitro shoot multiplication and rooting of *Dendrocalamus hamiltonii* Nees et Arn. Ex Munro: production of genetically uniform plants and field evaluation. *Acta Physiol Plant.* 2009; 31, 961–967. https://doi.org/10.1007/s11738-009-0311-6

148. Bhattacharya S, Bandopadhyay TK & Ghosh PD. High Frequency Clonal Propagation of *Cymbopogon martinii* var motia (palmarosa) Through Rhizome Culture and True to Type Assessment using ISSR Marker. *J. Plant Biochem. Biotechnol.* 2010; 19, 271–273. https://doi.org/10.1007/BF03263355
149. Tyagi P, Khanduja S & Kothari S. *In vitro* culture of *Capparis decidua* and assessment of clonal fidelity of the regenerated plants. *Biol Plant.* 2010; 54, 126–130. https://doi.org/10.1007/s10535-010-0019-x
150. Chandrika M, Ravishankar Rai V & Thoyajaksha. ISSR marker-based analysis of micropropagated plantlets of *Nothapodytes foetida*. *Biol Plant.* 2010; 54, 561–565. https://doi.org/10.1007/s10535-010-0100-5
151. Bose B, Kumaria S, Choudhury H. *et al.* Assessment of genetic homogeneity and analysis of phytomedicinal potential in micropropagated plants of *Nardostachys jatamansi*, a critically endangered, medicinal plant of alpine Himalayas. *Plant Cell Tiss Organ Cult.* 2016; 124, 331–349. https://doi.org/10.1007/s11240-015-0897-x
152. Tikendra L, Koijam AS & Nongdam P. Molecular markers based genetic fidelity assessment of micropropagated Dendrobium chrysotoxum Lindl. *Meta Gene*. 2019. https://doi.org/10.1016/j.mgene.2019.100562
153. Sun D, Li Q, Li HB, Ai J, Qin HY, Piao ZY. Plantlet regeneration through somatic embryogenesis in Schisandra chinensis (Turcz) Baill. and analysis of genetic stability of regenerated plants by SRAP markers. Bangladesh J. Bot. 2015; 44, 881–888.
154. Li X, Wang X, Luan C. *et al.* Somatic embryogenesis from mature zygotic embryos of *Distylium chinense* (Fr.) Diels and assessment of genetic fidelity of regenerated plants by SRAP markers. *Plant Growth Regul.* 2014; 74, 11–21. https://doi.org/10.1007/s10725-014-9892-1
155. Nautiyal BP, Prakash V, Chauhan RS, Purohit H and Nautiyal MC. Assessment of germinability, productivity and cost benefit analysis of Picrorhiza kurrooa cultivated at lower altitudes. *Current Science*. 2001; *81*(5): 579–585. http://www.jstor.org/stable/24105895

156. Sathiyakumar S, Samarakoon KW, Jayasekera SJBA, Ranasinghe P, & Dharmadasa RM. Sterilization protocol for the Sri Lanka green algae *Caulerpa racemosa* and its antioxidant activity. (2016). 254-258. http://repository.wyb.ac.lk/handle/1/824
157. Murashige T and Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 1962; 15: 473–497.
158. Bakhtiar Z, Mirjalili MH, Sonboli A. *et al. In vitro* propagation, genetic and phytochemical assessment of *Thymus persicus* — a medicinally important source of pentacyclic triterpenoids. *Biologia.* 2014; 69, 594–603.
159. Nilanthi D and Yang Y. Effects of Sucrose and Other Additives on *In Vitro* Growth and Development of Purple Coneflower (*Echinacea purpurea* L.), *Advances in Biology*. 2014; 4. https://doi.org/10.1155/2014/402309
160. Bakhtiar Z, Mirjalili MH, Sonboli A. *In vitro* callus induction and micropropagation of *Thymus persicus* (Lamiaceae), an endangered medicinal plant. Cropp Breeding and Applied Biotechnology. 2016; 16 (1): 48-54. https://doi.org/10.1590/1984-70332016v16n1a8
161. Loberant B and Altman A. Micropropagation of plants. Encyclopedia of industrial biotechnology: bioprocess, bioseparation, and cell technology. Wiley, New York: 2010; 3: 499-515. https://doi.org/10.1002/9780470054581.eib442
162. Gupta R, Sood H. Artificial Seed Production and Cryopreservation by Encapsulation Dehydration for Medicinal Herb of Himalayan Region, Swertia Chirayita. Cryoletters. 2022; Sep 1; 43(5): 295-302. **DOI:** https://doi.org/10.54680/fr22510110612
163. Doyle JJ and Doyle JL. Isolation of plant DNA from fresh tissue. Focus. 1990; 12: 13-15.
164. Tikendra L, Sushma O, Amon T, Devi NA, Paonam S, Bindyananda N, Potshangbam AM, Dey A, Devi RS, Nongdam P. Genetic clonal fidelity assessment of rhizome–derived micropropagated *Acorus calamus* L. – a medicinally important plant by random amplified polymorphic DNA and inter-simple sequence repeats markers. Phcog Mag. 2022; 18: 207-15.

165. Redenbaugh K, Fujii JA, Slade D, Viss P, Kossler M. Artificial seeds-encapsulated embryos. In: Bajaj YPS (ed) High technology and micropropagation I. Biotechnology in agriculture and forestry. 1991; 17, 395–416.
166. Redenbaugh K, Paasch BD, Nichol JW, Kossler ME, Viss PR, Walker KA. Somatic seeds encapsulation of asexual plant embryos. Biotechnology. 1986; 4:797–801.
167. Senapati SK, Aparajita S, Rout GR. An assessment of genetic fidelity of in vitro grown plantlets of rose (*Rosa hybrida*) through molecular markers. African Journal of Biotechnology Vol. 2012; 11(100): 16532-16538. https://doi.org/10.5897/AJB11.3978
168. Tabin S, Kamili A, Gupta RC. Micropropagation and conservation of Rheum webbianum collected from Zanaskar valley via tissue culture. Annals of Plant Sciences. 2018; 7. 2187. DOI:10.21746/aps.2018.7.4.4
169. Zhang X, Shi B, Qiu J. Research on key techniques of tissue culture of Qinghai Rhubarb. IOP Conference Series: Earth and Environmental Science. 2021; 647. 012-110. DOI:10.1088/1755-1315/647/1/012110

ANNEXURES

List of Publications:

- Upadhyay, S., Uniyal, A., Kumar, V., Gupta, S. 2023. Assessment of the genetic fidelity of true-to-type regenerants of medicinal plant *Rheum emodi* using RAPD and ISSR molecular markers. Research Journal of Biotechnology. 18 (9): 198-204.
- Upadhyay, S., Uniyal, A., Kumar, V., Gupta, S. 2022. Occurrence, distribution, conservation, ethanobotanical and pharmacological aspects of endangered medicinal herb belonging to genus Rheum. Research Journal of Chemistry and Environment. 26(7): 168-186.

List of Conferences:

- GangaAyurCon-2022. (International conference). Held in Gurukul Campus, Uttarakhand Ayurved University, Haridwar (UK) on 5th-6th September 2022.
  Topic: Molecular Analysis of micropropagated Rheum emodi plants using RAPD and ISSR markers for ascertaining clonal fidelity.
- BioSangam 2022. (International conference). Held in Department of Biotechnology MNNIT, Allahabad, Prayagraj, INDIA on March 10-12, 2022
  Topic: Efficient plant regeneration via direct somatic embryogenesis and synthetic seed production in *Rheum* species: a threatened herb species of Uttarakhand.
- Uttarakhand State Science and Technology Congress 2020-2022. Held in Graphic Era University Dehradun on 22nd- 24th June, 2022
  Topic: In vitro shoot regeneration from rhizome explants of *Rheum* sp.- An endangered medicinal plant of Uttarakhand.

Herbarium

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दिनांक / Dated 07.2022

प्रमाणपत्र / CERTIFICATE

प्रमाणित किया जाता है कि कु0/श्रीमति श्वेता उपाध्याय, **स्वामी रामा हिमालयन विश्वविद्यालय** देहरादून 248016, **उत्तराखंड** से प्राप्त निम्न पादप नमूना निम्नानुसार (वानस्पतिक नाम) इस कार्यालय के विद्यार्थी पादपालय में परिग्रहण **किया गया है ।**

Certified that the following plant sample received from **Ms. Sweta Upadhyay, Rama Himalyan University, Jolly Grant**, Dehradun 248016, Uttarakhand is accessioned at herbarium (BSD Student Herbarium) of this office:

पादप का नाम / Plant name	कुल / Family	परिग्रहण सं. / Acc. No.
1. ***Rheum webbianum*** **Royle** Syn: *Rheum emodi* Wall.	Polygonaceae	1145

29/07/2022

(एस.के. सिंह / S.K.Singh)
(वैज्ञानिक–ई / कार्यालयाध्यक्ष / Scientist-E/HoO)

Conference Certificates

उत्तराखण्ड राज्य

GangaAyurCon-2022

5th - 6th September, 2022

Organised by : Uttarakhand Ayurved University

Certificate of Presentation

This is to certify that SWETA UPADHYAY

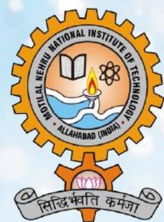
Molecular Analysis of Micropropagated Rheum emodi plants using RAPD and ISSR Markers for Ascertaining Clonal Fidelity has Presented 2nd Position

Scientific Paper in Session ...I...../Scientific Poster in

GangaAyurCon-2022

An International Conference on **Medicinal Plants of Ganga River Basin and Their Therapeutic Importance in India System of Medicine** held at Gurukul Campus Uttarakhand Ayurved University, Haridwar (U.K.)

Vice Chancellor, UAU	Registrar, UAU	Campus Director	Organizing Secretary	Organizing Secretary	Organizing Secretary
(Prof. Sunil Joshi)	(Dr. Rajesh Kumar)	(Prof. Pankaj Sharma)	(Dr. Ved Bhushan Sharma)	(Dr. Yadvendra Yadav)	(Dr. Kiran Vashisht)



BioSangam 2022

AN INTERNATIONAL CONFERENCE

Emerging Trends in Biotechnology

March 10-12, 2022

Organized by:

Department of Biotechnology

Motilal Nehru National Institute of Technology Allahabad, Prayagraj - India

CERTIFICATE

This is to certify that Dr./Mr./Mrs./Ms. Sweta Upadhyay

from Swami Rama Himalayan University, Dehradun

has participated / presented a paper (Oral /Poster) entitled Efficient plant regeneration via Direct Somatic Embryogenesis and Synthetic Seed production in Rheum species: a threatened herb species of Uttarakhand

in the International Conference (BioSangam 2022) during March 10 – 12, 2022 at the Department of Biotechnology, MNNIT Allahabad, Prayagraj, India.

Dr. Vishnu Agarwal
Chairman BioSangam & Head
BioSangam 2022

Certificate of Participation

This is to certify that

Sweta Upadhyay

has actively participated in 16th Uttarakhand State Science and Technology Congress 2020-22

22nd to 24th June, 2022

and presented a research paper (Oral/Poster) entitled

In-vitro shoot regeneration from rhizome explants of Rheum sp. – An endangered medicinal plant of Uttarakhand

under the discipline

Biotechnology, Biochemistry and Microbiology

at Graphic Era University, Dehradun

Ashutosh

Dr Ashutosh Mishra
Organizing Secretary - 16th USSTC
Council for Science and Technology
Uttarakhand

Dr Rajendra Dobhal, ***FNASc***
Chairman - 16th USSTC
Director General
Council for Science and Technology, Uttarakhand

Research Paper

Research Journal of Biotechnology Vol. **18 (9)** September **(2023)**
Res. J. Biotech.

Assessment of the genetic fidelity of true-to-type regenerants of medicinal plant *Rheum emodi* using RAPD and ISSR molecular markers

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Abstract
Rheum emodi commonly known as rhubarb is mainly found in Northern Himalayas. It is a valuable medicinal plant having major pharmacological activities such as antimicrobial, anticancer, antioxidant, anti-inflammatory and is used extensively as purgative, stomachic and astringent tonic and improves gastro related problems. This herb is used by the local communities for medicinal as well as common eating purpose. This leads to its immense decline in its natural habitat and now this herb falls under threatened species and demands conservation. In this prospective, an efficient in vitro propagation method from callus culture has been achieved using leaf explants excised from the juvenile plant of R. emodi. Murashige and Skoog (MS) basal medium was used for regeneration procedure with different concentration of phytohormones. Maximum frequency of callus formation (84.44±0.27%) was observed in MS+36.19μM (2, 4-D) in combination with 11.10μM (BAP). The highest percentage of adventitious shoot regeneration was observed as 75.56±0.27% and the maximum number of shoots per explant that is 3.67±0.27 was achieved on MS basal medium containing BAP (35.5 μM) and Kn (11.61 μM). The maximum frequency of rooting was observed in MS full strength media + IAA (28.55 μM) + BAP (8.88 μM). The highest frequency of roots per shoot was observed as 5.0±0.47 with an average root length of 11±1.25mm. For ascertaining the clonal fidelity, 20 ISSR markers and 15 RAPD markers were assayed and employed to validate the true-to-type regenerants of Rheum emodi.

Out of 15 RAPD and 20 ISSR markers, 7 markers and 15 markers produced distinct, clear and scorable bands with an average of 4.5 bands and 4.4 bands per marker respectively among the tissue cultured progenies. For each primer, the banding pattern was uniform and comparable to mother plant and showed about 99% homology. All the markers produce the monomorphic bands and no variation was detected among the micropropagated plants. Thus, the analysis of ISSR and RAPD patterns revealed that the bands were shared by both in vitro raised plants and parent clump confirming the genetic stability. DNA based molecular markers have proved to be versatile tools in diverse fields of biology. These markers proved to be model tools for routine analysis of clonal fidelity of micropropagated plants prior to commercialization.

Keywords: *Rheum emodi*, Medicinal plant, Clonal fidelity, *In vitro* propagation, RAPD and ISSR markers, Molecular markers.

Introduction

Rheum emodi (rhubarb), locally known by Dolu, belongs to family polygonaceae. It is an important medicinal herb extensively used in Unani and Ayurvedic traditional medicine systems. *Rheum* is distributed within the tropical and the sub-tropical areas of the world specifically in the Asian countries. This perennial herb is distributed in the Himalayan region having an altitude of 2800-3600 M height from the State of Kashmir to the Sikkim end in India. A variety of compounds are present in this herb such as anthraquinone, glycosides, flavonoids, tannins, saponins and volatile oil, which are mainly used as purgative and laxative. This herb is well known for its antimicrobial, antioxidant, wound healing, anticancerous, immune enhancing properties. Along with its medicinal use, its roots are used as natural dye in cosmetics, textiles and as food coloring agent. Besides this, its leaves are used for preparing vegetables and salads.

According to a recent study, ethyl acetate fraction of *Rheum* possesses the strongest antioxidant contents. The compounds associated with the antioxidant activity of *Rheum emodi* were identified as myricetin, myricitrin and myricetin 3-galloyl rhamnoside[9]. *Rheum* is also a major ingredient in many ayurvedic medicines such as Kankayanvati, Chiniumco tablet, Arjin tablet etc.[15] Due to these medicinal properties, this herb is extensively used by local communities which led to its decline and now this herb falls under threatened species and demands conservation[4].

In nature, the conventional propagation of this medicinal plant through vegetative or seed propagation is very slow as this process is very much hampered by the environmental conditions as well as poor germination power of seeds, even the death of seedlings at 2- leaved stage. In addition to this, these methods yield restricted number of propagules as it is dependent on age as well as size of plant and the specific time of the year. In the last two decades, plant tissue culture through *in vitro* regeneration has been increasingly applied for mass multiplication or clonal propagation of the highly valuable, medicinally important plants with different degree of success.

Advantages for *in vitro* propagation are its relatively short period of production, ability to generate plantlets throughout the year, its massive multiplication ability, production of healthy and disease-free plantlets. Plant propagation by the tissue culture method generally aims at the possible maximum multiplication rate without any mutation directly from the nodal segments, lateral or auxillary buds[1]. The

earliest references for the micropropagation of *Rheum* were identified in many researches[7,8].

Recent studies also support the micropropagation of *Rheum* species. High rate of callus induction was obtained from both the midrib and leaf explants[11]. These protocols have considerable implications showing the rapid multiplication of this herb. However, deployment of these propagation techniques on a commercial basis may be restricted or hindered because of the somaclonal variation as it has been reported in many crops[10].

Hence a quality checkup for the clonal fidelity at an early stage of propagation is considered to be very functional in plant tissue culture technique. Different molecular markers served as significant tool to assess the genetic similarity and the true to type nature of regenerated or micropropagated plantlets. Among the different applied DNA based markers, the AFLP (Amplified Fragment Length Polymorphism) and RFLP (Restriction Fragment Length Polymorphism) are among the most consistent markers for assessing of clonal fidelity in micropropagated plants.

Conversely these approaches engage the extensive care along with the use of radioactive labeling of very expensive enzymes and consequently incompatible under certain conditions whereas RAPD (Random Amplified Polymorphism DNA) and the ISSR (Inter Simple Sequence Repeats) markers require only a small quantity of DNA sample, do not involve any radioactive labels and are also fast, simple, easy to handle, cost effective with highly reliable techniques. These markers have been effectively applied to detect the somaclonal variation in many medicinal plants[2,12].

The objective of the present study was to establish the rapid propagation of *Rheum emodi* herb through the callus inducing propagation from leaf explant and to assess the clonal fidelity of regenerants using RAPD and ISSR molecular markers. Results of the study could prove that the selected molecular markers are the ideal tools for routine analysis of genetic fidelity of micropropagated plantlets prior to commercialization and thus provide a way for better conservation strategies for this threatened plant *Rheum emodi*.

Material and Methods

Plant Material: Juvenile leaves of *Rheum emodi* served as explants for callus initiation. The plants of *Rheum emodi* were collected from village Baniyakund, District Rudraprayag, Uttarakhand. Surface sterilization procedure includes 0.1% mercuric chloride for 1min, 70% ethanol 1 min. 10% NaOCl for 30 seconds, rinse 5 times with the double distilled sterile water to eliminate contaminants of disinfectant from explants.

Media composition and Culture Condition: MS (Murashige and Skoog medium) basal medium with vitamins, supplemented with 3% sucrose and 0.75% agar was used for regeneration. Different growth regulators (2, 4-D, BAP, Kinetin, IBA, IAA and TDZ) were filter sterilized before adding to the autoclaved cultured media[13]. All the cultures were maintained in a growth chamber at temperature 23°C with relative humidity as 70% and a photoperiod of 16/8- hr of light / dark with light supplied by the white cool fluorescent tube lights at an intensity of 50-60 umol m^{-2} s^{-1}.

Callus initiation and shoot regeneration: Juvenile leaves having midrib were cut in 2-3 cm pieces before culturing on the media[5]. All the explants were cultured in MS basal media supplemented with 2, 4-D (2, 4-dichlorophenoxyacecetic acid) ranging from 9 µM- 45 µM, IBA (Indole-3-butyric acid) ranging from 9 µM - 49 µM, BAP (6-Benzylaminopurine) ranging from 4 µM- 13 µM. These cultures were further subcultured on the same medium after the 15 days and were maintained up to 6 weeks. Media devoid of growth regulators were used as control.

For shoot regeneration, well established fragile calli obtained from the callus induction were used. After 6 weeks, proliferated fragile calli were transferred into the MS basal medium supplemented with different concentration of BAP (6-benzylaminopurine) as 8.8, 17.76, 26.64 and 35.52 µM or thidiazuron (TDZ) as 9.09, 18.18, 27.02 and 36.36 µM or kinetin as 6.97, 9.29, 11.61 and 13.94 µM for adventitious shoot regeneration. Subculturing was done once in every two weeks on fresh media and maintained for 5 weeks. Observation on the percentage of shoot organogenesis, average no. of shoots per callus and their mean shoot length were recorded after 5 weeks. Study consists of 10 explants for each replication and experiments were conducted 3 times.

Rooting and hardening of the Plantlets: 2-3 cm long shootlets were excised from shoot cultures and transferred onto rooting media (hormone free half MS/full MS) with combination of IBA (12.3, 24.6 µM) and IAA (14.2, 28.5 µM) for 3 to 4 weeks. After 15 days, the cultures that showed rooting were transferred to the fresh media and were maintained up to 4 weeks. After the observed time, the plantlets were successfully uprooted, washed one or two times with the autoclaved distilled water to remove the remaining basal callus and adhering media before transferring them to a pot containing sterilized autoclaved soil mixed with vermiculite in 1:1 ratio and pots were covered with perforated poly-bags and kept inside culture room for hardening for 2 weeks and then to greenhouse. Observation on the average number of roots per shoot, the percentage of rooting response and the mean root length were recorded after the 4 weeks of culture.

DNA extraction: Genetic stability of *in vitro* raised plantlets was analyzed by using RAPD and ISSR molecular markers. DNA was isolated from the leaves of both mother plant and the tissue cultured raised plants by modified CTAB method

suggested by Doyle and Doyle[3]. Further the quality and integrity of isolated DNA were confirmed by the gel electrophoresis on agarose gel of 0.8% (w/v) prepared in 1X TAE buffer. Ethidium bromide strain (1mg/L) was used to visualize the DNA. The quantification of DNA was measured by the spectrophotometric analysis using BioSpec-nano UV-VIS Spectrophotometer (Shimadzu). The isolated DNA dilution to 50ng/µl was performed and then finally stored at -20°C[14].

Analysis (PCR) of RAPD and ISSR markers: PCR was performed in thermocycler (Applied Biosystems by Thermo Fisher Scientific). 15 random decamer oligonucleotide RAPD and 20 ISSR markers were used for the amplification purposes. The RAPD reaction was prepared in 20µl reaction volumes containing 2 µl of genomic DNA, 2.0µl of 10 X taq polymerase buffer, 3mM $MgCl_2$, 1.6 µl of 0.2 mM dNTPs mix, 0.4 µl of RAPD primer, 1U/ µl of Taq DNA Polymerase and 12.65 µl of Mill-Q water. After initial denaturation at 94°C for 5 min, PCR were run for 40 cycles each of 1min at 94°C, annealing temperature was determined and changed according to the primers. The final step consisted of 1 cycle of 8 min at 72°C for complete polymerization.

The ISSR reaction was prepared in 20 µl reaction mixture having 2 µl of genomic DNA, 2 µl of 10X Taq polymerase buffer, 0.2 mM dNTPs mix, 0.4 µM of ISSR primer, 1U/µl of Taq DNA polymerase and 12.65 µl of Milli-Q water. After the initial denaturation at 94°C for 5 min, PCR cycle was run for 40 cycles consisting first of denaturation step of 30 sec at 94°C, in which annealing temperature differs according to the primers and finally an extension at 72°C for 1 minute. A final extension was followed at 72°C for 10 minutes[6].

Amplified PCR products were separated in 1% agarose gel using 1X TAE buffer and the gels were visualized using a gel documentation system (UVP Gel-Doc, Analytik Jena). Two independent amplifications (PCR) for each sample with ISSR and RAPD primers were performed and only scorable bands were considered for analysis.

Results and Discussion

Callus formation and shoot regeneration: Among the different auxins concentration tested, the highest percentage of callus formation (84.44± 0.27 %) was observed in MS medium 36.19 µM of 2, 4-D in combination with 11.10 µM of BAP after 6 weeks, with a fragile yellowish-brown callus. The results showed the combination of auxins with cytokinins as the most appropriate hormone combination for callus induction from the leaves explants of *Rheum emodi*. Further, decrease in callusing was observed in MS basal medium devoid of plant growth hormone. The callus was subcultured once in every 2 weeks before it showed the signs of regeneration.

Table 1
Effect of different cytokinins (treatments) on regenerative response of 6-week callus cultures derived from leaves explant of *R emodi*.

Phytohormones (PPM)			% of Shoot Regeneration	Mean No. of Shoots/Callus	Mean Shoot
BAP	Kn	TDZ	Mean ±SE	± SE	Length(mm)± SE
2	1.5	-	35.55± 0.27bc	1.33± 0.54a	6.00±2.49abc
4	1.5	-	53.33±0.47defgh	2.33±0.27ab	7.67±1.78abc
6	1.5	-	55.56± 0.27efghi	2.33±0.27ab	9.33±2.76abce
8	1.5	-	51.11±0.27cdefgh	1.33±0.27a	8.67±1.09abc
2	2	-	53.33±0.82defgh	1.00±0.47a	7.33±3.03abc
4	2	-	62.22±0.27ghij	1.67±0.72ab	10.33±4.53abce
6	2	-	66.67±0.47hij	2.00±0.47ab	16.67±0.98e
8	2	-	55.56±0.27efghi	1.67±0.27ab	9.33±1.44abce
2	2.5	-	48.89±0.27cdefg	2.00±0.47ab	10.67±0.54abce
4	2.5	-	71.11±0.27ij	2.33±0.72ab	10.33±2.60abce
6	2.5	-	75.56±0.27j	3.67±0.27b	19.00±1.70e
8	2.5	-	60.00±0.47ghi	2.33±0.72ab	14.00±2.9bce
2	3	-	44.44±0.27bcdef	1.67±0.72ab	9.00±3.74abc
4	3	-	46.67±0.47bcdefg	1.33±0.27a	15.33±2.37ce
6	3	-	48.89±0.54cdefg	2.00±0.82ab	12.33±1.91abce
8	3	-	42.22±0.72bcde	1.67±0.27ab	12.33±1.19abce
-	-	2	20.00±1.25a	1.33±0.54a	5.33±2.37ab
-	-	4	37.78±0.72bcd	1.00±0.47a	3.67±1.52a
-	-	6	31.11±0.72ab	0.67±0.27a	5.33±2.37ab
-	-	8	20.00±1.25a	0.67±0.54a	3.00±2.45a

Means of values having the same letters in columns are not significantly different by Duncan's multiple range test (P = 0.05).

Fig. 1: Shoot regeneration from the leaves (explants) of *Rheum emodi*. (A) Callus induction and Initiation of shoots; (B) Shoot initiation on the MS medium complemented with 36.19 µM of 2,4-D in combination with 11.10 µM of BAP after 6 weeks of calli; (C), (D) Multiple shooting from calli on MS+BAP (8.88 µM) after 6 weeks; (E) *In vitro* rooting on MS medium perforated with IAA (28.5 µM) + BAP (8.88 µM) after 3 weeks; (F) Plantlets under acclimatization phase

Table 2
Effect of different concentration and combination of auxins and strength of MS medium on rooting of adventitious shoots of *Rheum emodi*

Treatments	No. of roots per shoot Mean ±SE	Root length(mm) Mean ±SE
Half MS	1.33±0.27 a	7.66±0.54
Full MS	1.33±0.27a	9.33±0.98
MS+IBA(2.5µM) +BAP (2 µM)	3±0.47b	9.66±0.98
MS+IBA(5µM) +BAP (2 µM)	3.33±0.27bc	10.00±0.47
MS+IBA(2.5µM) +BAP (4 µM)	4.0±0bcd	10.0±0.47
MS+IBA(5µM) +BAP (4 µM)	4.33±0.27 cd	11.0±0.47
MS+IAA(2.5µM) +BAP (2 µM)	4.33±0.27cd	10.66±0.98
MS+IAA(5µM) +BAP (2 µM)	5.0±0.47d	11±1.25
MS+IAA(2.5µM) +BAP (4 µM)	3±0.47b	11.33±0.54
MS+IAA(5µM) +BAP (4 µM)	4.33±0.27cd	10.66±0.54

Mean Values that are followed by the same letters are not significantly different ($P \leq .05$), according to test (Duncan's multiple range test).

Shoot regeneration (Fig. 1) was observed. Table 1 shows the maximum frequency of adventitious shoot regeneration (75.56±0.27%) and the maximum number of shoots per explant (3.67±0.27) was observed on MS medium containing BAP (35.5µM) and Kn (11.61µM). No regeneration was observed in hormone–free medium.

Rooting and hardening: The shoots of about 15-20 mm height were transferred to hormone free MS basal medium or full-strength MS basal medium. The high frequency of rooting was observed in MS basal medium (full strength) + IAA (28.55 µM) in combination with BAP (8.88 µM) with the maximum frequency of roots per shoot of 5.0±0.47 with an average root length of 11±1.25mm (Table 2). The rooted plantlets were carefully transferred to pots having sterilized soil mixed with the vermiculite in 1:1 ratio and then pots were covered with the poly-bags with holes and were kept inside the culture room for acclimatization for 15 days.

Assessment of clonal fidelity of regenerated plants: The major drawback among the *in vitro* cultured plantlets is incidence of somaclonal variation that relies on the level of plant growth hormones, source of explant and the mode of their regeneration. Assessment of genetic homogeneity of *in vitro* raised plantlets is a precondition in the application of biotechnology for the micropropagation of true-to–type clones. The molecular markers could be proved as a innovative tool as effectively engaged to evaluate the genetic stability and true-to-type nature of the *in vitro* regenerated plants.

In the present study, to assess the genetic fidelity, randomly selected nine *in vitro* regenerated *Rheum emodi* plants with a control plant (mother plant) were taken and assessment was done by using RAPD and ISSR markers.

Out of 15 RAPD primers that were screened, 7 primers produced reproducible, scorable and resolvable 32 bands, having the length ranging from the 300 to 2500 bp and number of the distinct bands aimed for each primer set varied from 02 (REi7) to 07 (REi10) with an average of 4.5 bands per primer (Table 3). All the 7 RAPD markers showed the identical and similar DNA banding pattern among all the regenerated plants in respect to their mother plant, indicating the monomorphic nature. Similarly, among the 20 ISSR marker screened, 15 primers produced a total of 66 bands with the length ranging from 300 to 2700 bp. The number of scorable bands for each primer was wide-ranging from 02 (IRr13) to 10 (IRr15) with an average of 4.4 bands per primer (Table 4).

All the 15 ISSR markers showed scorable, resolvable, monomorphic banding pattern. Thus, the current study confirms that no polymorphism was detected during RAPD and ISSR analysis, revealing the genetic stability among the *in vitro* raised plantlets. Fig. 2 shows the monomorphic amplification pattern of *in vitro* generated *Rheum emodi* plantlets obtained with RAPD primer REi4 and REi6 and ISSR primer IRr17 and IRr16.

Table 3
RAPD primers used for assessing the genetic stability in micropropagated plantlets of *Rheum emodi*

Primer Code	Primer Sequence (5´-3´)	No. of total bands	Range of amplification(bp)
REi2	GGGTAACGCC	06	300-1300
REi4	AAGACCCCTC	05	600-2000
REi6	GAGTGGTGAC	03	600-1600
REi7	AACGCGTCGG	02	1600-1800
REi8	AAGGGCGAGT	06	600-1200
REi10	CAAACGTCGG	07	550-3000
REi11	GGACTGGAGT	03	1300-2500

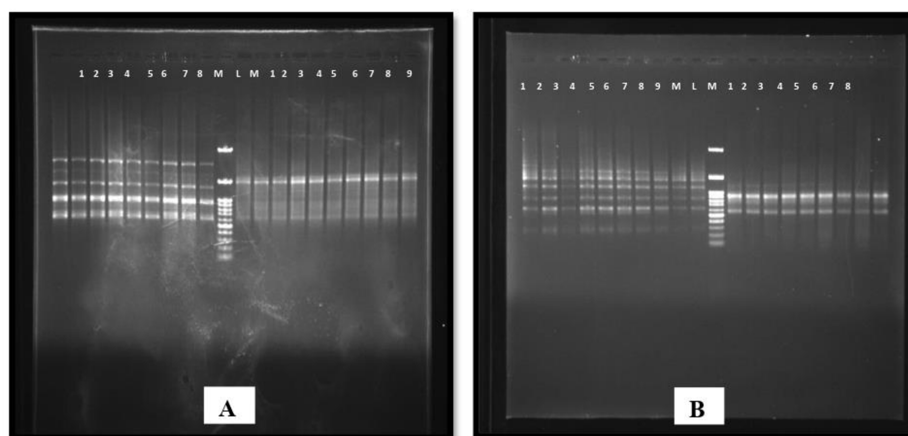

Fig. 2: RAPD and ISSR profiles of the *invitro* regenerated plants and mother plant and *Rheum emodi*. (A)PCR amplification products obtained with RAPD- REi4 (5'-AAGACCCCTC-3') and RAPD-REi6 (5'-GAGTGGTGAC-3') primer; (B) PCR amplification products obtained with ISSR-IRr17 (5'-AGTCGTAGTACACACACACACAC-3') and ISSR-IRr16 (5'-GAGAGAGAGAGAGAGACGACTC-3') primer. Lane L, 100 bp DNA ladder (Himedia); Lane M, mother plant; Lane 1-9, randomly selected micropropagated plants.

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Table 4
ISSR primers used for assessing the genetic stability in micropropagated plantlets of *Rheum emodi*

Primer Code	Primer Sequence (5´-3´)	No. of total bands	Range of amplification (bp)
IRr2	AGAGAGAGAGAGAGAGCTA	03	650-1400
IRr3	CTCTCTCTCTCTCTCTAGC	01	900
IRr6	AGAGAGAGAGAGAGAGCTT	04	500-1200
IRr9	GAGAGAGAGAGAGAGACTG	04	650-1500
IRr10	GAGAGAGAGAGAGAGAC	06	550-1800
IRr11	AGAGAGAGAGAGAGAGT	06	650-1700
IRr12	AGAGAGAGAGAGAGAGC	04	1100-2000
IRr13	AGAGAGAGAGAGAGAGG	02	700-1500
IRr14	GAAGAAGAAGAAGAAGAA	03	550-1700
IRr15	GAGAGAGAGAGAGAGACTT	10	550-2700
IRr16	GAGAGAGAGAGAGAGACGACTC	04	600-1200
IRr17	AGTCGTAGTACACACACACACAC	06	300-1600
IRr18	AGTACGAGTTGTGTGTGTGTGTG	03	350-800
IRr19	ACGAGTAGGGTGTGTGTGTGTGT	04	450-1600
IRr20	CTGAGTCGTCACACACACACACA	06	350-2000

Conclusion

Rheum emodi, a highly medicinal plant in Uttarakhand, now belongs to the endangered list. Local communities use rhizome and rhizome preparation for many medicinal properties as well as common eating purposes. Due to its overexploitation and climatic changes, the plant has been under tremendous pressure. All this leads to its decline in its natural habitat, thus this herb species demands conservation. *Rheum emodi* seeds have very low viability; they reduced their germination potential by half in six months. All these circumstances focus the interest to the plant regeneration through the tissue culture techniques as it can be proved to be a better option for its propagation, thus leading to its conservation and can be implemented for the mass propagation in forestry.

The current study focuses on the complete plant regeneration of *Rheum emodi*, which was obtained from MS media perforated with the 35.5 μM of BAP and 11.61 μM of Kinetin. This method is useful for those plants in which production of seeds is very less, the viability of seeds is low or the seeds are very expensive. These approaches could be used in the future for mass propagation of *Rheum emodi*. More studies and experiments should be performed for improved and better output. Development of medicinal plant sector should be done and dissemination of synthetic seeds should be promoted.

In current study, a total of 7 RAPD and 15 ISSR primer sets generate 98 distinct amplicons that give rise to the monomorphic patterns across all the randomly selected regenerated plantlets analyzed. Results shows that there is no polymorphism within the RAPD and ISSR analysis among the *in vitro* raised plantlets and the mother plant and thus confirms the genetic homogeneity of *in vitro* raised plantlets. These DNA based molecular markers have acted as versatile tools in different fields of biology. These markers could be proved to be an ideal tool for schedule analysis of genetic stability within the micropropagated plantlets prior to commercialization.

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References

1. Anandan R., Prakash M., Deenadhayalan T., Nivetha R. and Kumar N.S., Efficient *in vitro* plant regeneration from cotyledon-derived callus cultures of sesame (Sesamum indicum L.) and genetic analysis of True-to-Type regenerants using RAPD and SSR markers, *South African Journal of Botany*, **119**, 244–251 **(2018)**

2. Baro T. and Das S., *In vitro* propagation and somaclonal variation study of *Phlogacanthus thyrsiformis nees* an ethnic medicinal plant, *Plant Science Today*, **9(sp2)**, 18-23 **(2022)**

3. Doyle J.J. and Doyle J.L., Isolation of plant DNA from fresh tissue, *Focus*, **12**, 13-15 **(1990)**

4. Farooq U., Abaas G., Sagoo M.I.S. and Dar M.A., Ethnobotany of some selected monochlamydeae plant species from north western Himalaya, India, *Journal of Medicinal Plants Research*, **8**, 834–839 **(2014)**

5. Khan M.I., Shahzad A., Ganie I.B. and Pandith S.A., Organogenesis, direct somatic embryogenesis and shoot proliferation of *Rheum speciforme Royle:* An endemic and vulnerable medicinal herb from Indian Trans Himalayas, *In Vitro Cellular and Developmental Biology-Plant*, **58(1)**, 35-50 **(2022)**

6. Kumar S., Mangal M., Dhawan A.K. and Singh N., Assessment of genetic fidelity of micropropagated plants of *Simmondsia chinensis* (Link) Schneider using RAPD and ISSR markers, *Acta Physiol Plant*, **33**, 2541-2545 **(2011)**

7. Lal N. and Ahuja P.S., Propagation of Indian Rhubarb *Rheum emodi Wall.* using shoot-tip and leaf explants culture, *Plant Cell Reports*, **8**, 493-496 **(1989)**

8. Lal N. and Ahuja P.S., Assessment of liquid culture procedures for in vitro propagation of *Rheum emodi*. *Plant Cell, Tissue and Organ Culture*, **34**, 223-2226 **(1993)**

9. Park S.K. and Lee Y.K., Antioxidant Activity in *Rheum emodi* Wall (Himalayan Rhubarb), *Molecules*, **26**, 2555 **(2021)**

10. Rawat J.M., Rawat B., Agnihotri R.K., Chandra A. and Nautiyal S., *In vitro* propagation, genetic and secondary metabolite analysis of Aconitum violaceum Jacq.: a threatened medicinal herb, *Acta Physiol. Plant*, **35(8)**, 2589-2599 **(2003)**

11. Singh R. and Chaturvedi P., *In vitro* propagation and establishment of adventitious root cultures of *Rheum emodi* Wall. ex Meisn for quantification of emodin production, *In Vitro Cell Dev. Biol.-Plant*, **58**, 80–92 **(2022)**

12. Sultana K.W., Das S., Chandra I. and Roy A., Efficient micropropagation of Thunbergia coccinea wall. and genetic homogeneity assessment through RAPD and ISSR markers, *Scientific Reports*, **12(1)**, 1-11 **(2022)**

13. Tabin S. et al, *In Vitro* Culture of *Rheum emodi* Wall: An Endangered Medicinal Plant of Northwestern Himalaya, Proc. Natl. Acad. Sci., India, Sect. B Biol. Sci., **88**, 995–1006 **(2018)**

14. Tikendra L., Sushma O., Amon T., Devi N.A., Paonam S., Bindyananda N., Potshangbam A.M., Dey A., Devi R.S. and Nongdam P., Genetic clonal fidelity assessment of rhizome–derived micropropagated *Acorus calamus* L. – a medicinally important plant by random amplified polymorphic DNA and inter-simple sequence repeats markers, *Phcog Mag.*, **18**, 207-15 **(2022)**

15. Upadhyay S., Uniyal A., Kumar V. and Gupta S., Occurrence, Distribution, Conservation, Ethanobotanical and Pharmacological aspects of endangered medicinal herb belonging to Genus *Rheum*, *Res. J. Chem. Environ.*, **26(7)**, 168-186 **(2022)**.

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