Title

Autopolyploidization and in vitro regeneration of three highbush blueberry (Vaccinium corymbosum L.) cultivars from leaves and microstems

Abstract

Abstract: Blueberries are a fruit with an increasing global demand due to their phytochemical and bioactive compounds content. They are promoted worldwide because of their health benefits. For optimal growth and productivity, blueberry crops need acidic soil pH, specific chilling hours, and an adequate atmospheric temperature. This delicate production equilibrium is under severe threat from climate change, potentially leading to reduced yields and increased cultivation costs unless new cultivars are developed for each edafoclimatic zone. Therefore, considering varietal replacements with more productive cultivars offering higher quality and better adaptability to local conditions is imperative. In this study, we employ polyploidization and in vitro tissue culture to promote variability and lay the foundation for new cultivar development. We report the successful induction of octoploids in three blueberry cultivars, namely 'Biloxi', 'Legacy', and 'Duke', through whole-genome duplication. Leaves and microstem explants were exposed to 0.1% colchicine for 24 and 48 hours in in vitro culture. After analyzing the polyploid level of 160 regenerated shoots using DNA flow cytometry, we obtained a total of 18 mutants, consisting of 8 mixoploids and 10 octoploids. The number of chloroplasts in the stomata was analyzed by fluorescence microscopy, revealing the duplication of these organelles in the induced octoploid plants. To our knowledge, this represents the first successful induction of octoploids in three blueberry cultivars -'Biloxi,' 'Legacy,' and 'Duke'- achieved by exposing leaves and microstem explants to colchicine in in vitro culture. This technique holds promise as a valuable tool for the development of improved blueberry cultivars. Key message: This study presents the first successful induction of octoploids of three blueberry cultivars 'Biloxi',

'Legacy', and 'Duke' by inducing polyploidization exposing leaves and microstems

explants to colchicine in in vitro culture. © The Author(s), under exclusive licence to

Springer Nature B.V. 2024.

Authors

Jarpa-Tauler G.; Martínez-Barradas V.; Romero-Romero J.L.; Arce-Johnson P.

Author full names

Jarpa-Tauler, Gabriela (59204140100); Martínez-Barradas, Vera (57205673767);

Romero-Romero, Jesús Lucina (26647042900); Arce-Johnson, Patricio (6602667391)

Author(s) ID

59204140100; 57205673767; 26647042900; 6602667391

Year

2024

Source title

Plant Cell, Tissue and Organ Culture

V		Lo	m	0
·W	u	Iu		

158.0

Issue

1

Art. No.

18

DOI

10.1007/s11240-024-02810-9

Link

 $https://www.scopus.com/inward/record.uri?eid=2-s2.0-85197511246\&doi=10.1007\\ \%2fs11240-024-02810-9\&partnerID=40\&md5=fd6e6fc141899caa77838dc04a847f4\\ 3$

Affiliations

Facultad de Agronomía y Sistemas Naturales, Pontificia Universidad Católica de Chile, Santiago, Chile; Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile; Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, (CIIDIR- IPN), Unidad Sinaloa, Guasave, Mexico;

Instituto de Ciencias Aplicadas, Facultad de Ingeniería, Universidad Autónoma de Chile, Santiago, Chile

Authors with affiliations

Jarpa-Tauler G., Facultad de Agronomía y Sistemas Naturales, Pontificia Universidad Católica de Chile, Santiago, Chile; Martínez-Barradas V., Facultad de Agronomía y Sistemas Naturales, Pontificia Universidad Católica de Chile, Santiago, Chile, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, Chile; Romero-Romero J.L., Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, (CIIDIR- IPN), Unidad Sinaloa, Guasave, Mexico; Arce-Johnson P., Instituto de Ciencias Aplicadas, Facultad de Ingeniería, Universidad Autónoma de Chile, Santiago, Chile

Author Keywords

Adventitious shoot regeneration; Colchicine; DNA flow cytometry; Polyploidy breedin; Vaccinium corymbosumL; Whole genome duplication

Index Keywords

Colchicine; Cultivation; Development; Equilibrium; Explants; Fruits; Leaves; Tissue Culture; Climate change; Cultivation; Flow cytometry; Fruits; Plants (botany); Tissue culture; Adventitious shoot regenerations; Blueberry cultivars; Colchicine; DNA flow cytometry; Genome duplication; In-vitro; Polyploidy breedin; Vaccinia corymbosuml; Vitro culture; Whole genome duplication; Fluorescence microscopy

Funding Details

Pontificia Universidad Católica de Chile, UC; Agencia Nacional de Investigación y Desarrollo, ANID; Consejo Nacional de Humanidades, Ciencias y Tecnologías, Conahcyt, (739582); Consejo Nacional de Humanidades, Ciencias y Tecnologías, Conahcyt

Funding Texts

Funding text 1: The authors thank Alex Cabrera and Sergio Bustos for their technical assistance in the flow cytometry service, Pamela Naulin for her supervision in the plant experimentation unit center of the UC Faculty of Biological Science, and Nicole Salgado for her technical support with the confocal microscope in the advanced microscopy facility UMA UC, of the Pontificia Universidad Cat\u00F3lica de Chile.; Funding text 2: GJT was supported by Agencia Nacional de Investigaci\u00F3n y Desarrollo (Chile) 21191617 PhD scholarship and the 2018 Doctoral Scholarship from Vicerrector\u00EDa de Investigaci\u00F3n of Pontificia Universidad Cat\u00F3lica de Chile. VMB was supported by Consejo Nacional de Ciencia y 739582, Tecnolog\u00EDa (M\u00E9xico) and Agencia Nacional de Investigaci\u00F3n y Desarrollo (Chile) 21200394 PhD scholarships.

References

Adams K.L., Wendel J.F., Polyploidy and genome evolution in plants, Curr Opin Plant Biol, 8, pp. 135-141, (2005); Arumuganathan K., Earle E.D., Estimation of nuclear DNA content of plants by flow cytometry, Plant Mol Biol Rep, 9, pp. 229-241, (1991); Beaulieu J.M., Leitch I.J., Patel S., Et al., Genome size is a strong predictor of cell size and stomatal density in angiosperms, New Phytol, 179, pp. 975-986, (2008);

Bharadwaj D.N., Polyploidy in crop improvement and evolution, Plant Biology and Biotechnology. Volume I: Plant Diversity, Organization, function and improvement, (2015); Blakeslee A.F., Avery A.G., Methods of inducing doubling of chromosomes in plants by treatment with colchicine, I Hered, 28, pp. 393-411, (1937); Brazelton C., Fain C., Ogg M., Global State of the Blueberry Industry Report In, (2023); Broertjes C., Keen A., Adventitious shoots: do they develop from one cell?, Euphytica, 29, pp. 73-87, (1980); Clapa D., Nemes S.A., Ranga F., Et al., Micropropagation of Vaccinium corymbosum L.: an alternative procedure for the production of secondary metabolites, Horticulturae, 8, (2022); Comai L., The advantages and disadvantages of being polyploid, Nat Rev Genet, 6, pp. 836-846, (2005); Cui Y., Hou L., Li X., Et al., In vitro induction of tetraploid Ziziphus jujuba Mill. Var. Spinosa plants from leaf explants, Plant Cell Tissue Organ Cult, 131, pp. 175-182, (2017); De Jesus-Gonzalez L., Weathers P.J., Tetraploid Artemisia annua hairy roots produce more artemisinin than diploids, Plant Cell Rep, 21, pp. 809-813, (2003); Debnath S.C., A two-step procedure for adventitious shoot regeneration from in vitro-derived lingonberry leaves: shoot induction with TDZ and shoot elongation using zeatin, HortScience, 40, pp. 189-192, (2005); Debnath S.C., Propagation of Vaccinium in vitro: a review, Int J Fruit Sci, 6, pp. 47-71, (2007); Deng B., Du W., Liu C., Et al., Antioxidant response to drought, cold and nutrient stress in two ploidy levels of tobacco plants: low resource requirement confers polytolerance in polyploids?, Plant Growth Regul, 66, pp. 37-47, (2012); Dhooghe E., van Laere K., Eeckhaut T., Mitotic chromosome doubling of plant tissues in vitro, Plant Cell Tissue Organ Cult, 104, pp. 359-373, (2011); Dibyendu T., Cytogenetic characterization of induced autotetraploids in grass pea (Lathyrus sativus L), Caryologia, 63, pp. 62-72, (2010); FlowJo™ Software Version 10, 8, (2022); Gahan P.B., George E.F., Adventitious regeneration, Plant Propagation by Tissue Culture, 3Rd Editio. Springer Netherlands, pp. 355-401, (2008); Galbraith D.W., Harkins K.R., Maddox J.M., Et al., Rapid flow cytometric analysis of the cell cycle in intact plant tissues, Science, 220, pp. 1049-1051,

(1983); Ghanbari M.A., Jowkar A., Salehi H., Zarei M., Effects of polyploidization on petal characteristics and optical properties of Impatiens walleriana (Hook), Plant Cell Tissue Organ Cult, 138, pp. 299-310, (2019); Guerra D., Wittmann M.T.S., Schwarz S.F., Et al., Comparison between diploid and tetraploid citrus rootstocks: morphological characterization and growth evaluation, Bragantia, 73, pp. 1-7, (2014); He M., Yang N., Liu X., Zhang H., Establishment of Vaccinium Ashei Tissue Culture System and Polyploidy Induction, 23, pp. 59-61, (2019); Huber G., An Investigation of Highbush Blueberry Floral Biology and Reproductive Success in British Columbia, (2016); Kalt W., Cassidy A., Howard L.R., Et al., Recent research on the health benefits of blueberries and their anthocyanins, Adv Nutr, 11, pp. 224-236, (2020); Kashtwari M., Jan S., Wani A.A., Dhar M.K., Induction of polyploidy in saffron (Crocus sativus L.) using colchicine, | Crop Improv, 36, pp. 555-581, (2022); Lei L., Liu G., Yan D., Et al., Manipulation of ploidy for blueberry breeding: In vitro chromosome doubling of diploid Vaccinium duclouxii (Lévl.) Hand.-Mazz by trifluralin, Sci Hortic, 317, (2023); Li X., Zhang Z., Li Y., Et al., Study on polyploid induction of blueberry in vitro with colchicine treatment, J Northeast Agric Univ, 41, pp. 38-42, (2010); Lloyd G., McCown B., Commercially-feasible micropropagation of mountain laurel, Kalmia latifolia, by use of shoot-tip culture Comb, Proc. Int. Plant Propagators Soc., 30, pp. 421-427, (1980); Lu Y., Chen J., Xiao M., Et al., An overview of tubulin inhibitors that interact with the colchicine binding site, Pharm Res, 29, pp. 2943-2971, (2012); Ma Y., Xue H., Zhang L., Et al., Involvement of auxin and brassinosteroid in dwarfism of autotetraploid apple (Malus × Domestica), Sci Rep, 6, pp. 1-14, (2016); Marangelli F., Pavese V., Vaia G., Et al., In vitro polyploid induction of highbush blueberry through de novo shoot organogenesis, Plants, 11, pp. 1-10, (2022); Miyashita C., Ishikawa S., Mii M., In vitro induction of the amphiploid in interspecific hybrid of blueberry (Vaccinium corymbosum × Vaccinium ashei) with colchicine treatment, Sci Hortic, 122, pp. 375-379, (2009); Mu H.Z., Liu Z.J., Lin L., Et al., Transcriptomic analysis of phenotypic changes in birch (Betula

platyphylla) autotetraploids, Int J Mol Sci, 13, pp. 13012-13029, (2012); Nakel T., Tekleyohans D.G., Mao Y., Et al., Triparental plants provide direct evidence for polyspermy induced polyploidy, Nat Commun, 8, pp. 1-7, (2017); Nukaya T., Sudo M., Yahata M., Et al., The confirmation of a ploidy periclinal chimera of the Meiwa kumquat (Fortunella Crassifolia Swingle) induced by colchicine treatment to nucellar embryos and its morphological characteristics, Agronomy, 9, (2019); Podwyszynska M., Mynett K., Markiewicz M., Chromosome doubling in genetically diverse bilberry (Vaccinium myrtillus L.) accessions and evaluation of tetraploids in terms of phenotype and ability to cross with highbush blueberry (V. corymbosum L)., (2021); Qiu D., Wei X., Fan S., Et al., Regeneration of blueberry cultivars through indirect shoot organogenesis, HortScience, 53, pp. 1045-1049, (2018); Ren Y., Gao J., Dong X., Et al., In vitro tetraploid induction and plant regeneration of Pinellia ternata, a pearl-like herb, Plant Cell Tissue Organ Cult, 156, pp. 1-14, (2024); Ricci A., Capriotti L., Mezzetti B., Et al., Adventitious shoot regeneration from in vitro leaf explants of the peach rootstock Hansen 536, Plants, 755, (2020); Schindelin I., Arganda-Carreras I., Frise E., Et al., Fiji: an open-source platform for biological-image analysis, Nat Methods, 9, pp. 676-682, (2012); Sedov E.N., Apple breeding programs and methods, their development and improvement, Russ J Genet Appl Res, 4, pp. 43-51, (2014); Song G.Q., Hancock J.F., Vaccinium, Wild crop relatives: genomic and breeding resources, (2011); Tan F.Q., Zhang M., Xie K.D., Et al., Polyploidy remodels fruit metabolism by modifying carbon source utilization and metabolic flux in Ponkan mandarin (Citrus reticulata Blanco), Plant Sci, 289, (2019); Te Beest M., Le Roux J.J., Richardson D.M., Et al., The more the better? The role of polyploidy in facilitating plant invasions, Ann Bot, 109, pp. 19-45, (2012); Van Drunen W.E., Husband B.C., Whole-genome duplication decreases clonal stolon production and genet size in the wild strawberry Fragaria vesca, Am J Bot, 105, pp. 1712-1724, (2018); Wang W., He Y., Cao Z., Deng Z., Induction of tetraploids in impatiens (Impatiens walleriana) and characterization of their changes in morphology and resistance to downy mildew, HortScience, 53, pp. 925-931, (2018); Xue H., Zhang B., Tian J.R., Et al., Comparison of the morphology, growth and development of diploid and autotetraploid 'Hanfu' apple trees, Sci Hortic, 225, pp. 277-285, (2017); Yahata M., Katsumi I., Katori C., Et al., Induction and characterization of chromosome-doubled Nagabo-Natsuhaze (Vaccinium Sieboldii Miq.), a wild species native to Japan, by colchicine and oryzalin treatments of in vitro shoots, Hortic Res, 21, pp. 1-10, (2022); Zeldin E.L., McCown B.H., Towards the development of a highly fertile polyploid cranberry, Acta Hortic, pp. 175-180, (2002); Zhang X.Y., Hu C.G., Yao J.L., Tetraploidization of diploid Dioscorea results in activation of the antioxidant defense system and increased heat tolerance, J Plant Physiol, 167, pp. 88-94, (2010)

Correspondence Address

P. Arce-Johnson; Instituto de Ciencias Aplicadas, Facultad de Ingeniería, Universidad Autónoma de Chile, Santiago, Chile; email: patricio.arce@uautonoma.cl

Publisher

Springer Science and Business Media B.V.

ISSN

01676857

CODEN

PTCED

Language of Original Document

English				
Abbreviated Source Title				
Plant Cell Tissue Organ Cult.				
Document Type				
Article				
Publication Stage				
Final				
Source				
Scopus				
EID				
2-s2.0-85197511246				