Review Article

AComprehensiveReviewonRoleofPlantTissueCultureinOrnamentalCrops:CultivationFactors,ApplicationsandFutureAspects.

Abstract

Ornamentalplantsaregrownlargelyfortheirartisticvalue,floriculturistsmustprioritizetheproliferationandimprovementofqualitytraits,aswellastheproductionofuniquediversity.Micropropagation,clonalreliabilityandconservationareallcrucialfactorstoconsider.Applicationofinvitrotechniquesinornamentalplantsuchasinvitroembryorescue,somatichybridization,invitropollinationandinvitroploidymanipulationbuttoenhance,techniqueslikeasembryorescueandsomatichybridizationarecommonlyemployed.Thecreationofsyntheticseedallowsforseason-independentseedproducingandlong-termseedpreservation.Manyfactorsinfluenceornamentalplanttissueculture,includingplantgenotype,explanttype,andthephysicalenvironment(light,temperature,humidity,andCO2),inadditiontomediumcompositionandgrowthregulators.Wecompiledandreviewedanoverallupdateoncultivationfactors,applicationproceduresinornamentalplanttissueculture,invitroplantenhancementapproachesandfutureprospectsinthisstudy.

Keywords:explanttype,ornamentals,invitroculture,embryorescue,hybridization,temperature,light.

Introduction

Ingeneral,thephrase"ornamentalplant"or"ornamental"referstoplantsthataregrownprimarilyfortheiraestheticallypleasanttraitssuchasshape,bark,leaves,flowers,fruit,oranycombination.Globally,thefeasibleoutputofdecorativeplantsisexpanding[1].TheornamentalandfloriculturesectorintheUnitedKingdomisprojectedtobeworth£2.1billionin2005,withoverseascommerceof£60-75billion.Itseconomicworthhasskyrocketedoverthepreviousttwodecades,andthereisastronglikelihoodoffurtherexpansioninboththelocalandinternationalmarkets[2].Tissueculturesysteminornamentalflowerslikeroseshasbeenestablished[3,4,5,6].Recently,ininvitroflowerinductioninrosewasdemonstrated[7,8].Tissueculturetechniquesareusedformicropropagationandthegenerationo

fpathogen-freeplants[9].Planttissueculturehasemergedasapromisingmethod,formingthebaseofplantbiotechnology.Growerswanthigh-qualityplantingmaterialtoincreaseproduction.Theeffectivenessoftheclonalpropagationmethodisdependentonavarietyofparameters,includinggenotype,medium,plantgrowthregulators,andexplanttype,allofwhichshouldbeexperientialduringtheprocess[10].Naphthaleneaceticacid(NAA)anddbenzyladenine(BA)arethemostoftenutilizedgrowthregulatorsforornamentalplantmicropropagationviaorganogenesis,embryogenesis,andaxillaryproliferation.Thehighfrequencyofdirectembryogenesishasbeenstudiedinthinlayerculturesofhybridseedgeranium(Pelargonium).[11].Plantsraisedthroughmicropropagationareofuniformquality,pathogenfree,andcanbeproducedmuchmorequickly,withnewcultivarsbecomingcommerciallyavailablewithin2to3yearsofdevelopmentratherthanthe5to10yearsrequiredforconventionalpropagation.Theyalsoproduceuniformlysuperiorseedsandhaveimprovedvigorandquality.Propagationviameristemhasbeenidentifiedasamechanismofrapidregenerationin Caladiumandtheplantsproducedthroughthistechnologyhaveahighexportpotentialbecausetheycanbeshippedinternationallywithfewquarantinerestrictionsandhavethepotentialtodevelopnewcultivarsofthespecies.Severalstudieshavebeenconductedinrecentyearsinorder toproducesemi-automaticsystemsthatexploittheprincipleofdevelopmentintemporaryimmersionwiththegoalofavoidingtissuehyperhydricity.Somesystemsoftemporaryimmersion(TIS)havebeenemployedontropicalplants[12,13]andfruittrees[14].

1.ApplicationofInVitroTechniquesinOrnamentals

Planttissuecultureiswidelyknownforitsabilitytoproducedisease-freeplantletsthroughclonalreplication.Invitrocultivationprovidesseveralopportunitiesformodifyingplantmaterialstoincreasetheirquality.Forhybridization,invitroproceduresincludingasmicropropagation,embryorescueandsomatichybridizationareutilized.

1.1.PlantImprovementbytheApplicationofInVitroEmbryoRescue

Developingaviableplantfromanembryoisreferredtoasembryocultureorembryorescue(Figure1).Hannigpioneeredtheembryoculturetechniquebygrowingmatureembryosofafew Brassicaceaeplantsonsugar-supplementedsaltmedia[15].Dietrichrevealedin1924thatbothmatureandimmatureembryosmaybesaved.[16].In1925,thefirstinterspecifichybridizationwasdescribedintheperennialflax(*Linum perenne*L.x*Linumaustriacum*L.)throughembryorescuefromnonviableseeds[17].Sinceitsdiscover

y,embryorescuehasbeenemployedforinterspecifichybridizationinawiderangeofcrops,includingfloral,decorative,medicinal,andwoodyplants[18,19].

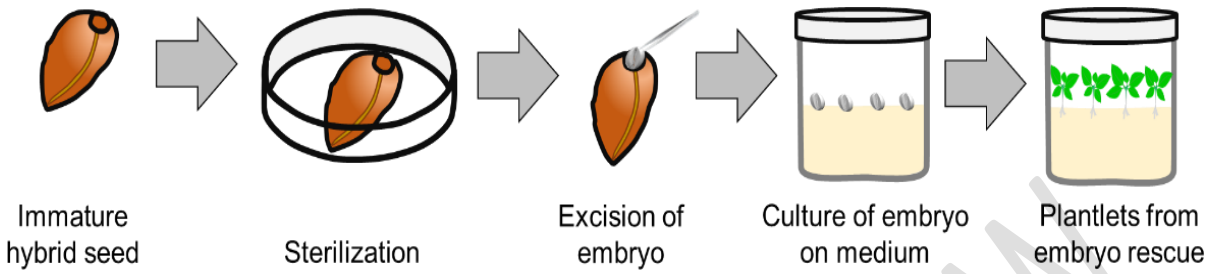

Figure1.Processofembryorescuefromimmature(ornon-viable)seedafterhybridization

Itallowsfortheculture oftheovary,ovule,andembryo[20,21].Thesuccessofembryorescuedependsonvariousfactors,suchassizeandageoftheembryo,intactnessofembryo,excisionprocedure,sterilization,culturemedium,supplementationinculturemedium,light,temperature,etc.Ithasbeenusedincropimprovementbyintraspecific/interspecific/intergenerichybriddevelopment,haploid/doublehaploidproduction,overcomingembryoabortion,overcomingseeddormancy,overcomingselfandcross-incompatibility,shorteningthebreedingcycle,propagatingrareplants,etc.[22,23].Forexample,breedingcycleswereshortenedbyembryorescueinrose[24],andlily[25].Interspecifichybridsweredevelopedinchrysanthemumsbyembryorescuetolerant[26,27],salt-tolerant[28],aphidresistance[29],andheterotic[30,31]characteristics.Anewflowershapeandcold-tolerantintraspecific(*Campanulacarpatica*'White')andinterspecific(*C.medium*and*C.formanekiana*)hybrid,respectively,weredevelopedinbellflowers[32].Interspecifichybrids,haploids,ordoublehaploidsweredevelopedinrose[33],tulip[34],lisianthus[35],lily[36]andornamentalalliums[37,38].Embryrescuehasbeenwidelystudiedforcropimprovement,whileitscurrentresearchhasbeenreducedbytherapidevolutionofadvancedmolecularbreeding.

Furthermore,embryorescueiscommonlyemployedtoovercomepost-fertilizationbarriersinplants,althoughmanyornamentalshavepre-fertilizationbarriersthatmaybeovercomebyin-vitropollination.Plantreproductivecells(stigmaandanther)areseparatedandunitedundercontrolledcircumstancestoformazygoticembryowithin-vitropollination.Thein-vitroapproachhasbeenusedforbloomingandpollinationinseveralornamentals[39,40].

1.2.PlantImprovementbySomaticHybridizationandInVitroPollination

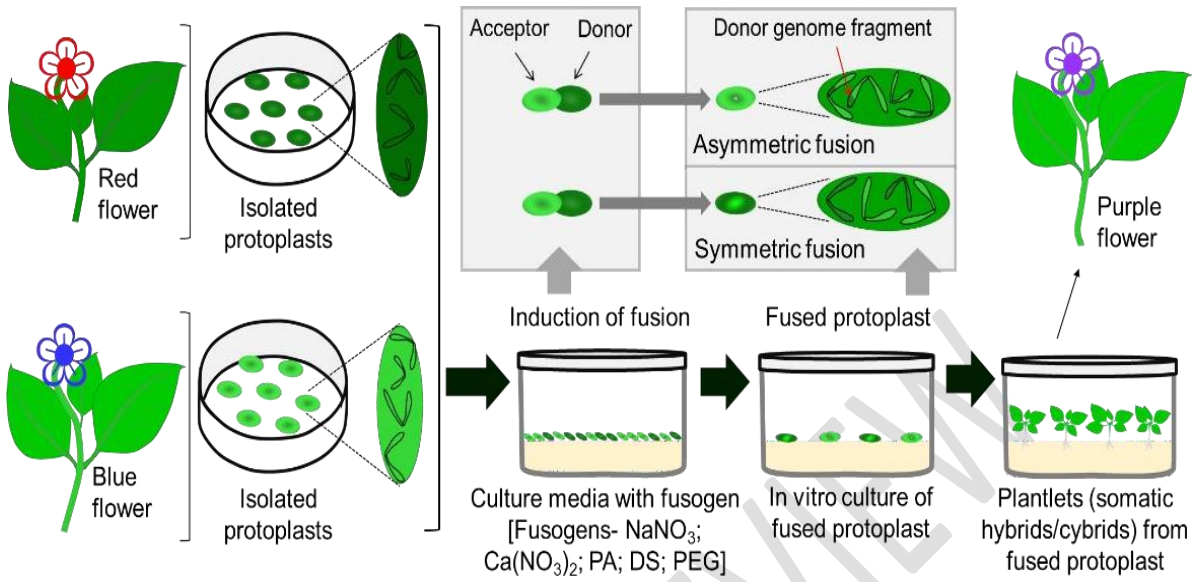

Figure2.Illustrationofsomatichybridorcybriddevelopmentthroughprotoplastfusion.Here, $NaNO_3$;sodiumnitrate,$Ca(NO_3)_2$;calciumnitrate,PA;polyvinylalcohol,DS;dextransulfate,polyethyleneglycol(PEG).

Somatichybridizationhasbeenshowntobeasignificantsourceofgeneticdiversity,alsoknownassomaclonalvariation.Manysomaclonesarethoughttobebetterhybrids.Thedonor-recipientapproachandcytoplast-protoplastfusionarethetwomostcommonstrategiesforproducingsomatichybrids.Incytoplast-protoplastfusion,protoplastsareallowedtofuse

inordertocombinesomaticcellsfromvariouscultivars,species,orgenera(Figure2).

Somatichybridizationoccurswhenthenucleargenomeofoneparentiscombinedwiththemitochondrialand/orchloroplastgenomeoftheotherparent.Thedonor-recipientfusionapproach,whichtransfersparticulargenesorchromosomes,isanalternativeandbetterwayofsomaticincompatibility[41,42].Fusogensarechemicalsthatareutilizedforprotoplastfusions.Commonfusogensincludesodiumnitrate(NaNO3),calciumnitrate(CaNO3),dextransulfate,polyvinylalcohol,andpolyethyleneglycol[43].Somatichybridizationthroughprotoplastfusioncanproducesymmetricorasymmetrichybrids,knownassomatichybridsorcybrids(Fig.2).Thefirstasymmetrichybridwasdiscoveredbysomatichybridizationof Nicotianatabacum(tobacco)andPetroseli

umhortense(parsley)[44,45].Manywildplantspecieshaveimportantfeatures,includingdiseaseand pathogenresistance,whichcanbetransmittedtocultivatedcropspecies.Somatichybridizationallow sdesiredfeaturestobetransferredtoboostyield,resistance,tolerance,andsoon[46,47].Itenablesbree derstogenerateuniquehybridsusinganasexualtechniqueratherthantraditionalbreeding(Figure2).

Somatichybridizationhasbeenappliedforthegeneticimprovementsofvariousfloweringan dornamentals,suchasrose[48],Dendrobium[49],chrysanthemum[50],dianthus[51],gentin[52],ir is[53]andSaintpaulia[54].

Somaclonalvariantsorsomatichybridscanbeconfirmedbymorphological,biochemical,pr oteinmarker,cytogenetic,andmolecularanalyses.Restrictionfragmentlengthpolymorphism(RFL P),simplesequencerepeat(SSR),amplifiedfragmentlengthpolymorphism(AFLP),methylation-sensitiveamplificationpolymorphism(MSAP),transposon-basedmarkersystems,andNext-GenerationSequencing(NGS)havebeenappliedforthevalidationofsomatichybridsatthemolecula rlevelinseveralornamentals.Somaclonalvariationishighlydependentonthe PGRs[55].Theproble msinseparatingprotoplasts(mentionedinSection3.2),creatingunanticipatedanduselessvariations, newlycreatedvariantsthatarenotoriginal,andsoonarethefundamentallimitsofsomatichybridizatio n[56].

1.3.ProductionofSyntheticSeeds

Asyntheticseedorartificialseedisanyencapsulatedplanttissue,somaticembryos,orothermi cropropagules(Figure3).Syntheticseedshaveseveraladvantagesovernaturalseeds,includingseas on-independentseedproduction,geneticuniformity,maintaininghybridvigor,long-termstoragecapacity,rapidmultiplication,freedomfromvegetativeandseed-bornepathogens,high-volumelow-costpropagation,ensuringqualityplantmaterialsandshorteninglifecycles.[57,58].Inornamentals, somaticembryos,nodalsegments,andbranchtipsarecommonlyemployedasexplantsforthegenerat ionofsyntheticseeds,althoughcallusisseldomusedandPLBsaremostlyusedinorchidstoproducesy ntheticseeds.Syntheticseedshavebeengeneratedin*Caladiumbicolor*(caladium),*Eustomagrandifl orum*(lishianthus),*Pinuspatula*(pine),*Genistamonosperma*(bridalbroom),*Hyoscyamusmuticus*(Egyptianhenbane),and*Clitoriaternatea*(bluepeaorbluebellvine)fromthesomaticembryo;*Gypsop hilapaniculata*(gypsophila),*Saintpauliaionantha*(saintpaulia),*Urgineaaltissima*(tallwhitesquill),and*Taraxacumpieninicum*(Mniszekpieninski)fromshoottip;*Rosadamascena*f.*trigintipetala*(D amaskrose),*Syringavulgaris*(lilac),*Neriumoleander*(oleander),*Centellaasiatica*(Asiaticpennyw ort),*Ecliptaalba*(falsedaisy),*Eryth-*

rinavariegata(tiger'sclaw),*Photiniafraseri*(redtipphotinia),*Rutagraveolens*(rue),*Salixtetrasperma*(Indianwillow)fromaxillarybuds/nodes,*Anthuriumandreanum*(anthurium)fromcallus,*Liliumlongiflorum*(easterlily)frombulb,anddifferentspeciesoforchidsfromPLBs(*Cymbidiumgiganteum*,*Vandacoerulea*,*Geodorumdensiflorum*,*Coelogynebreviscapa*,*Cremastraappendiculata*,*Flickingerianodosa*,*Spathoglottisplicata*,etc.)[57,58].

Invitrosyntheticseedsinornamentalsenableseason-independentseedsynthesis,long-termstorage,andtimelysupplytogrowers.Concentrationsofsucrose,sodiumalginate(Na-alginate),andcalciumchloride(CaCl2)arecriticalforthesynthesisofartificialseedsinornamentals.Concentrationsof2-3%sucrose,2-3%Na-alginate,and50-100mMCaCl2werediscoveredtobebeneficialforsyntheticseedformationinornamentals[57,58].

Syntheticseedshavesomelimitationsovertheadvantages:lowefficientrootsystems,development-synchronousseedsfromthesomaticembryo(themosteffectiveplantmaterialforsyntheticseeddevelopment),deviationfromthenormalstructure,lossofembryogenicpotentialwithtime,etc.Syntheticseedtechnologycanbeusedmoreeffectivelyinthecommercialornamentalplantpropagationsectoraferresolvingtheselimitations.

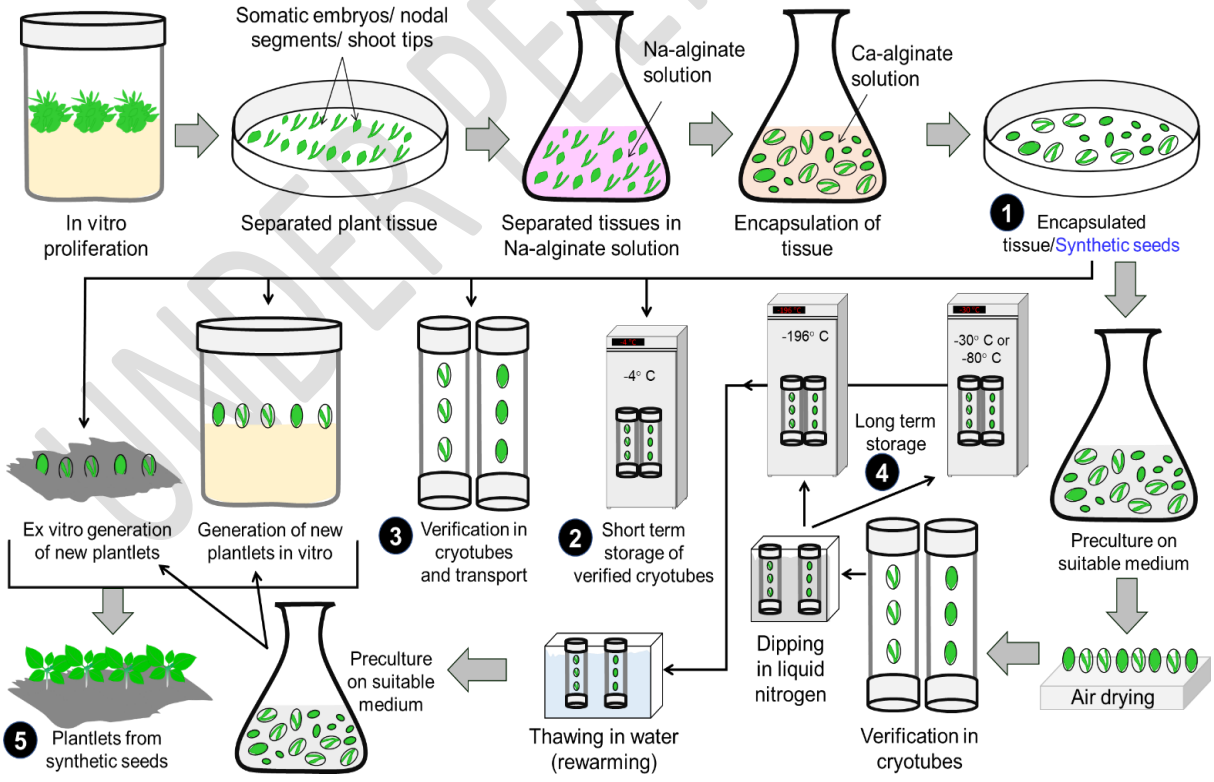

Figure3.Productionandapplicationofsyntheticseeds.Thenumbersinthefigurerepresenttheendingpointofeachstep,suchastheproductionofsyntheticseeds(1),short-

termstorageofsyntheticseeds(2),syntheticseedsfortransportation(3),long-termstorageofsyntheticseeds(4),andplantletgenerationfromsyntheticseeds(5).

1.4.InVitroPloidyManipulation

Invitroploidymodificationisamethodofpromotinggeneticdiversitybyraisingorloweringthenumberofchromosomes(Figure4).Polyploidyinductionisemployedfororna mentalcropdevelopmentandcanextendbreedingchancestoenhancedecorativefeatures,environmentaltolerances,andrestorefertilityinbroadhybrids[59].Forchromosomaldoubling,themostoftenutilizedantimitoticdrugsarecolchicineandoryzalin[60].Twogingerlilylines,*Hedychiumgardnerianum*ShepardexKerGawl.and*H.coronarium*J.Koenig,wereemployedforchromosomaldoublingusingcolchicineororyzalin,andthetetraploidgingerlilywaseffectivelycreated[61].Forty—eighttetraploidsweredevelopedinornamentalaroidplantsusingcolchicine(Caladium×hortulanumBirdsey)thatshowedvariationinleafshape,color,andthicknesscomparedtothewildtype[62].Tetraploidanisehyssop(AgastachefoeniculumL.)wasinducedbytheapplicationofcolchicine,whichshowedawiderangeofvariationcomparedtodiploidplantsintheirmorphophysiologicalcharacteristics[63].PolyploidhasalsobeeninductedinDendrobium,Phalaenopsis,Epidendrum,andOdontiodaorchidsbytheapplicationoforyzalin[64].Invitro-generatedpolyploidsofrose,lilies,phlox,petunia,bellflowers,rhododendronandotherplantsshowedawiderangeofphenotypicdifferences.Asidefromantimitoticagents,ploidymodificationisaffectedbyspecies,explantkinds,antimitoticagentexposuretechnique,antimitoticagentexposurelength,a

ndsoon.

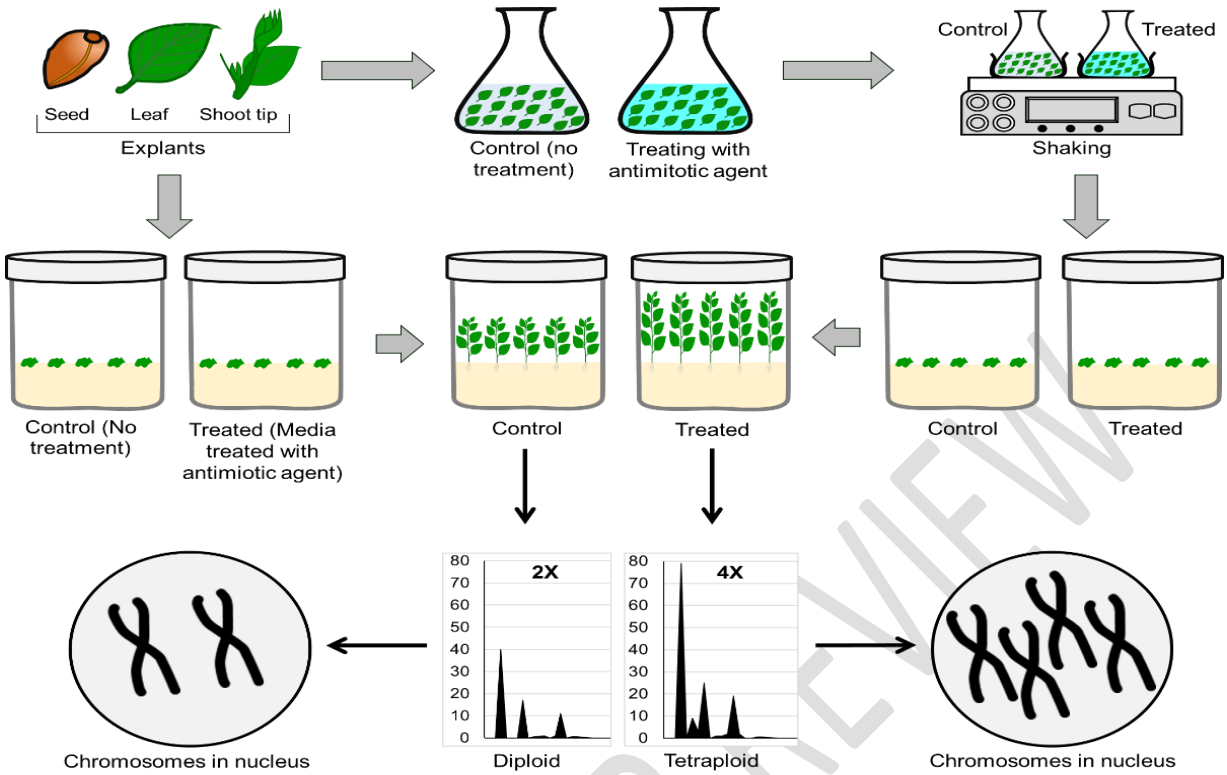

Figure4. Invitrochromosomedoubling(ploidymanipulation)forgeneticdiversification

2.FactorsAffectingonTissueculturetechniques

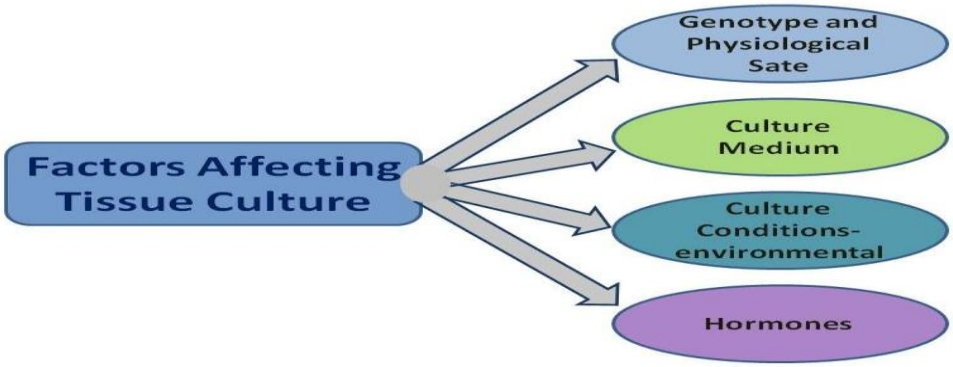

Fig 5.Factors Affecting on Tissue culture techniques

2.1.Genotype

Genotypeisoneofthemostimportantfactorsaffectingtissueculture[65].Genotypicdifferen cesbetweensixgenotypesofPrimulavulgariswereachievedincallusinductionrate,typeofcallus,roo

tformationduringthecallusphase,andshootregenerationrate[66].Shen*etal.*(2008)foundsignificantdifferencesincallusandshootformationfromleafexplantsamongfourDieffenbachiacultivars[67]. Also,GheisariandMiri(2017)observedthathormonalrequirementforcallusinductionanddirectbubletregenerationoftwolisianthusvarietieswasdifferent.

2.1.1.Sourceofexplant

Explantsource,bothinvitroandinvivo,isalsovitalforregeneration.Invitroexplantisthought tobethebestmethodfororganogenesis.Cactusplantsgrownoutdoorsoringreenhousescanbeutilized asexplantsourcestogenerateinvitrocultures;invitroplantsraisedfromseedscanalsobeusedasstarting materialforcactusmicropropagation[68].

2.1.2.Typeofexplant

Theprecise selectionofexplantmaterialcanhaveasignificantimpactontissueculturesuccess [69,70].Thismightbeowingtothevaryinglevelsofendogenoushormonesfoundinvariousplantsections.Explanttypessuchasleaf,petiole,hypocotyl,epicotyl,embryo,internode,androothadasubstantialimpactonplanttissueculture[71,72].Bulbscalesegmentsarethemostcommonlyutilizedexplantsourceforinvitromultiplicationofgeophytesornamentalplantssuchas*Fritillariaimperialis*,*Hyacinthusorientalis*and*Polianthestuberose*.Explantsuppliessuchasovary,flowerstalk,leafstalk,andmatureseedsarealsoutilisedforbulbgrowth[73].PetalexplantofP.tuberosewasimplementedtoinitiateglobularandheartsomaticembryosthatafter3weeksdevelopedastorpedoandcotyledonaryembryos. Axillarybranchingusingaxillarybudandstemnode,asinotherplants,isthemajoritycommonexplant typeutilizedfordirectshootpropagationofDieffenbachia[74].Themajorityofthestrategiesestablishedforinvitromultiplicationof Phalaenopsisincludeseedlingpropagationorculturedthedormantbudslocatedatthebaseoftheinflorescence.Themostwidelyutilizedexplantforregenerationistheleaf[75].Thegreatestrateofregenerationwasseeninchrysanthemumcv.Boramileafexplants[76].

2.1.3.Orientationofexplant

Orientationofmotherplantintheculturemediumalsoaffectstheshootproliferationandregeneration effectiveness.Ingeneral,regenerationefficiencyishigherinhorizontalpositionascomparedtoverticalduetolittlecontactofexplanttomediuminverticalposition.Theeffectofexplantlocationinthemultiplicationof*Dieffenbachia*wasevaluated.Thehighestnumberofshootswasobtainedwithsub-apicalsegmentsplacedvertically[77].

2.2.MediumFactors

2.2.1.Media

Thetypeoftissueculturemediumselecteddependsuponthespeciestobecultured.Somespeciesaresensitivetohighsaltsorhavedifferentrequirementsformacro-andmicronutrients[78].Eventissuesfromdifferentareasofaplantmayhavedifferinggrowingneeds.Formicropropagation,manybasalmediasuchasWhite,Nitsch&Nitsch,andB5(Gamborg)havebeenused,butthemostgenerallyusedculturemediumis(MSmedium),sinceitincludesalloftherequirednutrientsforinvitrogrowth.Incomparisontomanyotherformulations,itisclassedasahighsaltmedium,withhighquantitiesofnitrogen,potassium,andseveralmicronutrients,notablyboronandmanganese.However,duetothehighsaltconcentration,thisnutrientsolutionisnotalwaysappropriateforinvitroplantgrowthanddevelopment.B5mediumprovedtobesuitableordirectshootregenerationoflisianthus.Also,theuseofdilutemediaformulationshassometimespromotedbetterformationofshootandespeciallyroots,sincehighconcentrationofsaltsmayinhibitrootgrowth,eveninpresenceofauxinsintheculturemedium.Phalaenopsisorchidexplants'capacitytocreateshoots.Theyconcludedthat12MSculturemediumprovidedoptimalaxillaryshootmultiplicationandseedlingdevelopment[79,80].

2.2.2.Carbonsource

Carbohydrateisafundamentalcomponentofanynutritionalmedium,anditsinclusionisrequiredforinvitroculturegrowthanddevelopment[81].Sucroseisbyfarthemostoftenutilizedcarbonsourceforavarietyofreasons.Itisinexpensive,widelyaccessible,autoclavable,andeasilydigestedbyplants.MSmediumsupplementedwith0.1mg/lNAA+0.1mg/lBAand60g/lsucrosewasshowntobebetterforLiliumledebouriibulbletregeneration.Othercarbohydrates,suchasglucose,maltose,andgalactose,aswellasthesugar-alcoholsglycerolandsorbitol(particularlyintheRosaceaefamily),canbeemployed.Primulasp.seedgerminationrateandpercentageareregreaterinMSmediumcontaining10g/lsucrosethaninglucose[82].

2.2.3.Complexorganiccompounds

Theseareagroupofundefinedsupplementssuchascaseinhydrolysate,coconutmilk,orangejuice,tomatojuice,grapejuice,pineapplejuice,bananapuree,etc.Thesecompoundsareoftenusedwhennoothercombinationofknowndefinedcomponentsproducesthedesiredgrowthordevelopment.Someofthemareusedasorganicsourcesofnitrogensuchascaseinhydrolysate,peptone,tryptone,andmaltextract.Thesemixturesareverycomplexandcontainvitaminsaswellasaminoacids.Thehighestseedgerminationpercentage,PLBdevelopmentandseedlinggrowthofPhalaenopsisobtainedwith

MSor½MSmediacontaining100mg/lcoconutwaterand1-2g/lpeptone[83].Polyamines,particularlyspermineandspermidine,aresometimesbeneficialforsomaticembryogenesisanddirectregeneration.

2.3.CultureConditionsandenvironmentfactors

2.3.1.Gasexchangeandrelativehumidity

Theculturevesselistypicallyaclosedsystem,althoughdependingonthekindofvessel,theclosure,andhowfirmlytheyaresealedtogether,somegasexchangemayoccur.Thevessels'sealingmustallowforenoughventilationtopreventsevereethylenebuildupandCO_2depletion.Theuseoftightlyclosedcontainersthatimpedegasexchangemayhaveadetrimentalimpactonproperplantgrowthanddevelopmentduringinvitroculture.Severalstudieshavedemonstratedthebenefitsofutilizingclosureswithfiltersorventedcontainers,whichenablegasexchangeandhenceincreasephotosyntheticcapability,multiplicationrate,andplantsurvivalfollowingtransfertoexvitrosettings[84].

Therelativehumiditywithintheculturevesselsistypicallyquitehigh,resultinginapoorlyestablishedepicuticularwaxlayeranddysfunctionalplantletstomata.Asaresult,variousmethodsforreducingrelativeairhumidityinsidethevesselhavebeentested,suchasopeningculturecontainersforafewdayspriortoacclimatization,usingspecialclosuresthatfacilitatewaterloss,orcoolingcontainerbottoms,whichincreasescondensationofwatervaporsonthegelsurface.Furthermore,relativehumiditymanagementduringinvitroacclimationisanimportantroleinimprovingthemorphologicalcharacteristicsofplantletswhentransplantedinvivo[85].

2.3.2.Light

Thethreefeaturesoflight,whichinfluence*invitro*growthcharacteristicssuchasstemelongation,leafsizeandplantanatomy,arewavelength,fluxdensityandphotoperiod.Tapingkaeinvestigatedtheeffectsoflightqualityandquantityongrowthanddevelopmentof*Anigozanthosbicolor*and*Zieria fraseria*grown*invitro*.Threewhitelightintensitiesof40,80and200µmol$m^{-2}s^{-1}$;lightoffivespectralquantities:white(390-760nm),blue(450-492nm),green(492-550nm),yellow(550-588nm)andred(647-770nm);andfivelight-darkcycles:4L2D,6L6D,12L12D,16L8Dand24Lwereapplied.Totalrhizomenumberanddryweightof*A.bicolor*plantletsafter6weeksofgrowthwaspositivelyaffectedbyhighlightintensitiesof80to200µmol$m^{-2}s^{-1}$andshortlight-darkcycleof4L2D.Thelightspectrumwasnotcriticalforrhizomeproductionin*A.bicolor*.Shootdryweightof*Z.fraseria*washighestinplantletsgrownunder200µmol$m^{-2}s^{-}$

[1]irradiance.Shootlengthwaspositivelyaffectedbybluelight.Lightcyclehadnoeffectontheproductivitybutshorterphotoperiodsreducedshootlength.TheRed(R)andBlue(B)LEDlightonthe*invitro*multiplicationof*Rosa*sp.wereusedandresponsescomparedwithexplantgrownunder16/8h(light/dark)fluorescentlight.ShootsunderRBLED(16:4)showedthegreatestgrowthandelongation[86].

Severalstudiesfoundthatlightpromotedrootdevelopmentandshootgrowth,whiledarknesspromotedrootformation.Lisianthuscalligrowninthelight(1200lux)hadahigherfreshweightandweregreen,butcallicultivatedintheshadewereyellow.ThebreakdownofendogenousIAAcausesthediminishedrootinginthepresenceoflight.Shootsfromproliferatingculturesweresometimesmovedtorootinductionmediawithauxinfor4-7daysinthedarkbeforebeingswitchedtothesamemediumwithoutauxinandincubatedunderlightforrootelongation.

2.3.3.Temperature

Temperatureinfluencesseveralphysiologicalactivities,includingrespirationandphotosynthesis.Themostfrequenttemperaturerangeforcultivationhasbeen20°Cto27°C,howeveridealtemperaturesvarygreatlydependingongenotype.Priortoinvivotransplanting,airtemperatureisstatedasasignificantelementindeterminginvitrogrowthanddevelopmentofPhalaenopsisplantlets.PlantletsofPhalaenopsisacclimatizedtovariedairtemperatures(152,252,and352°C)weretransplantedimmediatelytoinvivohabitatsfor14days.Plantletsacclimatizedtolowairtemperatureretainedhigherlevelsofchlorophylla,chlorophyllb,totalchlorophyll,andtotalcarotenoidcontentthanthoseacclimatizedtohightemperature.

2.4.Plantgrowthregulators(PGRs)

PGRsregulateandgovernthebeginningandgrowthofshootsandrootsonexplantsandembryos,aswellascelldivisionandexpansion.Plantgrowthregulatorsareclassifiedintovariousgroups,includingcytokinin,auxins,gibberellins,ethylene,andabscisicacid.Theproductionofadventitioussho otsandrootsfrequentlyneedabalanceofauxinandcytokinin.Highamountsofauxinrelativetocytokininincreasedrootdevelopment,whereashighlevelsofcytokinincomparedtoauxinstimulatedshootformation.Inlisianthusandgladioli,forexample,maximalcallusinductionwasobservedonMSmediumsupplementedwith2and10mg/lNAA,respectively[87],whereasthehighestshootregeneration wasachievedwithMSmediumcontaining5mg/lBA+0.1mg/lNAAand4mg/lKin+0.5mg/lNAA(19,39).Similarresultshavebeenobtainedforcallusinductionoffritillaryandbulbletregenerationofhyacinth.Thebalanceofgrowthregulatorsdependsontheobjectiveofthecultivationinvitro(ase.g.,sho

ot,root,callusorsuspensionculture)andonthemicropropagationphaseconsidered(initiation,multiplicationorrooting)[88]

3.Futureaspects

Tissueculturetechniqueshavebeenadjustedinrecentdecadestopromoteplantgrowth,biologicalactivity,transformationandsecondarymetabolitesynthesisduetodevelopmentandadesiretogrowonalargescale.Aconsiderableadvancementinstrategieshasbeensoughttodealwiththeproblemoflowconcentrationsofsecondarymetabolitesinentireplants.Thesterileplantletswillsolvethecontaminationproblemandshortenthesterilizingprocedure.Secondarymetabolitesandmedicinallyrelevantchemicalshavefoundinvitropropagationtobequiteeffectiveforselectivemetaboliteformation.

Inrecentyears,researchershavebeguntoexamineornamentalsatthemolecularlevel,includinggeneticmodification,utilizinginvitrotechnologies[89].Agrobacteriumtumefaciens-mediatedtransformationhasbeenusedtocreatetransgenicornamentalspeciesinaround40genera[90],butonlyafewornamentals,suchasPhalaenopsisandpetunia,haveacceptableandeffectivetransformationstrategies.Manygenesandtranscriptionsareinvolvedintheinvitroorganogeniccallus,shoot,root,somaticembryos,andPLBs,andtheirtranscriptionsarealsocontrolledbytheexogenousadministrationofvariousgrowthregulators[91].

References

1. JainSM.Feedingtheworldwithinducedmutationsandbiotechnology ProceedingsInternationalNuclearConference-GlobaltrendsandPerspectives.Seminar1:agricultureandbioscience.BangiMalaysiaMINT.2002;6(2)1–14.
2. FrankenbergerEA,HasegawaPMandTigchelaarEC.Influenceofenvironmentalanddevelopmentalstateontheshootingcapacityof Cordylinegenotypes.PlantPhysiology1981;102(4)221-232.
3. HsiaCNandKorbanSS.Organogenesisandsomaticembryogenesisincallusculturesof RosahybridaandRosachinensisminima.PlantCellTissueandOrganCulture.1996;44(10)1-6.

4. KintziosS,ManosCandMakriO.Somaticembryogenesisfrommatureleavesofrose(*Rosasp*.).PlantCellReports.1999;18(6)467-472.
5. IbrahimRandDeberghPC.Factorscontrollinghighefficiencyofadventitiousbudformation andplantregenerationfrominvitroleafexplantsofroses(*Rosahybrida*).ScientiaHorticultur ae.2001;88(2)41-44.
6. KimCK,ChungJD,JeeSOandOhJY.Somaticembryogenesisfrominvitrogrownleafexplan tsof*Rosahybrida*L.AfricanJournalofPlantBiotechnology.2003;5(3)169-172.
7. RoutGR,MohapatraAandMohanJainS.Tissueculture ofornamentalpotplant:Acriticalrevi ewonpresentscenarioandfutureprospect.BiotechnologicalAdvances.2006;24(6)531-560.
8. WangGY,YuanMFandHongY.Invitroflowerinductioninroses.InvitroCellularandDevelo pmentalBiology-Plant.2002;38(5)513-518.
9. VuNH,AnhPHandNhutDT.Theroleofsucroseanddifferentcytokininsintheinvitrofloralm orphogenesisofrose(hybridtea)cv.‘FirstPrize’.PlantCellTissueandOrganCulture.2006;8 7(1)315-320.
10. KavianiB,HesarAAandKharabian-MasouleyA.InvitropropagationofMatthiolaincana(Brassicaceae)-anornamentalplant.PlantOmicsJournal.2011;4(7)435-440.
11. GillR,GerrathJandSaxenaPK.High-frequencydirectembryogenesisinthinlayerculturesofhybridseedgeranium(Pelargonium) .CanadianJournalofBotany.1992;71(6)408–413.
12. AlvardD,CoteFandTeissonC.Comparisonofmethodsofliquidcultureforbananamicropro pagation.PlantCellTissueandOrganCulture.1993;43(1)55-60.

13. LorenzoJC,GonzalesBL,EscalonaM,TeissonC,EspinosaPandBorrotoC.Sugarcaneshootsformationinanimprovedtemporaryimmersionsystem.PlantCellTissueandOrganCulture.1998;54(1)197-200.
14. DamianoC,LaStarzaSR,MonticelliS,GentileA,CaboniEandFrattarelliA.1stSymp.LiquidSystemsforinVitroPropagationofPlants.2002;(As,29/5-2/6)140-141.
15. HannigE.Zurphysiologiepflanzlicherembryonen.I.Ueberdieculturvoncruciferen-embryonenausserhalbdesembrysacks.Bot.Ztg.1904;62,45–80.
16. DieterichK.U¨berkulturvonEembryonenausserhalbdessamens.Flora1924,117,379–417.
17. LaibachF.Dastaubwerdenvonbastardsamenunddiekunstlicheaufzuchtfruhabsterbenderbastardembryonen.Z.Bot.1925;17,417–459.
18. RaghavanV.Onehundredyearsofzygoticembryocultureinvestigations.Vitr.CellDev.Biol.Plant.2003;39,437–442.
19. Marasek-CiolakowskaA,NishikawaT,SheaDJ,OkazakiK.Breedingofliliesandtulips-Interspecifichybridizationandgeneticbackground.Breed.Sci.2018;68,35–52.
20. SharmaDR,KaurR,KumarK.Embryorescueinplants:Areview.Euphytica1996;89,325–337.
21. DengY,TengN,ChenS,GuanZ,SongA,ChangQ.Reproductivebarriersintheintergenerichybridizationbetween*Chrysanthemumgrandiflorum*(Ramat.)Kitam.andAjaniaprzewalskiiPoljak.(Asteraceae).Euphytica2010;174,41–50.
22. SahijramL,RaoBM.Hybridembryorescueincropimprovement.InPlantBiologyandBiotechnology;Bahadur,B.,VenkatRajam,M.,Sahijram,L.,Krishnamurthy,K.,Eds.;Springer:NewDelhi,India,2015;Chapter18,pp.363–384
23. PramanikK,SahooJP,MohapatraPP,AcharyaLK,JenaC.Insightsintotheembryorescue—Amodernin-

vitrocropimprovementapproachinhorticulture.PlantCellBiotechnol.Mol.Biol.2021;22,20–33.

24. CaserM,DenteF,GhioneGG,MansuinoA,GiovanniniA.,ScariotV.Shorteningofselectiontimeof*Rosahybrida*byinvitrocultureofisolatedembryosandimmatureseeds.Propag.Ornam.Plants2014;14,139–144.
25. YuanMS,WuMC,ShiiCT.Shorteningbreedingcyclesofspiderlilies(*Lycorisspp.*)throughembryocultureanddikaryotypehybridizationbetween*Lycorisaurea*and“a”karyotypespecies.ActaHortic.2003;620,345–352.
26. SunCQ,ChenFD,TengNJ,LiuZL,FangWM,HouXL.Factorsaffectingseedsetinthecrossesbetween*Dendranthemagrandiflorum*(Ramat.)Kitamuraanditswildspecies.Euphytica2009;171,181–192.
27. SunCQ,ChenFD,TengNJ,LiuZL,FangWM,HouXL.Interspecifichybridsbetween*Chrysanthemumgrandiflorum*(Ramat.)Kitamuraand*C.indicum*(L.)DesMoul.andtheirdroughttoleranceevaluation.Euphytica2010;174,51–60.
28. ZhuWY,JiangJF,ChenSM,WangL,XuLL,WangHB,LiPL,GuanZY,ChenFD.IntergenerichybridbetweenChrysanthemum×morifoliumandArtemisiajaponicaachievedviaembryorescueshowssalttolerance.Euphytica2013;191,109–119.
29. DengYM,ChenSM,LuAM,ChenFD,TangFP,GuanZY,TengNJ.Productionandcharacterisationoftheintergenerichybridsbetween*Dendranthemamorifolium*and*Artemisiavulgaris* exhibitingenhancedresistancetochrysanthemumaphid(*Macrosiphoniellasanbourni*).Planta2010;231,693–703.
30. ChengX,ChenS,ChenF,DengY,FangW,TangF,LiuZ,ShaoW.Creatingnovelchrysanthemumgermplasmviainterspecifichybridizationandbackcrossing.Euphytica2011;177,45–53.

31. TangF,ChenF,ChenS,Teng,N,FangW.IntergenerichybridizationandrelationshipofgenerawithinthetribeAnthemideaeCass.*Dendranthemacrissum*kitam.×*Crossostephiumchinense*(L.)Makino).Euphytica2009;169,133–140.
32. RöperAC,LütkenH,HegelundJN,PetersenKK,ChristensenB,MüllerR.EffectofdifferentovuleisolationtimesontheembryodevelopmentofCampanulahybrids.ActaHortic.2012;953,161–166.
33. HolemanDJ.SimpleEmbryoCultureforPlantBreeders:AManualofTechniquefortheExtractionandIn-VitroGerminationofMaturePlantEmbryoswithEmphasisontheRose,1sted.;RoseHybridizersAssociation:Hartford,CT,USA,2009;pp.1–34.
34. PuangkritT,NontaswatsriC.IntersubgenerichybridizationbetweenParacurcumaandEucurcumaviaembryorescue.ActaHortic.2014;1025,37–42.
35. WangQ,ZhangY,KawabataS,LiY.DoublefertilizationandembryogenesisofEustomagrandiflorum.J.Jap.Soc.Hortic.Sci.2011;80,351–357.
36. Marasek-CiolakowskaA,SochackiD,MarciniakP.BreedingaspectsofselectedornamentalbulbouscropsAgronomy2021;11,1709.
37. NomuraY,MaedaM,TsuchiyaT,MakaraK.Efficientproductionofinterspecifichybridsbetween*Alliumchinense*andedible*Alliumspp*.throughovaryculture andpollenstorage.Breed.Sci.1994;44,151–155.
38. DubouzetJG,ArisumiKI,TakeomiE,MaedaM,SakataY.StudiesonthedevelopmentofnewornamentalAlliumthroughinterspecifichybridizationIII.Hybridizationofautumn-floweringspeciesthroughpull-stylepollination,cutflowercultureandembryorescue.Mem.Fac.Agric.KagoshimaUniv.1994;30,35–42.

39. ZulkarnainZ,TapingkaeT,TajiA.ApplicationsofinvitrotechniquesinplantbreedingInAdvancesinPlantBreedingStrategies:Breeding,BiotechnologyandMolecularTools;Al-Khayri,J.M.,Jain,S.M.,Johnson,D.V.,Eds.;Springer:Cham,Switzerland,2015;Chapter10,pp.293–328.
40. MurthyKSR,KondamudiR,RaoPVC,PullaiahT.Invitroflowering—Areview.J.Agric.Technol.2012,8,1517–1536.
41. YamashitaY,TeradaR,NishibayashiS,ShimamotoK.AsymmetricsomatichybridsofBrassica:PartialtransferofB.campestrisgenomeintoB.oleraceabycellfusion.Theor.Appl.Genet.1989;77,189–194.
42. TrickH,ZelcerA,BatesGW.Chromosomeeliminationinasymmetricsomatichybrids:Effectofgammadoseandtimeinculture.Theor.Appl.Genet.1994;88,965–972.
43. AnthonyP,MarchantR,BlackhallNW,PowerJB,DaveyMR.Chemicalfusionofprotoplasts.InPlantTissueCultureManual;Lindsey,K.,Ed.;Springer:Berlin,Germany,1995;Chapter1,pp.1–15.
44. SmithHH,KaoKN,CombattiNC.InterspecifichybridizationbyprotoplastfusioninNicotiana.Confirmationandextension.J.Hered.1976;67,123–128.
45. DuditsD,FejerO,HadlaczkyG,KonczC,LazarGB,HorvathG.Intergenericgenetransfermediatedbyprotoplastfusion.Mol.Gen.Genet.1980;179,283–288.
46. WangJ,JiangJ,WangY.ProtoplastfusionforcropimprovementandbreedinginChina.PlantCellTissueOrganCult.2013;112,131–142.
47. Ranghoo-SanmukhiyaVM.Somaclonalvariationandmethodsusedforitsdetection.InPropagationandGeneticManipulationofPlants;Siddique,I.,Ed.;Springer:Singapore,2021;Chapter1,pp.1–18.

48. PatiPK,SharmaM,AhujaPS.Roseprotoplastisolationandcultureandheterokaryonselectionbyimmobilizationinextrathinalginatefilm.Protoplasma.2008;233,165–171.
49. KanchanapoomK,JantaroS,RakchadD.Isolationandfusionofprotoplastsfrommesophyllcellsof*Dendrobiumpompadour*.ScienceAsia.2001;27,29–34.
50. FurutaH,ShinoyamaH,NomuraY,MaedaM,MakaraK.Productionofintergenericsomatichybridsofchrysanthemum[*Dendranthema*×*grandiflorum*(Ramat.)Kitamura]andwormwood(*Artemisiasieversiana*JFEhrh.ex.Willd)withrust(PucciniahorianaHenning)resistancebyelectrofusionofprotoplasts.PlantSci.2004;166,695–702.
51. NakanoM,MiiM.Somatichybridizationbetween*Dianthuschinensis*and*D.barbatus*throughprotoplastfusion.Theor.Appl.Genet.1993;86,1–5.
52. TomiczakK,SliwinskaE,RybczynskiJJ.ProtoplastfusioninthegenusGentiana:Genomiccompositionandgeneticstabilityofsomatichybridsbetween*G.kurroo*Royleand*G.cruciata*L.PlantCellTissueOrganCult.2017;131,1–14.
53. ShimizuK,MiyabeY,NagaikeH,YabuyaT,AdachiT.Productionofsomatichybridplantsbetween*Irisensata*Thunb.and*I.germanica*.Euphytica1999;107,105–113.
54. Afkhami-SarvestaniR,SerekM,WinkelmannT.Protoplastisolationandculturefrom*Streptocarpus*,followedbyfusionwith*Saintpauliaionantha*protoplasts.Europ.J.Hort.Sci.2012;77,S249–S260.
55. ChinCK,LeeZH,MubbarakhSA,AntonyJJJ,ChewBL,SubramaniamS.Effectsofplantgrowthregulatorsandactivatedcharcoalonsomaclonalvariationsofprotocorm-likebodies(PLBs)of*Dendrobium*SabinBlueorchid.Biocatal.Agric.Biotechnol.2019;22,101426.
56. Ranghoo-SanmukhiyaVM.Somaclonalvariationandmethodsusedforitsdetection.InPropagationan

dGeneticManipulationofPlants;Siddique,I.,Ed.;Springer:Singapore,2021;Chapter1,pp. 1–18.

57. QahtanAA,Abdel-SalamEM,AlatarAA,WangQC,FaisalM.Anintroductiontosyntheticseeds:Production,techniques,andapplications.InSyntheticSeeds;Faisal,M.,Alatar,A.A.,Eds.;Springer:Cham, Switzerland,2019;Chapter1,pp.1–20.
58. MaqsoodM,KhusrauM,MujibA,KalooZA.Syntheticseedtechnologyinsomeornamentalandmedicinalplants:Anoverview.InPropagationandGeneticManipulationofPlants;Siddique,I.,Ed.;Springer:Singapore,2021;Chapter2,pp.19–31.
59. TouchellDH,PalmerIE,RanneyTG.Invitroploidymanipulationforcropimprovement.Front.PlantSci.2020;11,722.
60. DhoogheE,vanLaereK,EeckhautT,LeusL,vanHuylenbroeckJ.Mitoticchromosomedoublingofplanttissuesinvitro.PlantCellTissueOrganCult.2011;104,359–373.
61. HabibiM,ShukurovaMK,WatanabeKN.Testingtwochromosomedoublingagentsforinvitrotetraploidinductiononginger lilies,*Hedychiumgardnerianum*ShepardexKerGawlandHedychiumcoronariumJ.Koenig.Vitr.CellDev.Biol.Plant2022;58,489–497.
62. CaiX,CaoZ,XuS,DengZ.Induction,regenerationandcharacterizationoftetraploidsandvariantsin‘Tapestry’caladium.PlantCellTissueOrganCult.2015;120,689–700.
63. TalebiSF,SaharkhizMJ,KermaniMJ,SharafiY,RaoufFardF.Effectofdifferentantimitoticagentsonpolyploidinductionofanisehyssop(*Agastachefoeniculum*L.).Caryologia2017;70 ,184–193.
64. MiguelTP,LeonhardtKW.Invitropolyploidinductionoforchidsusingoryzalin.Sci.Hortic. 2011;130,314–319.
65. KumarN,Reddy.Invitroplantpropagation:areview.JournalofForestScience.2011;27(2),61-72.

66. SchweenG,SchwenkelHG.Effectofgenotypeoncallusinduction,shootregeneration,andphenotypicstabilityofregeneratedplantsinthegreenhouseofPrimulassp.PlantCell,TissueandOrganCulture.2003;72:53-61.
67. ShenX,KaneME,ChenJ.Effectsofgenotype,explantsource,andplantgrowthregulatorsonindirectshootorganogenesisinDieffenbachiacultivars.InVitroCellular&DevelopmentalBiology–Plant,2008;44(4),282-288.
68. Pérez-Molphe-BalchE,Santos-DíazMS,Ramírez-MalagónR,Ochoa-AlejoN.Tissueculturefornamentalcacti.ScientiaAgricola2015;72(6),540-561.
69. GheisariM,MiriSM.Effectofgrowthregulatorsonregenerationofhyacinthcv.BlueJacket. NationalConferenceonNewConceptsinAgriculture,Tehran,Iran.
70. SoheiliSh,MiriSM,GhazijahaniN.Effectofexplanttypeandgrowthregulatorsoncallusinductionofhyssop(*Hyssopusofficinalis*).M.ScThesisofHorticulture,IslamicAzadUniversity-KarajBranch.
71. RezaeiNazariM,MiriSM,GhazijahaniN.Highfrequencysomaticembryogenesisin*Hypersicumperforatum*.3rdInternationalConferenceonResearchinScienceandTechnology,Berlin,Germany.2016
72. RezaeiNazariM,MiriSM,GhazijahaniN.Somaticembryogenesisin*Hypersicumperforatum*byusingpetioleasexplant.3rdInternationalConferenceonResearchinEngineering,ScienceandTechnology,Batumi,Georgia.2018
73. HabibiMM,MiriSM,MirmasumiSM.Effectsofbenzyladenine(BA)andnaphthaleneaceticacid(NAA)onproductionbulblettwolocallymassfritillary.2ndNationalConferenceonNewConceptsinAgriculture,Saveh,Iran.2013.
74. Elsheikh,A.M.,Daffalla,H.M.,andKhalfala,M.M.,(2013).InVitromicropropagationofthe ornamentalplantDieffenbachia-Areview.UniversalJournalofPlantScience,1(3):91-99.

75. MousaviES,BehbehaniM,HadaviE,MiriSM.Callusinductionandplantregenerationinlisianthus(*Eustomagranditlorum*).TrakiaJournalofSciences.2012;10(1),22-25.
76. LimKB,KwonSJ,LeeSI,HwangYJ,NaingAH.Influenceofgenotype,explantsource,andgellingagentoninvitroshootregenerationofchrysanthemum.Horticulture,Environment,andBiotechnology.2012;53(4),329-335.
77. MogollonJN.Effectofthreecytokininsandthelocationoftheexplantinvitromultiplicationofthe*Dieffenbachiamaculata*Schott.cv.Sublime.The46thAnnualMeetingoftheInterAmericanSocietyforTropicalHorticulture,Miami,USA.2000.
78. AbdalidehdeziA,AzadiP,MiriSM.Studyonregenerationoftuberose(Polianthestuberose)byinvitroculture.2013;M.ScThesisofHorticulture,IslamicAzadUniversity-KarajBranch.
79. AbbaszadehSM,MiriSM,NaderiR.OptimizationofasymbioticgerminationofPhalaenopsisorchidininvitroconditions.InternationalConferenceonAgricalturalScience,MedicinalPlantsandTraditionalMedicine.2018.
80. SoleimaniV,MohammadiA,MiriSM.StudyofinvitropropagationofPhalaenopsisorchid.2014;M.ScThesisofHorticulturalScience,IslamicAzadUniversity-KarajBranch.
81. TrigianoRN,GrayDJ.PlantTissueCulture.2011;CRCPress.
82. RafieeM,NaderiR,MiriSM.Disinfectionandgerminationofprimrose(*Primulasp.*)seeds.4thInternationalConferenceonAppliedResearchinChemistryScienceandBiology,Tehran,Iran.2017.
83. AbbaszadehSM,MiriSM,NaderiR.OptimizationofinvitroseedgerminationofPhalaenopsisorchid.2018;M.ScThesisofHorticulturalScience,IslamicAzadUniversity-KarajBranch.
84. MiriSM.EffectofIAA,IBAandNAAauxinsoninvitrorootingofM.9andM.26.CellularandMolecularPlantBiologyJournal.2018;12(4),15-23.

85. BayanatiM,DavoodiD,JafarkhaniKermaniM.Effectofagaranddifferentculturemediaonthemicropropagation*ofRosahybrida*cv.'BlackBaccara'.JournalofOrnamentalPlants.2015;5(2),75-81.
86. AzadiP,Khosh-KhuiM.Micropropagationof*Liliumledebourii*(Baker)Boissasaffectedbyplantgrowthregulator,sucroseconcentration,harvestingseasonandcoldtreatments.ElectronicJournalofBiotechnology.2007;10(4).
87. ZivyarS,MiriSM,RahimiMeydaniA.Theeffectofplantgrowthregulatorof BAPandNAAoncallusformationfromcormexplantsofGladiolus.2014;1stNationalOrnamentalCongress.
88. KumarN,ReddyMP.Invitroplantpropagation:areview.JournalofForestScience.2011;27(2),61-72.
89. GiovanniniA,LauraM,NesiB,SavonaM,CardiT.Genesandgenomeeditingtoolsforbreedingdesirablephenotypesinornamentals.PlantCellRep.2021;40,461–478.
90. KoetleMJ,FinnieaJF,BalázsabE,StadenJV.AreviewonfactorsaffectingtheAgrobacterium-mediatedgenetictransformationinornamentalmonocotyledonousgeophytes.S.Afr.J.Bot.2015;98,37–44.
91. BullT,MichelmoreR.Moleculardeterminantsofinvitroplantregeneration:Prospectsforenhancedmanipulationoflettuce(*Lactucasativa*L.).Front.PlantSci.2022;13,1211.