小红参组织培养的褐变因素及防止措施

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摘要　介绍不同消毒剂、消毒方法、外植体、基本培养基、活性炭浓度、暗培养时间、光照强度、培养方式对小红参组培褐变影响的研究。

关键词　小红参;组织培养;褐变因素;防止措施

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Browning Factors and Preventing Measures in the Tissue Culture of Rubia yunnanensis

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Abstract　The researches on the effects of different disinfectors, sterilizing methods, explants, basic media, active carbon concn., culture time in darkness, illumination intensities and culture modes on the browning in the tissue culture of Rubia yunnanensis were introduced.

Key words　Rubia yunnanensis; Tissue culture; Browning factors; Preventing measures

小红参为茜草科(Rubiaceae)植物云南茜草 *Rubia yunnanensis* (Franch.) Diels 的全草,其性味甘、苦,微温,主要以根入药,有多种医疗效果[1-5]。小红参极易褐化,组织培养往往由于外植体的严重褐变而受阻,笔者针对影响小红参组培褐变的几个因素进行对比试验,旨在探求简单有效的防褐措施。

1　材料与方法

1.1　试验材料　供试材料为经过 1~2 年人工种植的小红参(*Rubia yunnanensis* (Franch.) Diels),取自楚雄农业学校的药园,连根挖起带土移栽至实验室内预培养,每 2~3 d 用多菌灵溶液喷布,15 d 后取材进行试验。

1.2　试验方法

1.2.1　消毒剂和消毒方法。选取健壮、无病虫的顶芽或侧芽,经清洗后,分别用 75% 酒精、5% 次氯酸钠;75% 酒精、0.2% 升汞两组不同消毒剂和消毒方法处理。接种于 MS + BA 5 mg/L + NAA 0.5 mg/L 培养基中,暗培养 5 d 后置于1 500 lx 的光照强度下培养 25 d 时观察外植体褐变度情况。

1.2.2　不同类型外植体。用小红参的根、茎、叶、顶芽、花、果实为外植体,消毒后接种于 MS + BA 0.2 mg/L + NAA 3.0 mg/L培养基上,暗培养 5 d,再置于 1 500 lx 的光照强度下培养25 d时观察各种外植体类型褐变度情况。

1.2.3　不同活性炭浓度。用小红参的顶芽(或侧芽)为外植体,消毒后接种于添加了活性炭为 0、1、3、5 g/L 的 MS + BA 5 mg/L + NAA 1.5 mg/L 培养基上,暗培养 5 d,再置于 1 500 lx 的光照强度下培养 25 d 时观察外植体褐变度情况。

1.2.4　不同基本培养基。用小红参的真叶为外植体,消毒后分别接种于基本培养基为 MS、1/2 MS、N6 添加 BA 0.2 mg/L + NAA 3.0 mg/L 的培养基上,暗培养 5 d,再置于 1 500 lx 的光照强度下培养 25 d 时观察外植体褐变度情况。

1.2.5　不同暗培养时间。用小红参的顶芽(或侧芽)为外植体,消毒后接种于 MS + BA 3 mg/L + NAA 0.5 mg/L 培养基上,分别进行 0、5、10、15 d 暗培养时间的对比,并在培养 25 d 时观察外植体褐变度情况。

1.2.6　不同光照强度。用小红参的顶芽(或侧芽)为外植体,消毒后接种于 MS + BA 5 mg/L + NAA 1.5 mg/L + 活性炭 1 g/L 的培养基上,暗培养 5 d 后,分别置于 1 000、2 000、3 000、4 000 lx 的光照强度下培养,并在培养 25 d 时观察外植体褐变度情况。

1.2.7　不同培养方式。用小红参的顶芽(或侧芽)为外植体,消毒后接种于 MS + BA 5 mg/L + NAA 1.5 mg/L 的固体培养基(添加活性炭 1 g/L)和纸桥液体培养基上,暗培养 5 d 后,置于 1 500 lx 的光照强度下培养对比,并在培养 25 d 时观察外植体褐变度情况。

2　结果与分析

2.1　不同消毒剂和消毒方法的消毒效果　由表 1、2 分析可知,用升汞对小红参进行消毒处理效果要显著优于次氯酸钠($P < 0.05$),其中 75% 酒清浸泡 10 s 后,再用 0.2% 升汞浸泡 8 min 的方法是最佳的,污染率 15%,成活率 70%。随着酒精和升汞浸泡时间延长,褐化较重,虽然污染减轻,但成活率却明显下降。

2.2　植株不同部位的离体培养效果　诱导效果好的部位是真叶和顶芽(或侧芽),花、果实、根茎的出愈率都比较低,褐化程度较重。而根和茎的诱导率均为零。由表 3 分析表明,真叶与顶芽(或侧芽)诱导效果差异不显著($P > 0.05$)。

2.3　活性炭浓度对诱导不定芽形成的影响　由表 4 分析可知,活性炭浓度 0 与 1 g/L 差异极显著($P < 0.01$),1 g/L与 3 g/L、3 g/L 与 5 g/L 差异不显著($P > 0.05$),1 g/L 与 5 g/L 差异显著($P < 0.05$)。添加活性炭比不添加活性炭诱导效果更

表 1　75%酒精和 5%次氯酸钠不同消毒时间的消毒效果

Table 1　Sterilization effect of 75% alcohol and 5% NaClO for different sterilization time

酒精消毒 Alcohol sterilization s	次氯酸钠消毒 NaClO sterilization min	接种数 No. of inoculated 块	污染数 No. of contamination 块	成活数 No. of survival 块	成活率 Survival rate %	污染率 Contamination rate %	褐化率 Browning rate %
5	10	20	15	4	20	75	80
	12	20	12	3	15	60	85
10	10	20	13	5	25	65	75
	12	20	10	8	45	50	55
15	10	20	10	7	35	50	65
	12	20	7	8	40	35	60

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表 2 75%酒精和 0.2%升汞不同消毒时间的消毒效果

Table 2 Sterilization effect of 75% alcohol and 0.2% $HgCl_2$ for different sterilization time

酒精消毒 Alcohol sterilization s	升汞消毒 $HgCl_2$ sterilization min	接种数 No. of inoculated 块	污染数 No. of contamination 块	成活数 No. of survival 块	成活率 Survival rate %	污染率 Contamination rate %	褐化率 Browning rate %
5	8	20	10	9	48	45	52
	10	20	7	12	56	32	44
	12	20	5	11	55	25	45
10	8	20	5	13	70	15	30
	10	20	3	15	64	12	36
	12	20	2	10	52	10	48
15	8	20	4	12	60	14	40
	10	20	2	13	55	10	45
	12	20	1	9	40	5	60

表 3 不同植株部位外植体的褐化差异 %

Table 3 Differences of browning for explants from different plant positions

分项 Subentry	根 Root	根茎 Rhizoma	真叶 Leaf	顶芽(侧芽) Apical bud (lateral bud)	花 Flower	果实 Fruit	茎 Stem
出愈率 Callus forming ratio	0	15	90	85	8	6	0
褐化死亡率 Frequency of browning and death	100	85	10	15	92	94	100

表 4 不同活性炭浓度对褐化的影响

Table 4 Effects of different active carbon concentration on browning

活性炭浓度 Active carbon g/L	外植体数 No. of explants 块	分化数 No. of differentiation 块	诱导率 Induction rate %	褐化死亡率 Rate of browning and death %	出芽时间 Sprouting time d	植株色泽 Colour and lustre of plant	长势 Growth potential
0	20	9	45	55	10	黄绿 Olivine	慢 Slow
1	20	17	85	15	8	绿 Green	快 Quick
3	20	15	75	25	7	绿 Green	快 Quick
5	20	12	60	40	5	绿 Green	较慢 Relatively slow

好,其中以添加 1 g/L 活性炭的处理效果最好。

2.4 不同基本培养基对离体培养的影响 由表 5 分析可知,MS 与 1/2 MS 差异极显著($P<0.01$),与 N6 差异显著($P<0.05$)。MS 培养基愈伤诱导效果优于其他两种培养基,诱导率达 95%,褐化较轻,出愈时间提前 2~3 d,生长量最大。

2.5 不同暗培养时间对离体培养的影响 由表 6 经分析可知,暗培养 5 d 与 0、15 d 差异显著($P<0.05$),暗培养 5 d 与 10 d 差异不显著($P>0.05$)。可见,暗培养 5~10 d 比直接光照培养好,褐化较轻,出芽时间早、诱导率高、长势快、植株色泽浓绿;随着暗培养时间延长,诱导率逐渐下降,长势也较弱。

2.6 光照强度对不定芽形成的影响 由表 7 经分析可知,小红参顶芽(或侧芽)暗培养 5 d 后,放置于 1 000 lx 弱光条件下,不定芽出芽数量多,芽长势好,生长快,叶色浓绿。随着光照强度的增强,出芽数量下降,长势较弱,叶色较淡。统计分析:1 000 lx 与 2 000 lx 差异不显著($P>0.05$),1 000 lx 与 3 000、4 000 lx 差异极显著($P<0.01$)。

表 5 不同基本培养基对褐化的影响

Table 5 Effects of different basic media on browning

基本培养基 Basic medium	接种数 No. of inoculated 块	形成愈伤数 No. of callus 块	愈伤诱导率 Ratio of callus induction %	褐化死亡率 Frequency of browning and death %	出现愈伤 Callus appeared d	生长量 Growth	颜色 Color
MS	20	19	95	5	11	大 Large	橙红色 Salmon color
1/2 MS	20	12	60	40	15	小 Small	橙色 Orange
N_6	20	15	75	25	13	中 Medium	橙色 Orange

表 6 暗培养对褐化的影响

Table 6 Effects of dark treatment time on browning

暗培养时间 Dark treatment time d	出现时间 Appeared time d	诱导率 Induction ratio %	褐化死亡率 Frequency of browning and death %	不定芽长势 Growth potential of adventitious buds	植株色泽 Colour and lustre of plant
0	8	45	55	较慢 Relatively slow	绿 Green
5	4	78	22	快 Quick	浓绿 Deep green
10	12	63	37	较慢 Relatively slow	绿 Green
15	13	50	50	慢 Slow	黄绿 Olivine

表 7 不同光照强度对不定芽形成的影响

Table 7 Effects of different light intensity on browning

光照强度 Light intensity lx	诱导率 Induction ratio %	褐化死亡率 Frequency of browning and death %	不定芽长势 Growth potential of adventitious buds	植株色泽 Colour and lustre of plant
1 000	76	24	快 Quick	绿 Green
2 000	60	40	快 Quick	绿 Green
3 000	32	68	较慢 Relatively slow	浅绿 Reseda
4 000	12	88	慢 Slow	黄绿 Olivine

2.7 不同培养方式对顶芽(或侧芽)萌发形成不定芽的影响 由表 8 经分析可知,固体培养与纸桥液体培养在侧芽诱导率和褐化死亡率两方面均差异不显著($P>0.05$)。从操作方便而言,采用固体培养方式较适宜。

3 讨论

通过不同消毒剂和消毒方法、外植体、基本培养基、活性炭浓度、暗培养时间、光照强度、培养方式对小红参组培褐变的影响进行研究。结果表明:0.2%升汞消毒处理效果要显著优于 5%次氯酸钠,其中 75%酒精浸泡 10 s 后,用 0.2%升

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6643 - 6653.
[3] MICHEAL CAREY S T S. Transcriptional regulation in eukaryote concepts, strategies and techniques[M]. [s.l.]: Cold Spring Harbor Laboratory Press, 2001.
[4] HUANG W W, BATEMAN E. TATA elements direct bi-directional transcription by RNA polymerases II and III[J]. Nucleic acids Res, 1996, 24(6): 1158 - 1163.
[5] JACK J L, RICHARD H K, JARO S. An inverted TATA box directs downstream transcription of the bone sialoprotein[J]. Gene Biochen J, 1995, 310: 33 - 40.
[6] ZHU Q, LAMB C. TATA box and initiator functions in the accurate transcription of a plant minimal promoterin vitro[J]. Plant Cell, 1995, 7(10): 1681 - 1689.
[7] COLGAN J M J. Cooperation between core promoter elements influences transcriptional activity in vivo[J]. Proc Natl Acad Sci USA, 1995, 92(6): 1995 - 1999.
[8] O'SHEA GREENFIELD A S S. Roles of TATA and initiator elements in determining the start site location and direction of RNA polymerase II transcription [J]. J Biol Chem, 1992, 267(2): 1391 - 1402.
[9] FLEDLER U, PHILLIPS J, ARTSSENKO O, et al. Optimazation of scFv antibody production in transgenic plants[J]. Immunotechnology, 1997, 3: 205 - 216.
[10] CHANDRASEKHARAN M B, BISHOP K J, HALL TC. Modulespecific regulation of the beta-phaseolin promoter during embryogenesis[J]. Plant J, 2003, 33: 853 - 866.
[11] WU C Y, HARUHIKO W, YASUYUKI O, et al. Quantitative nature of the Prolamin-box, ACGT and AACA motifs in a rice glutelin gene promoter: minimal ciselement requirements for endosperm-specific gene expression[J]. The Plant J, 2000, 23(3): 415 - 421.
[12] REIDT W, WOHLFARTH T, ELLERSTROM M, et al. Gene regulation during late embryogenesis: The RY motif of maturation-specific gene promoters is a direct target of the *FUS*3 gene product[J]. Plant J, 2000, 21(5): 401 - 408.
[13] SHIRSAT A H, MEAKIN P J, GATEHOUSE J A. Sequences 5′ to the conserved 28 bp Leg box element regulate the expression of pea seed storage protein gene *legA*[J]. Plant Mol Biol, 1990, 15: 685 - 693.
[14] RACHAEL L N, ROBERT W W, RAYMOND L R. Eukaryotic DNA fragments which act as promoters for a plasmid gene[J]. Nature, 1979, 277: 324 - 325.
[15] DONNA M W, ELIZABETH J D, PAUL S L. Cloning restriction fragmentsthat promote expression of a genein *Bacillus subtilis*[J]. J Bacterol, 1981, 146: 1162 - 1165.
[16] MARK M Z, DAIRENA F G, DENIS S, et al. Chromogenic identification of genetic regulatory signals in *Bacillus subtilis* based on expression of cloned Pseudomonas gene[J]. Proc Natl Acad Sci USA, 1993, 80: 1101 - 1105.
[17] SCOPIONE R C, DECAMARGO S S, SCHENBERG A C, et al. A new promoter-probe vector for *Saccharomtces cerevisiae* using fungal glucoamylase cDNA as the reporter gene[J]. Yeast, 1993, 9: 599 - 605.
[18] SIDHU R C, MATHEWES S, BOLLON A P. Selection of secretory protein-encoding genes by fusion in *Saccharomyces cerevisiae*[J]. Gene, 1991, 107: 111 - 118.
[19] FORDOR I, KRANIKOVA O V, BERETS E, et al. Cloning structure and features of a *Saccharomyces cerevisiae* DNA fragment causing the expression of reporter genes[J]. Mol Biol, 1990, 24: 1411 - 1418.
[20] LUO X M. Cloning and characterization of three *Aspergillus niger* promoters [J]. Gene, 1995, 163: 127 - 131.
[21] SU N, SUN M, LI Y N, et al. Isolation and modification of rice chloroplast 16S promoter, construction of expression vector and transformation[J]. Chinese Bulletin of Botany, 2003, 20(3): 295 - 301.
[22] WANG L J, FAN S H, GUO A G. Cloning and function analysis of gene *ats*1*A* promoter from *Arabidopsis thaliana* [J]. Acta Botanica Boreali-Occidentalia Sinica, 2004, 4(10): 1856 - 1860 .
[23] LI X B, CAI L, CHENG N H, et al. Molecular characterization of the cotton *GhTUB*1 gene that is preferentially expressed in fiber[J]. Plant Physiol, 2002, 130(2): 666 - 674.
[24] LIU Y G, ROBERT F W. Thermal asymmetric Interlaced PCR: Automatable amplification and sequencing of insert end fragments from PI and YAC clones for chromosome walking[J]. Genomics, 1995, 25: 674 - 681.
[25] SUNILKUMAR G, CONNELL J P, SMITH C W, et al. Cotton α-globulin promoter: isolation and functional characterization in transgenic cotton, *Arabidopsis*, and tobacco[J]. Transgenic Research, 2002, 11(4): 347 - 359.
[26] HWANG Y S, YANG D, MCCULLAR C, et al. Analysis of the rice endosperm-specific globulin promoter in transformed rice cells[J]. Plant Cell Reports, 2002, 20(9): 842 - 847.
[27] QU L Q, TAKAIWA F. Evaluation of tissue specificity and expression strength of rice seed component gene promoters in transgenic rice[J]. Plant Biotechnology Journal, 2004, 2(2): 113 - 125.
[28] MORCILLO F, HARTMANN C, DUVAL YT, et al. Regulation of 7S globulin gene expression in zygotic and somatic embryos of oil palm[J]. Physiologia Plantarum, 2001, 112(2): 233 - 243.
[29] MEI C, WASSOM J J, WIDHOLM J M. Expression specificity of the globulin - 1 promoter driven transgene (chitinase) in maize seed tissues[J]. Maydica, 2004, 49(4): 255 - 265.
[30] RUSSELL D A, FROMM M R. Tissue-specific expression in transgenic maize of four endosperm promoters from maize and rice[J]. Transgenic Research, 1997, 6(2): 157 - 168.
[31] XUE G P, PATEL M, JOHNSON J S, et al. Selectable marker-free transgenic barley producing a high level of cellulase (1, 4-β-glucanase) in developing grains[J]. Plant Cell Rep, 2003, 21: 1088 - 1094.
[32] RADKE S E, ANDREWS B M, MOLONEY M M, et al. Transformation of *Brassica napus* L using *Agrobacterium tumefaciens*: Developmentally regulated expression of a reintroduced napin gene[J]. Theoretical and Applied Genetics, 1988, 75(5): 685 - 694.
[33] TOMLINSON K L, MCHUGH S. LABBE H, et al. Evidence that the hexose-to-sucrose ratio does not control the switch to storage product accumulation in oilseeds: Analysis of tobacco seed development and effects of overexpressing apoplastic invertase[J]. J Exp Bot, 2004, 55, 2291 - 2303.
[34] KLUTH A, SPRUNCK S, BECKER D, et al. 5′deletion of a *gbss*I promoter region leads to changes in tissue and developmental specificities[J]. Plant Molecular Biology, 2002, 49(6): 669 - 682.
[35] FIEDLER U, PHILLIPS J, ARTSAENKO O, et al. Optimization of scFv antibody production in transgenic plants[J]. Immunotechnology, 1997(3): 205 - 216.
[36] LINDGREN L O, STALBERG K G, HOGLUND A S. Seed-specific overexpression of an endogenous *Arabidopsis* phytoene synthase gene results in delayed germination and increased levels of carotenoids, chlorophyll, and abscisic acid [J]. Plant Physiology, 2003, 32(2): 779 - 785.

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表 8　不同培养方式对褐化的影响

Table 8　Effects of different culture methods on browning

培养方式 Culture method	出芽时间 Sprouting time d	诱导率 Induction ratio %	褐化死亡率 Frequency of browning and death %	侧芽数 No. of lateral buds 个	长势 Growth potential	颜色 Color
固体培养 Solid culture	6 ~ 7	85	15	4 ~ 5	快 Quick	绿 Green
纸桥液体培养 The liquid medium with filter paper	5 ~ 6	65	35	3 ~ 4	快 Quick	浅绿 Reseda green

汞浸泡 8 min 的方法是最佳的；真叶和芽的褐化率比根、根茎、花、果实、茎要显著低；MS 培养基褐变比 N6 和 1/2 MS 显著低；添加 1 g/L 活性炭、接种后暗培养 5 ~ 10 d、在 1 000 ~ 2 000 lx 弱光下培养有利于减轻褐变。纸桥液体培养与固体培养差异不显著。

参考文献

[1] 兰茂. 滇南本草[M]. 昆明：云南人民出版社，1975：349.
[2] 杨宇. 小红参的临床运用[J]. 中国民族民间医药杂志，2000(2)：119 - 120.
[3] 代夫，王正文，王朝凤. 小红参对银屑病活血化瘀作用的研究[J]. 昆明医学院学报，1994，15(4)：70.
[4] 符光利. 小红参临床运用点滴[J]. 中国民族民间医药杂志，2001(51)：246 - 247.
[5] 吴煜秋，张荣平. 中药小红参的研究概况(综述)[J]. 中国民族民间医药杂志，2003(64)：259 - 264.