植物组织培养过程中器官发生途径再生植株分子机制研究进展

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摘要　综述了植物组织离体培养过程器官发生途径所经历的3个阶段的分子机制,包括已分化器官脱分化、根器官再生和芽器官再生。重点概述了禾谷类作物组织培养再生途径中的细胞遗传学机理。分析了当前植物再生性状分子基础研究存在的3个主要问题,即具有稳定表现的高再生性能植物品种有限,再生性状相关主效基因发掘不足,已鉴定的再生相关基因功能不明显。提出应加强高再生性能植物品种选育,为发掘、控制植物离体组织培养再生关键基因提供丰富的基因资源,推动植物再生性状的分子机制研究。

关键词　离体培养;植株再生;再生相关基因

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Research progress on molecular mechanism of plant regeneration from cultured tissues *via* organogenesis

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Abstract　The progress of plant regeneration *in vitro* in molecular level is reviewed in this paper, including the main stages of plant tissue culture of explants, the molecular mechanisms of dedifferentiation of differentiated tissues, shoot regeneration and root regeneration, as well as the cytogenetic basis of regeneration traits in cereal crops. It is summarized that the limitation of plant genotypes with high and stable regeneration performance, shortage of cloned key genes associated with regeneration traits, and weak function of identified genes related to somatic embryogenesis in other plants are the main problems in the investigation of plant regeneration in molecular biology. In future the identification or development of the plant genotypes with desirable regeneration ability should be stressed by using various strategies to expand the gene resource for the exploration of the corresponding phenotype. The aim of this review is to deepen the awareness of the importance of molecular investigation in plant regeneration, and to boost the characterization of more plant regeneration-relevant genes for the further understanding of molecular mechanism of underlying plant organogenesis during plant tissue culture *in vitro* and the development of plant genetic engineering breeding.

Keywords　tissue culture *in vitro*; plant regeneration; genes relevant to regeneration

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植物胚胎发生是植物整个生活史中的一个重要发育过程,涵盖了从受精卵发育、分化到产生单个幼苗的全过程,是植物细胞全能性(totipotency)或多能性(pluripotency)的具体体现[1]。植物离体细胞胚胎发生(也叫成体细胞胚胎发生,somatic embryogenesis)过程包括已分化器官脱分化、芽器官形态建成及根器官形态建成3个重要过程[2]。随着这些生命活动过程相关基因的克隆和鉴定,对高等植物离体组织体外培养再生过程的认识已上升到分子水平。本文从上述3个方面对所涉及的分子机制研究进展作一综述。

1 植物组织离体培养再生植株经历的主要阶段

从本质上来说,植物组织离体培养再生单个植株的过程是一个无性繁殖过程,包括外源植物激素信号应答、已分化细胞的脱分化、静止细胞的再分裂以及特定组织或器官原基或分生组织的形成等过程[3]。图1为小麦离体组织再生过程的主要培养阶段。概括起来,植物离体再生过程主要包括3个阶段:首先细胞发生脱分化作用进而获得胚胎发生能力或器官再生能力,随之通过对外源激素响应为分化特定器官做准备,最后是不依赖植物激素的器官再生或胚胎发生过程。对于器官发生途径的再生过程而言,第一阶段还可细分为2个亚阶段:细胞增殖及根器官建成能力获得和芽器官发生能力形成[4]。

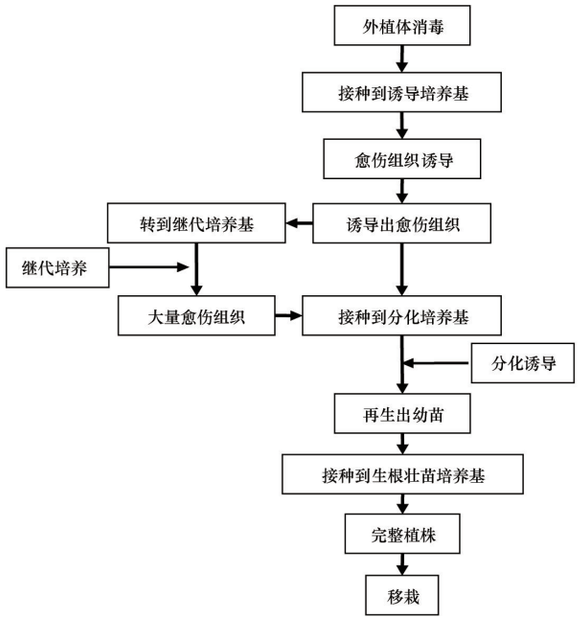


图1 小麦离体组织培养再生植株流程

Fig. 1 Protocol of wheat regeneration during tissue culture *in vitro*

2 已分化器官脱分化的分子机制

已分化细胞的脱分化是多细胞有机体全能性、再生及新的干细胞群形成的基础。生物体的脱分化过程通常被认为与细胞周期活动的重建密切相关,典型特点是原来的分化状态消失及新的"干细胞"出现[5]。研究表明,胚胎发生能力的获得在很大程度上依赖脱分化过程,在此过程中发生既有的转录和翻译机制的消除或改变,取而代之的是细胞进入新的发育模式[6]。生长素和细胞分裂素是诱导植物细胞进入脱分化状态的重要植物激素。植物原生质体培养是研究细胞脱分化过程极好的实验材料[7],经去壁处理的植物细胞(原生质体)受不同外源信号诱导后,出现3种发育途径,即表现为单纯的生长素诱导脱分化过程、生长素及细胞分裂素共同指导的细胞增殖过程和不添加任何激素导致细胞死亡过程[8]。活跃的细胞分裂对于细胞脱分化状态的维持以及新器官发生是必须,同时也是体细胞胚胎发育前的胚胎发生准备过程以及外植体直接发育产生的实生苗过程所必须的。在植物体内,仅当成熟的分化过程引发极端的表型变化后,如管状分子(木质部组分)原生质体的死亡及筛状分子(韧皮部组分)核丢失,植物细胞才永久地失去再生潜力[9]。

Ozawa等[10]认为,静息细胞重新进行细胞分裂与编码细胞周期蛋白(cyclins)及细胞周期蛋白依赖性激酶(cyclin-dependent kinases,CDKs)的基因表达具有很大的相关性。在细胞周期蛋白依赖性激酶中,PSTAIRE类蛋白的研究最为深入[11]。根据含有保守的PSTAIRE结构域的不同,分为具有高度保守的PSTAIRE结构域的$p34^{cdc2}$同源型及含有部分保守PSTAIRE结构域的CDKs,前者在整个细胞增殖周期中持续表达,后者则在细胞周期特定阶段表达。*cdc2aAt*(拟南芥PSTAIRE类CDKs)组织表达分析表明,该基因在处于增殖周期的植物细胞及根组织非分裂细胞中都活跃表达,此外,植物组织培养脱分化过程的伤害处理能快速诱导*cdc2aAt*的表达[12,13]。Shaul等[14]认为,*cdc2aAt*的表达与细胞增殖能力的获得具有高度相关性。对与细胞增殖无关的细胞周期基因(如*CYCD* 1-3)的表达研究表明,在经碳源、生长素和细胞分裂素胁迫处理的静止期细胞中,*CYCD2*和*CYCD3*分别受到碳源及细胞分裂素的诱导表达,这些基因的表达也被认为是细胞周期得以活化的必要条件[15]。

植物细胞脱分化过程伴随许多基因表达的变化[16,17],因此,对细胞脱分化过程基因表达的研究有助于解析细胞脱分化的机制。研究认为,染色质超螺旋的去组装能够确保已分化的细胞快速而有序地重塑基因表达过程[18]。William等[19]研究发现,伴随细胞多能性的出现,组蛋白H3发生翻译后修饰,异染色体蛋白1(heterochromatin protein 1,HP1)再分布,同时随着核仁区18S核糖体DNA的凝集,发生核仁崩解。Duval等[20]发现,染色体特定区段的去凝集能够激活诸如*VIP1*及*NO APICAL MERISTEM*(*NAM*)等基因表达,后者与植物体中新生的"干细胞"群的形成有关(图2)。Souer等[21]研究发现,NAM突变导致矮牵牛(*Petunia hybrida* L.)胚芽顶端分生组织不能正常发育。泛素作为蛋白质降解的标记物,在细胞脱分化过程中的表达存在一定的组织特异性。研究表明,当转化的拟南芥叶肉细胞发生脱分化时,泛素基因的表达与细

胞发育过程密切相关,且在幼叶及分裂旺盛的细胞中最为明显[16],此现象也在烟草(*Nicotiana tabacum* L.)原生质体脱分化过程中发现[7]。Zhao等[7]认为,泛素介导的蛋白裂解系统是植物激素诱导原生质体进入细胞分裂S期所必需,其作用方式可能是通过一种称为细胞周期调控的泛素蛋白酶复合物E3(Skp1-Cdc53-F-box,SCF)介导的G1/S过渡期的特异蛋白底物磷酸化作用实现[22]。研究认为,泛素基因上调表达是细胞结构重建的一个重要特征,通过系统选择性地降解维持细胞原始形态的蛋白,从而激活细胞增殖必需蛋白的表达[23,24]。

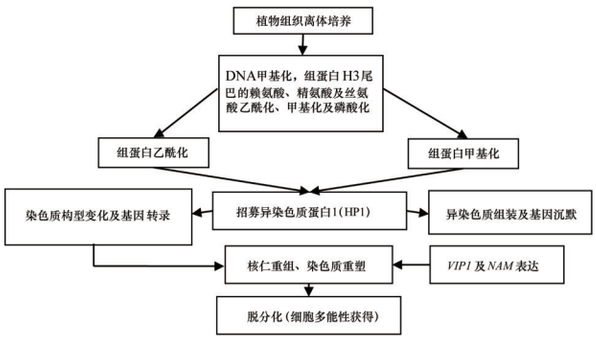


图2　植物细胞脱分化分子机制[25,26]

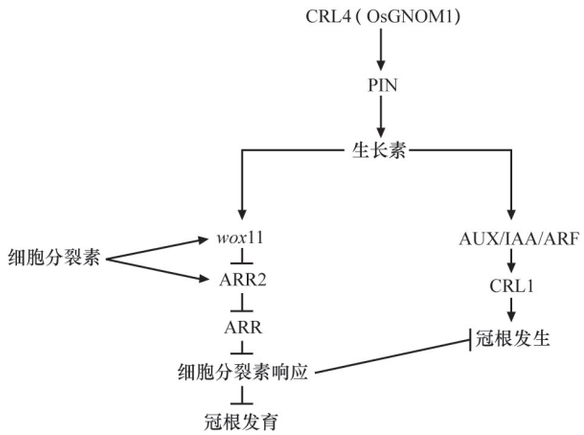
Fig. 2　Molecular mechanism of plant cell dedifferentiation[25,26]

3　根器官建成的分子基础

植物根器官的建成是一个高度可塑过程,对培养环境中的营养、湿度、温度、光照及其他培养条件高度敏感[27]。植物主根形成于种子萌芽后不久,并随着根的成熟,中柱鞘的静息细胞开始分裂并通过一个精确的分裂机制形成侧根原基[28],接着侧根延长并进一步产生分支。再生植株的移栽成活与形成不定根的能力密切相关,然而不同植物形成不定根的能力不同[29]。谷类作物的根主要是由不定根发育而来的须根系组成,也叫节根或冠根,其发育过程包括:胚胎中的根顶端分生组织(RAM)首先发育成幼根,随后在胚芽鞘的节点处产生数个胚性的冠根;在胚胎发育后期,幼根和冠根继续分化产生分枝,即发育长侧根和短侧根[30]。

在水稻中,目前已发现CRL4(crown rootless)和OsGNOM1两种冠根缺失突变体,其特征是冠根完全缺失或产生较少量的侧根、根向地性弱及异常的生长素运输[31,32],推测由CRL4/OsGNOM1介导的生长素极性运输对于冠根的形成、发育及侧根的分化是必须的。同样的情况也发生在ARL1(adventitious rootless 1)和CRL1突变体中。拟南芥侧根形成过程包括两个重要步骤:木质部中柱鞘细胞周期的再激活及新分生组织的形成[28],在此过程中生长素通过调控相关基因的转录水平发挥作用[33-35],其中,G1/S检测点(check point)是生长素诱发侧根形成的靶位点,生长素通过在转录水平调控CDKs抑制蛋白KRP2的表达进而阻断G1/S期转变[35]。研究表明,生长素在绝大多数植物侧根及不定根形成中起积极作用[36]。提高内源或外源生长素浓度能明显促进侧根及不定根的形成[37],而因基因突变或者抑制因素导致的生长素浓度降低或运输阻断可抑制根的诱发及延长[38,39],如在番茄(*Lycopersicon esculentum* L.)中,高浓度生长素能促进侧根[40]及不定根[41]的形成,而生长素运输抑制子(auxin transport inhibition)则抑制两者的形成[41]。在生长素不敏感的横生性(diageotropic,dgt)突变体中,侧根的形成完全受到抑制[40]。

除生长素外,细胞分裂素对胚胎侧根的形成和发育也有影响[42,43]。水稻的*wox*11参与冠根的形成和发育调控,并干扰细胞分裂素的信号通路。*wox*11的表达受生长素及细胞分裂素的诱导,过表达*wox*11后抑制A型RR2(A type response regulator 2,ARR2)及其他ARRs的表达,而ARRs属细胞分裂素诱导表达型[44]。在拟南芥的RAM中,*WUS*基因抑制ARR5-7及ARR15的表达[45]。生长素及细胞分裂素对谷类作物冠根形成的调控路径如图3所示。



箭头所示为正调控,射线表示负调控。ARF:生长素响应因子;ARR:A型响应调节子;AUX/IAA:生长素/吲哚-3-乙酸;CRL:冠根缺失;OsGNOM1:水稻膜介导ADP核糖基化G蛋白-嘌呤核苷酸交换因子(ARF-GEF),参与生长素运载体运输调控;*wox*11:WUSCHEL相关的同源框1

图3　植物冠根形成及发育的调控路径[46]

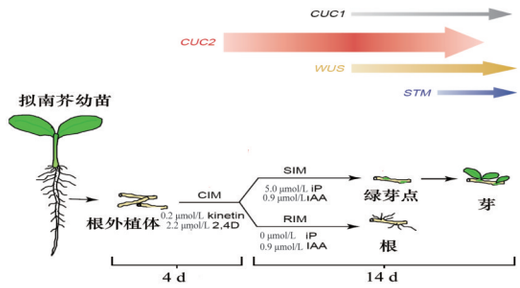
Fig. 3　Gene regulatory network controlling crown root initiation and development in plant[46]

对乙烯生物合成及信号通路的研究发现,乙烯在拟南芥的侧根形成中也发挥重要作用[47,48]。如在CTR1和ETO1突变体中,增强的乙烯信号[49]和乙烯合成[50]均显著降低侧根的数目。此外,经乙烯前体1-氨基环丙烷羧酸(ACC)处理的拟南芥,其侧根形成受到明显抑制,而对ACC处理不敏感的拟南芥,侧根形成不受影响,认为这是由于乙烯与生长素互作改

变了后者的运输所致[48]。在对番茄侧根及不定根形成的研究中也发现,乙烯通过调控生长素的运输促进不定根的形成,但抑制侧根形成[51]。

4 芽器官发生的分子基础

植物芽器官形成能力在脱分化过程已经确立,并受到细胞分裂素的诱导[3,52],其调控路径不仅体现在促进芽的发育,同时在特定条件下也表现为抑制芽的发生。图4为模式植物拟南芥的芽器官发生过程。在愈伤组织诱导培养基(callus induction medium,CIM)上培养拟南芥和小麦的根,经一段时间诱导后(拟南芥为4 d,小麦为8 d)离体培养的根组织就表现出对激素处理的响应,随之在再生芽诱导培养基(shoot induction medium,SIM)或根诱导培养基(root induction medium,RIM)上,通过不同种类和浓度的激素处理,拟南芥愈伤组织很容易诱导出芽或根,而小麦愈伤组织只诱导出根。



CIM:愈伤组织诱导培养基;SIM:芽诱导培养基;
图上方带颜色及明暗的箭头表示基因的相对表达量

图4 拟南芥组织培养芽器官形成过程[52]

Fig. 4 Developmental events during shoot regeneration from root explants in *Arabidopsis thaliana* L.[52]

芽分生组织活力的保持是通过协调器官发生及干细胞活力维持之间的遗传互作实现的。在拟南芥中,*WUSCHEL*(*WUS*)主要负责干细胞活力维持,而*CLAVATA*1-3(*CLV*)则与器官发生有关。芽分生组织能进行自我调控,*CLV*基因能抑制*WUS*基因转录,并通过二者的互作在干细胞及其组织中心间形成反馈调节[53]。研究证明,*LEC*1、*LEC*2、*FUS*3和*ABI*3等基因在拟南芥体细胞胚胎发生过程中上调表达,而*WUS*基因则在胚性愈伤组织中尚不能根据形态或分子检测鉴定体细胞胚时,就已启动表达,并通过调节外源生长素浓度实现其正确表达,对于体细胞胚的诱导至关重要,同时,在特定区域生长素梯度的建立与*WUS*基因的诱导表达密切相关,能够增加体细胞胚的数量[54]。生长素梯度的建立促进了*PIN*1在胚性愈伤组织中的极性定位,后者可能负责生长素的极性运输,以及在芽分生组织和体细胞胚中的积累,*WUS*和*PIN*1通过调节下游基因表达发挥其诱导胚性发生的作用[54]。进一步发现,*WUS*基因的表达由细胞分裂素和生长素的比例决定,*CLV*3、*TFL*1、*FLY*1对于拟南芥体细胞胚发生和花序离体再生同样起着重要作用[55]。过表达拟南芥*WUS*基因的棉花,其胚性细胞的发生和竞争力增强[56]。

在植物芽器官发生相关基因表达研究中,没有得到一致结果,如对再生性能差异的2个烟草品种芽发生过程进行SDS-PAGE分析,发现了2个特异的蛋白表达条带,而在矮牵牛芽器官发生过程中未能发现这种特异性的差异[57]。尽管如此,仍发现一些芽器官发生过程中特异表达基因(表1)。Che等[58]利用芯片技术研究拟南芥根组织在CIM及SIM上培养过程中的基因表达谱,发现芽发育过程中有2%~3%的基因表达上调,主成分分析表明绝大多数基因只在特定的阶段上调表达,且这些基因主要是编码转录因子或激素信号通路的组分。研究发现,细胞分裂素及KNAT1(KNOTTED-1-in *Arabldopsis thaliana*,与STM有关的调控基因)在芽发育过程的调

表1 高等植物离体培养芽发生过程中的基因表达

Table 1 Genes in response to shoot organogenesis during *in vitro* culture of higher plants

基因	描述	参考文献
ARP	Asymmetric leaves1/rough sheath2/phantastica	[62]
*ARR*5	A type response regulator gene	[3],[58]
*CKI*1	Conferring cytokinin-independent phenotype	[61]
*CRE*1	A cytokinin receptor	[58]
*CUC*1, *CUC*2	CUP-SHAPED COTYLEDON	[63]
*CYCD*3	Acquisition of competence for organogenesis	[64]
*ESR*1	Enhance shoot regeneration, vegetative-to-organogenic transition	[65]
*ETR*1	An ethylene receptor	[66]
*IBC*6, *IBC*7	Putative response regulator genes induced by cytokinin	[67]
*KN*1	Responsible for the knotted leaf phenotype	[68]
SERK	Somatic embryogenesis receptor-like kinase	[69]
SRD	Shoot redifferentiation	[70]
STM	Shoot meristemless	[71]

控网络错综复杂、相互促进[59],并发现一些不依赖于细胞分裂素的芽发育调控路径[60,61]。Katayama等[62]在对河苔草科植物芽再生机制研究时,发现无SAM参与的芽再生过程,而由新形成的叶或苞片充当SAM,分化出新的芽-叶混合器官。与三裂科植物(有SAM)的比较研究发现,河苔草科中*STM*(shoot meristemless)和*WUS*主要在新生叶或苞片中表达,而在老叶或苞片中仅*STM*表达,且限于基部,而*ARP*则替代*STM*在远端表达,但在花发育过程中,*STM*及*WUS*在花分生组织均表达,而在花中不表达。

5　植物器官再生的细胞遗传学研究

小麦、玉米、水稻、大麦、高粱及燕麦(*Avena sativa* L.)中存在广泛的遗传变异,其中有许多变异影响组织培养的效应,包括愈伤组织诱导、愈伤组织生长和植株再生[72]。细胞遗传学研究表明,绝大多数控制或影响组织培养效应(tissue culture response,TCR)的基因属显性或部分显性,且能够稳定遗传。小麦中存在丰富的易位系、代换系及附加系等遗传工程材料,为研究小麦TCR性状提供了便利。

Reisch等[73]研究发现,2个显性基因控制苜蓿芽分化,且仅当二者共存时才能促进苜蓿的芽再生,再生率达75%。在玉米幼胚的再生研究中也发现了显性或不完全显性控制的情况[74]。Ma等[75]发现高粱幼胚的植株再生至少由2个互补的显性基因控制。在小麦再生性状遗传力的研究中发现,小麦幼胚愈伤组织生长和植株再生也受到显性或部分显性基因控制。根据小麦单体系Wichita与高再生力株系ND7532的杂交,发现控制小麦幼胚的TCR属质量性状且可遗传[72]。Mathias等[76]发现矮秆基因*Rht*对小麦幼胚愈伤组织诱导及形态建成存在显著影响。在中国春/黑麦附加系中,发现黑麦4号染色体具有促进花药培养的效应,而黑麦染色体6和7拥有控制幼胚再生的效应,认为小麦花药与幼胚的诱导培养间无相关性,可能具有不同的遗传调控机制[77]。Henry等[78]研究发现,黑麦染色体1RS具有控制花药培养的效应。用小麦品种Cappelle-Desprez的4B染色体替换中国春的4B染色体后,后者的愈伤组织诱导率由68.3%上升到92%,再生率由48.4%提高至58.2%[79]。Felsenburg等[80]研究发现,小麦6BL染色体丢失严重,延缓愈伤组织生长,染色体2BS与愈伤组织分化具有相关性。Galiba等[81]对来自不同培养环境的中国春/"Cheyenne"系列代换系幼胚再生的遗传力进行研究,发现幼胚愈伤组织诱导率不受代换系的影响,温室生长材料的再生能力受染色体5A、4B、7B、1D、2D、3D及7D的影响,大田生长材料则受6A、7B、1D、4D、6D及7D的影响。据此,染色体7B、1D及7D可能存在控制小麦幼胚再生性状的关键因素。综上所述,小麦中许多染色体与TCR有关,进一步揭示和定位这些基因将有助于通过生物技术手段提高小麦TCR。根据小麦再生性状QTL定位结果,发现在2B染色体上存在3个与TCR有关的QTL,其中,TCR-B1及TCR-B2影响绿芽点数及芽再生率,而TCR-B3仅影响再生率[82]。此外,在染色体2、3、5及7中,发现7个染色体区段(percent embryos forming embryogenic callus,PEFEC)与胚性愈伤组织诱导率有关,1个染色体区段与愈伤组织分化率有关(QPefec.nau-3B.2,percent callus pieces regenerating plantlets,PCRP),QPefec.nau-7D及QPcrp.nau-3A与幼胚的TCR有关[83]。进一步对这些影响小麦组织培养植株再生性能QTL的精确地位,将对后续主效再生相关基因的分离具有重要意义。

6　植物再生性状分子基础研究存在的主要问题

目前,试图从分子角度解析和提高植物离体组织培养再生能力的策略已逐步开展,并从几种植物中克隆、鉴定了与胚性愈伤组织发生和植株再生相关的基因,如*AGP*、*SERK*、*NiR*、*WUS*、*LEC*等[84-89]。纵观已有研究结果,主要存在以下几方面的不足:

1)具有稳定表现的高再生性能植物品种有限。尽管分子生物学技术已日益成熟,并已广泛应用在植物分子生物学研究领域,具有极端性状差异品种对于分子标记开发、功能连锁群体构建及核心功能基因分离至关重要。然而,截至目前,具有稳定表现的各类高再生植物品种十分有限,限制了利用分子生物学手段研究再生性状的进程。

2)对控制植物再生性状关键基因的发掘不足。依据控制模式植物(如拟南芥)某一性状主效基因,利用比较基因组学及功能基因组学方法鉴定控制该性状的基因,是当前植物功能基因研究的一个重要手段。然而,目前已报道的控制植物再生性状的基因十分稀缺,限制了利用比较基因组学进行再生相关基因的分离及功能鉴定研究。

3)已鉴定的植物再生相关基因功能不明显。目前已鉴定的再生相关基因主要来自拟南芥、菊苣、水稻、小麦、大麦和玉米等少数几个植物,将这些基因在其他植物中进行功能鉴定,或根据这些基因序列从其他植物中分离出同类基因进行功能分析,其促进胚性愈伤组织发生和植株再生的作用并不明显。原因可能是这些基因的作用具有物种特异性,也可能是这些基因并非再生性状的关键基因。

未来需要加强对稳定的高再生性能植物品种的鉴定力度,利用各种途径创造和筛选高再生性能的植物品种,为有效发掘、控制植物离体组织再生性能的关键基因提供丰富的基因资源,推动植物再生性状分子基础研究,促进转基因植物新品种培育。

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·学术动态·

中国科学技术协会

中国科协第93期“新观点新学说学术沙龙”聚焦“非常规地质”

2014年10月25—26日，主题为“观念与创新：非常规地质”的中国科协第93期“新观点新学说学术沙龙”在北京举行。中国工程院院士、中国地质科学院研究员赵文津，中国工程院院士、西藏自治区国土资源厅总工程师多吉，中国地质大学(北京) 教授张金川担任领衔科学家。非常规油气、地热能及干热岩、非常规资源及行星地质等学科的50余位专家、学者与会交流。

近年来，以页岩气、致密砂岩油气、煤层气、天然气水合物等为代表的非常规油气地质研究、地热能和干热岩的勘探开发、非常规资源及行星地质的研究迅猛发展，涌现出许多新观点、新学说，开辟了新的能源资源、星际地质研究领域。与会专家围绕中国页岩气可持续发展、页岩油和致密油、非常规油气勘探、天然气水合物资源及环境、地热能开发利用、干热岩、非常规铀资源、火星浅表水与有机质等议题展开交流。

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