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西瓜组织培养与遗传转化研究进展

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摘 要:西瓜是我国重要的经济作物,其面积和产量居世界水果前列。由于西瓜资源的遗传背景相对狭窄,对于一些重要病害缺乏可利用的抗性材料,采用转基因手段有助于创制新种质。转基因技术是生物遗传改良和基因功能验证的重要方法,而组织培养和遗传转化是植物转基因成功与否的2个前提条件。西瓜遗传转化采用的方法有多种,而农杆菌介导法应用最广泛。大量的研究表明,西瓜是被公认比较难于转化的作物,目前为止转化效率低仍然是阻碍西瓜转基因的主要瓶颈。本文分析了西瓜组织培养的影响因素,如西瓜的种子贮存时间、基因型、苗龄、外植体类型、激素组合、培养条件;同时分析了农杆菌介导法影响西瓜遗传转化效率的主要因素,包括筛选标记基因、农杆菌菌株类型、预培养时间、农杆菌侵染时间和浓度、共培养时间;探讨了目前西瓜组织培养和遗传转化体系所存在的问题,提出了今后的研究方向。

关键词:西瓜;组织培养;遗传转化

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Research advances on watermelon tissue culture and genetic transformation

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Abstract: Watermelon is one of the economically significant cucurbits in China for its production yield and areas rank the first in the world. There are many agricultural problems in the cultivation of watermelon for which traditional breeding method often does not have already explained, such as lacking of resistance cultivar against viral diseases. However, there are inadequate germplasm resources to meet the needs of watermelon breeders. Other important issues requiring improvement are fruit quality, nutrition, flavour, tolerance of storage of fruits and resistance to abiotic stresses. The above problems are difficult to solve through time-consuming conventional breeding methods, but biotechnology shed more light on solving this issue. Transgenic technology is a powerful tool for gene functional validation and genetic improvement of plants. Tissue culture and genetic transformation are the two basic components for that. Tissue culture is a prerequisite to successful genetic transformation which introduce significantly interest genes into the plant genome while preserving genetic identity of plants. Recent advances in this technology have resulted in successful development of commercially disease and herbicide resistant plants which enhanced tolerance to environment stresses, increased crop productivity and reduced the usage of harmful pesticides. The plants have been engineered for safe and inexpensive production in large quantities produced in transgenic plants, as well as plants which possess enhanced nutritional traits to date. In this paper, we analyzed the major factors affecting watermelon tissue culture which is aimed to regenerate explants of cotyledons, hypocotyls, apical buds, anthers, ovaries, protoplasts and leaves. But most watermelon tissue culture

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used cotyledons as the explants, induction of adventitious shoots was obtained from proximal parts of the cotyledon incubated on MS medium containing different concentrations of benzylaminpurine 1-7 mg·$L^{-1}$and lower concentrations of auxin 0-3 mg·L^{-1} or 2 mg·L^{-1} of 2,4-D. Regeneration of watermelon in tissue culture has been achieved for various watermelon cultivars. The various factors that influenced tissue culture of watermelon including the seeds storage time, genotypes, age of seedlings, type of explants, composition of culture medium and environmental conditions. However, an efficient system for plant regeneration is essential for in vitro techniques which is useful in procedure of transgenic genes. Recently most transgenic watermelon researches focused on improving regeneration of plantlets from callus and adventitious shoots directly, in addition to pursuing efficient transformation methods of foreign genes into watermelon. *Agrobacterium*-mediated genetic transformation currently was the common method in the watermelon transgenic technology. In 1994, Choi in Korea reported the first transgenic watermelon plants regenerated from cotyledons explants by *Agrobacterium* inoculation. Since then, by using modified *Agrobacterium*-mediated genetic transformation, resistance to various plant diseases has been achieved in transgenic watermelon through the incorporation of genes coding for the coat protein gene of various plant viruses, including *Zucchini yellow mosaic virus* (ZYMV), *Watermelon mosaic virus* (WMV), *Cucumber mosaic virus* (CMV) and *Cucumber green mottle mosaic virus* (CGMMV). Other researchers have reported the development of genetically engineered watermelon plants through transformation with ACC oxidase antisense gene, antifungal proteins and chitinases etc. *Agrobacterium*-mediated genetic transformation technology is a highly complex and evolved process involving genetic determinants of both the bacterium and the host plant cell. The watermelon is still acknowledged recalcitrant crop for transformation. Its transformation efficiency is still very low so far. Here we summarized key factors influencing *Agrobacterium*-mediated watermelon genetic transformation, including genotypes of the plant, types of explants, plasmid vectors, bacterial strains, the selection markers genes, the *Agrobacterium* strains, the pre-culture time, the infection concentrations and immersing time of Agrobacterium, the co-culture time. A number of researches indicated that three to ten days cotyledons are used as suitable explants for watermelon transformation, kanamycin is the usual selection marker, and herbicide resistance *Bar* gene and hygromycin B resistance gene are also used. Some *Agrobacterium* strains are applied to produce genetic transformation watermelon, such as EHA105, LBA4404, EHA101 and GV3101. There is no evidence to prove which strain is more effective. One to five days pre-culture or without can produce adventitious shoots, the co-cultivation time of watermelon explants with *Agrobacterium* from one to five days also produce adventitious shoots. The efficiency of plant transformation can depend also on the *Agrobacterium* density, ranging from 1×10^{6} to 1×10^{10} cfu·mL^{-1} can increase transcient transformation, however, it is not always correlated with higher stable transformation. It also discussed that some questions about tissue culture and genetic transformation of watermelon. It has not yet been solved that vitrification, yellowing, the top necrosis phenomenon in the process of subculture. Cotyledons is the mainly used explants affects the ploidy level of watermelon regenerated from tissue culture that high frequency of tetraploid regenerants is a common phenomenon. Some fruit characters are significantly different in morphology, and there is gene escape in transgenic watermelon plants system. The disorganization of meristematic structures following the exposure of explants to *Agrobacterium* remains the major obstacle to develop efficient transformation technology. These problems seriously restrict the usage of watermelon transgenic technology. In 2013, a powerful tool CRISPR (clustered regulatory interspersed short palindromic repeat)/Cas9 (CRISPR associated proteins) gene editing technology in plants was developed.

Since then, the CRISPR/Cas9 system has been used in various plant species for targeted genome editing, such as arabidopsis, tobacco, wheat, rice, maize, cucumber and watermelon. It is able to achieve highly flexible and specific targeting. This system can edit multiple target genes simultaneously. We also expect new watermelon germplasm with novel desired traits will be created by this tool in coming years.

Key words: *Citrullus lanatus*; Tissue culture; Genetic transformation

西瓜(*Citrullus lanatus*)属于葫芦科双子叶植物，味甘汁多，清爽解渴，富含葡萄糖、苹果酸、番茄红素、维生素等多种营养物质，赋有“盛夏之王”的美誉[1-3]。据FAO最新数据统计，西瓜种植面积占世界水果第七位，产量位居第一。我国的西瓜约占世界种植面积的52.7%，产量达70%，是世界上最大的西瓜生产国[4]。由于我国不是西瓜的原产地，导致同源性较高，遗传基础狭窄[5-7]，难以通过常规育种手段选育既有优良农艺性状和商品性状、同时又抗病抗逆的亲本材料。目前，市场上西瓜品种繁多，但是抗病虫害和抗逆境胁迫的品种远不能满足产业发展的需求。通过转基因技术获得新种质从而改良或者增加优良性状具有重要的现实意义。1983年世界上第一例转基因烟草在美国问世之后[8]，转基因技术在许多作物上广泛应用并获得了巨大成功。经过30多年的发展，这项技术逐渐应用成熟并完善。但直到1994年，首例转基因西瓜材料才被报道[9]，到目前为止，转基因技术应用于西瓜的突破性进展仍显不足，大部分研究依然集中在提高遗传转化效率这个核心问题，与新品种选育还有一定的距离。

笔者主要针对近20年来国内外西瓜组织培养和遗传转化的研究进展作一综述，重点对农杆菌介导的遗传转化各个环节进行探讨，同时提出未来的研究方向。

1 西瓜组织培养

高效组织培养是植物遗传转化体系成功的首要条件。西瓜组织培养的研究最早开始于1971年，Andrus等[10]用无籽西瓜下胚轴诱导获得了丛生芽，开启了西瓜组织培养的前奏。随后，西瓜的顶芽、子叶、花药、小孢子、原生质体、叶片等都被用来诱导丛生芽的再生并获得成功[11-14]。受基因型、子叶苗龄、叶片年龄、组织来源、生理状态等培养条件和时间等因素的限制，西瓜的再生能力具有很大差异。

1.1 种子贮存时间

西瓜组织培养最常用的外植体是成熟种子。试验表明，采收当年的种子和贮存4 a(年)以上的种子，在培养基上萌发时发芽率和发芽势不整齐，随着贮存年限的增加，种子的发芽率和发芽势逐渐降低，在培养基上萌发的时间逐渐增长，诱导丛生芽效率降低[15]。贮存时间小于半年的种子，虽然活力指数和发芽指数高，但发芽率和发芽势较低，可能是由于贮存时间太短，西瓜种子还未度过休眠期[16]。贮存1~2 a的种子，发芽率和发芽势较高，在培养基上萌发整齐，有效外植体数量多，诱导丛生芽效率高。笔者实验室的研究结果与前人的结论一致：用于西瓜组织培养的种子贮存时间在半年以上2年以内，种子的活力指数高，幼苗生长整齐、健壮，诱导丛生芽效果最好[17]。

1.2 基因型

基因型是影响西瓜再生的内在因子，研究表明，不同的基因型在相同的培养条件下，丛生芽再生率差别很大。孙治图等[18]对23种西瓜材料进行再生筛选，发现丛生芽再生频率变化范围为0~90%。郝立新等[19]建立的5个西瓜再生体系的丛生芽诱导率为46.6%~94%，Compton等[20]以4个不同西瓜品种子叶为外植体，丛生芽再生率介于11%~66%，其他学者[21-23]应用不同的西瓜品种，再生率也不相同。基因型差异是影响西瓜组织培养广泛应用的最大限制因素，仍需寻找再生能力强的基因型；或者选择适合不同基因型的广谱培养基。

1.3 苗龄

苗龄是影响西瓜再生的另一个重要因素，它决定着外植体的生理状态。因为苗龄不同，同一部位的外植体丛生芽再生率差异明显。据报道大多基因型的西瓜苗龄为2~5 d时，外植体诱导丛生芽效率高[9,11,24-27]，也有个别基因型的西瓜苗龄7~10 d[28]时，外植体诱导不定芽的分化，而苗龄超过10 d的西瓜外植体则不能诱导不定芽的分化。很多学者研究发现，通过子叶的颜色可以判断最佳外植体，子叶颜色由黄变绿时丛生芽再生能力最强。颜色发黄以及变为深绿色的子叶分化效果均不理想[11,16,29-31]。这种

方法避免了因基因型、种子质量、种子贮存时间、消毒剂、培养基等条件的差异而造成的用天数计算苗龄的误差，具有参考价值。

1.4 外植体类型

不同类型的外植体诱导丛生芽再生的能力不同，西瓜最常用的外植体是子叶[9,11,16,24-27,29-31]，除子叶外，茎尖、胚轴[32]、果实[33]、叶片[12]等也运用到西瓜再生的研究中，经过诱导均可获得丛生芽，这些报道虽然较少，但是为西瓜组织培养工作提供了更多的研究途径，使用叶片作为外植体有助于解决倍性的干扰问题。

1.5 子叶部位

子叶部位是影响西瓜丛生芽再生率的又一因素，完整子叶、二分之一子叶基端、四分之一子叶基端、子叶顶端均可获得较高的丛生芽诱导率[9,11,16,24-27,29-31,34-36]。大部分研究采用靠近下胚轴的子叶基端作为外植体，这个部位分化能力相对较强，能够直接诱导大量丛生芽。而Choi等[9]研究发现‘Sweet Gem’和‘Gold Medal’这2个基因型则是子叶的上半部分诱导丛生芽能力强，丛生芽诱导率分别是子叶基端的1.25和2倍。万勇等[34]认为完整子叶诱导丛生芽效率高。不同基因型西瓜子叶的最佳外植体部位要结合实验条件来确定。

1.6 激素组合

不同的激素及组合对西瓜丛生芽的诱导作用不同。西瓜各部分组织分化丛生芽的能力因为内源激素水平不同，导致对外源激素水平的要求也不同。BA是诱导西瓜子叶外植体分化必不可少的生长调节剂，诱导子叶丛生芽BA的浓度范围为1~10 mg·L^{-1}。IAA、IBA、NAA等生长素类生长调节剂常用的浓度范围为0~3 mg·L^{-1}[9,11,20,25,28,37-38]。Chaturvedi等[39]认为IAA浓度低于0.18 mg·L^{-1}，容易诱导丛生芽直接分化，而IAA浓度高于0.18 mg·L^{-1}，容易诱导愈伤组织，丛生芽必须及时转接到继代培养基，否则不仅加速愈伤组织形成，还会形成更多的畸形芽。只有Tabei等[26]研究认为使用介于10~20 mg·L^{-1}高浓度的IAA，诱导西瓜子叶丛生芽效果较好。目前仅有1篇文献报道[28]使用浓度为2.5 mg·L^{-1}的激素2,4-D，诱导西瓜叶片获得丛生芽。

1.7 培养条件

西瓜丛生芽诱导过程中培养条件很重要，不同的研究学者结论不一致，一些研究结果[11,13]表明，西瓜种子在光照条件下培养5 d，子叶由黄转绿时作为外植体，诱导丛生芽，再生率较高（16 h光照27 ℃，8 h黑暗23 ℃）。而另一些研究结果显示，西瓜种子在温度为25~28 ℃黑暗条件下培养3~5 d，然后将子叶切下诱导，获得高频率的不定芽[9,25,27-8,35,40]；而也有的试验得出结论：西瓜种子先在暗处培养2~3 d，然后移到光下培养，子叶外植体才能诱导不定芽形成[38]。笔者经过试验发现，如果持续光照培养而不经暗培养，只有个别种胚萌发，大部分种胚不能转绿呈白色，胚根不伸长；完全黑暗培养，短于2 d的种胚，种皮不容易除去，3~6 d的子叶都能不同程度地诱导不定芽形成，但生成的不定芽颜色容易由绿变黄，不健壮；将种子先在28 ℃恒温箱暗培养2 d，再在25 ℃培养室光照培养3 d，子叶由黄转绿时诱导丛生芽的效率最高，但在光下培养超过3 d，子叶颜色逐渐转为深绿色时，诱导率逐渐下降，甚至没有丛生芽形成[17]。

2 西瓜遗传转化

目前西瓜遗传转化使用基因枪法[41-42]、花粉管通道法[43-44]、DNA浸胚法[45-48]、农杆菌介导法[5,6,9,11,13,17,24-30,37,40,49-61]等转化不同的目的基因。其中应用最多的是农杆菌介导的遗传转化方法。因其培养条件和使用工具相对简单、目的基因插入染色体的拷贝数低、转化效率相对较高、具有广泛的应用价值。西瓜的遗传转化工作起始于20世纪90年代初，1994年，韩国Choi等[9]首次报道了利用农杆菌将*GUS*标记基因和*npt*II抗性基因转入西瓜。随后，陆续有学者对西瓜开展了遗传转化研究，将各种外源基因导入西瓜，并获得了转基因材料。目前，应用于西瓜遗传转化的外源基因主要有以下几类：一是标记基因，如β-葡糖苷酸酶基因（*GUS*），新霉素磷酸转移酶基因（*npt*II）[9]；二是病毒基因，包括西瓜花叶病毒（WMV）、小西葫芦黄化花叶病毒（ZYMV）、黄瓜花叶病毒（CMV）、黄瓜花叶绿斑驳病毒（CGMMV）等[25,40,49-53]；三是总DNA，包括高抗枯萎病的南瓜总DNA、高抗枯萎病的瓠瓜总DNA、银杏总DNA等[45-48]；四是真菌病害基因，包括葡聚糖酶基因、几丁质酶基因、硫堇蛋白基因等[53-57]；此外，还有与果实贮藏相关的ACC合成酶及其抗逆相关的基因等[24,58]。

外源基因导入植物受多种因素的影响，包括基

因型、筛选标记、农杆菌菌株类型、预培养时间、共培养时间、农杆菌侵染浓度和时间等，遗传转化的每一个步骤都会影响转化效率。研究认为影响西瓜遗传转化的关键因素是基因型，染色体上的基因能够调控外植体的再生能力，选择适合的转化受体非常重要[59-65]。

2.1　筛选标记基因

植物遗传转化使用的筛选标记基因主要是除草剂、抗生素、氨基酸等类型[66-67]。其中卡那霉素（Kan）在西瓜转基因研究中应用最多[9, 24, 42, 59, 68-69]。Kan能干扰植物叶绿体细胞，导致植物黄化，最终死亡，使转化体容易从非转化体中筛选出来。Kan也能够清除转化后多余的农杆菌[70-71]。除此之外，Kan对于鉴定转基因后代也起重要作用[72]。将转基因材料的种子播种在含有Kan的培养基上，可以初步判断外源基因的拷贝数；鉴定转基因种子的纯度；对于转基因幼苗植株，直接喷施一定浓度的Kan，根据子叶和真叶颜色也可以筛选抗性材料[73]。

抗除草剂基因（*bar*）和潮霉素抗性基因（*hyg*）也有应用。有研究指出*bar*基因可能比*npt*II基因有效，*npt*II基因相比*hyg*基因容易筛选转基因丛生芽[74]，但*npt*II基因筛选的外植体容易产生嵌合芽[37]。也有研究指出*npt*II基因和*hyg*基因筛选转化材料的程度相似，而*hyg*基因筛选的阳性植株适应外界环境的能力差，移栽到温室后，不容易恢复活力，死亡率较高[25]。张志忠[29]研究表明，西瓜子叶对潮霉素敏感，而*npt*II基因会导致外植体玻璃化，不适合筛选西瓜转化体。

2.2　农杆菌菌株

利用农杆菌介导法转化西瓜子叶外植体，选择对西瓜敏感的农杆菌菌株可以提高转化效率。不同研究采用的农杆菌菌株不同得出的结论不同，有研究对比2个农杆菌菌株LBA4404和EHA105转化野生西瓜，发现这2个农杆菌菌株不影响转化效率[25]；有的试验结果认为EHA101的侵染效率比LBA4404高[37,75]。也有的试验得出结论：农杆菌菌株EHA105比LBA4404以及AGL-1侵染效率高。Cho等[28]研究表明，农杆菌菌株EHA101侵染效率为1.16%，高于GV3101（0.33%），而LBA4404效率最差，转化率为0。而有的研究则认为LBA4404对西瓜子叶块进行侵染的效率最高。利用不同的农杆菌菌株均获得一定数量的阳性植株[40,53,76]，适合转化西瓜的最佳农杆菌菌株目前没有统一结论，需要进一步研究转化过程中农杆菌与宿主细胞相互识别等机制，明确适合转化西瓜的农杆菌菌株[77-78]。

2.3　预培养

外植体被农杆菌侵染时，在诱导不定芽的培养基上经过一定时间的预培养，可以增强对农杆菌的耐受能力，外植体经过预培养可以利用培养基中的植物激素促进细胞分裂，活跃的分裂细胞更容易感知和整合T-DNA[79-81]，从而提高转化效率。预培养也可以减少农杆菌或者筛选试剂对细胞分化的干扰[82]。不同研究学者对转基因西瓜的预培养时间结论不一致：1~10 d都有报道[9,25,28,37]。而不经过预培养获得转基因西瓜材料也有报道[40,53,76]，可能是西瓜的子叶细胞富含营养物质，具有较强的分生能力[83]。

2.4　侵染时间和菌液浓度

农杆菌侵染外植体的目的是使较多的农杆菌附着在外植体伤口处，便于侵染。农杆菌侵染的时间和浓度，以及外植体对农杆菌的敏感程度都影响转化效率。当农杆菌浓度介于1×10^6到1×10^{10} cfu·mL^{-1}时，可以增加外植体的瞬时转化率，但不一定能获得稳定高效的转化率[84-85]。侵染时间短，农杆菌不能充分附着到外植体表面；而侵染时间长，农杆菌会过度繁殖，其分泌物对外植体具有毒害作用，抑制外植体的生长。在转化过程中，要根据不同的农杆菌种类，繁殖快的菌株侵染时间要缩短，而繁殖慢的菌株侵染时间要适当延长[63]。西瓜的子叶块相对肥厚，对农杆菌侵染不敏感，所以使用的侵染时间相对较长，西瓜转基因研究中侵染时间主要集中在10~30 min[25,40,53,76]。

2.5　共培养

共培养是农杆菌入侵外植体伤口将T-DNA转入受体细胞，从而成功转化目的基因的关键。理论上，农杆菌与外植体共培养时间越长，受侵染的细胞越多，目的基因的转化频率越高。但是试验发现，延长共培养时间，导致农杆菌过度增殖，外植体会受到农杆菌的毒害，分化受到抑制，而共培养时间太短（小于16 h），农杆菌不能完成T-DNA的转移过程，在创伤部位不能形成肿瘤，同样不能获得转基因材料[63,65,86]。确定合适的共培养时间是提高转化率成功的关键因素。西瓜转基因共培养时间研究主要集中在2~4 d[9,25,27,37,76]，均获得了不同数量的转基因植株。

3 问题与展望

3.1 西瓜组织培养出现的问题

西瓜的玻璃化现象相对突出，严重影响组培苗的成活。玻璃化苗经过继代培养和移栽，不容易恢复形成正常苗。玻璃化现象呈现为无菌苗叶片变薄、半透明水渍状、表面无角质蜡质层、颜色深绿；茎畸形、肿胀、细长、易脆易碎；解剖组织结构异常、表皮空洞、断裂、中空等症状[87-88]。导致西瓜组培苗玻璃化的原因很多，继代培养时间长、营养不足、光照时间短、温度不适、培养基成分、生长调节剂、琼脂浓度、组培瓶空气湿度等因素都有影响[89]。通过提高琼脂和蔗糖浓度，降低培养基中的可利用水和容器的相对湿度，添加一些激素或降低部分离子浓度，都可以起到降低玻璃化的作用，但到目前为止，这一问题并未彻底解决。

西瓜黄化死亡的现象鲜有报道。而笔者发现西瓜的黄化现象比玻璃化现象更加严重，丛生芽继代培养15 d以后，叶片开始出现失绿黄化趋势，有的整个植株叶片逐渐黄化，直至褐化死亡，而有的丛生芽会从茎尖生长点处开始褐化，逐渐死亡。叶绿素降低、光合作用下降[90]可能是发生这种现象的原因。通过增加铁盐的浓度，可延缓黄化现象的发生[91]，但不能彻底改善这一情况。

西瓜虽然能够诱导生根，但是无菌苗根系不发达，移栽时容易断裂，难以成活[17,92]，而且西瓜的根系抗病性差，容易遭受枯萎病以及炭疽病等多种病害，移栽的试管苗后期，容易感染各种病害而死亡[93-94]。将西瓜的试管苗嫁接到南瓜或瓠瓜砧木上，利用砧木根系发达、抗病能力强等特点，可以克服西瓜组培苗移栽到田间后出现的不易存活的问题，同时增加幼嫩西瓜植株的抗病性[92-96]。

西瓜组织培养中有关污染的防治方法鲜有报道。有的西瓜品种种子着生内生菌，利用表面消毒剂难以清除。常规消毒后，种子播种到萌发培养基上，一开始肉眼看不见细菌污染，但是经过进一步操作，将子叶切好放在诱导丛生芽培养基上时，3 d内子叶周围陆续出现黏液状细菌，严重阻碍试验的进展[91]。消除细菌发生是西瓜组织培养成功的前提。通过添加抗生素例如青霉素、乳酸、头孢霉素等可以降低细菌的扩散[97]，但不能彻底消除内生菌的危害，这类种子目前没有方法消除细菌，最好弃去不用。

西瓜组织培养工作到目前为止，已经研究了40多年，相关的研究报道很多，但是还存在以上问题需要解决。其次，长期继代，西瓜的后代生活力下降等问题也普遍存在，说明西瓜的组织培养工作仍有待加强研究。

3.2 西瓜遗传转化存在的问题

西瓜是较难利用农杆菌介导法获得转基因材料的葫芦科植物。科学家们一直在尝试利用基因工程的方法培育具有优良特性的西瓜品种[11]，但是西瓜继代过程出现的丛生芽玻璃化、叶片黄化、植株顶端褐化坏死等问题一直阻碍试验继续深入开展[43]；另外，西瓜外植体对农杆菌的敏感程度、诱导丛生芽的能力以及再生芽的分化方式等因素都限制了西瓜转基因材料的成功获得。

西瓜转基因材料后代发生基因丢失的情况，遗传性状不稳定。研究发现含有3种病毒CWV、CGMMV和WMV外壳蛋白的转基因西瓜，T0代转基因材料对这3种病毒都具有抗性，而T1代转基因材料对2种病毒CMV和WMV具有抗性，而对CGMMV已经失去了抗性，CGMMV CP片段可能在T0代传递到T1代过程中丢失，以至其功能丧失。F_1代的基因组结构复杂，很难确定什么因素导致片段丢失。插入基因转录引起基因沉默；外壳蛋白与病毒RNA互作、阻止病毒复制等这些因素都可能是导致基因片段丢失的原因。

转基因还会导致西瓜体细胞无性系发生变化，导致转基因材料形态学上产生变异[98]，例如转基因植株下胚轴对照野生型明显增长，果皮厚度、果肉硬度、果柄长度等都有变化[99-100]，可能是由于转基因的多重效应引起的[101]。转基因表达水平与很多因素相关，如T-DNA插入基因组的位置、染色体的结构、甲基化状态、转录后调控、拷贝数等[102-104]。引起性状改变的原因尚需研究。

遗传转化还会导致西瓜转基因植株发生倍性变化，形成四倍体、甚至八倍体[100,105]。原因可能是较高浓度的Kan筛选压、以及在培养基中添加的各种抑制农杆菌生长的抗生素导致植株发生变异[106]。

西瓜遗传转化技术存在以上诸多问题，到目前仍然没有取得重大突破，获得转基因植株数量少，严重制约后续基因功能等方面的研究。

3.3 展 望

转基因研究是解决人口、耕地及环境日益冲突的重要手段之一，是未来农业研究的热点和重

点[107-108]。而葫芦科作物开展转基因研究相对缓慢，制约的瓶颈就是遗传转化的效率问题。今后需要继续优化影响遗传转化的各个因素，包括农杆菌菌株、菌液浓度、预培养、共培养、筛选继代培养、生根培养等，通过优化其中的条件，寻找各个因素的最佳组合，找到更加适合转化的基因型材料，探究农杆菌与外植体侵染的机制等，完善西瓜遗传转化的方法，提高转化效率。

目前主要利用来源于外来物种的基因导入西瓜，获得抗病、抗逆等性状的转基因西瓜，而转化西瓜自身的优良基因鲜有报道。西瓜品质改良等方面的研究以及转基因西瓜的安全性问题涉及较少[109-110]。转入多个目的基因代替单个基因、无标记基因转化、利用自身来源的基因转化以及对转基因产品的安全性评价等都是未来研究的热点。除了选择标记基因策略，生物安全标记基因以及转化后剔除标记基因的方法已经应用到玉米[111]、小麦[112]、水稻[113]等作物上，这些方法对提高转基因植物安全性具有实际意义，也是未来转基因西瓜标记基因安全性研究的方向。

目前使用的转基因方法都是随机插入植物染色体组，会导致植物内源基因破坏等不利结果，而CRISPR/Cas9基因编辑技术自2013年成功应用以来，已经迅速应用于拟南芥、烟草、玉米、水稻、小麦、黄瓜、西瓜等多种作物[114-117]，它可以对植物基因组特定的序列进行精准替换、缺失、插入，后代通过遗传分离能够获得不含转基因元件的突变植株，相比传统育种安全性更高，它的出现将在植物基因组定向编辑中发挥重大作用。

2013年西瓜基因组测序结果发表之后[118]，西瓜功能基因组的研究工作广泛开展，随着西瓜遗传转化体系的进一步完善，西瓜基因功能验证将成为可能，将有更多西瓜的基因被挖掘应用，西瓜的品种改良有望取得突破性进展。

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