林木三倍体育种研究进展及展望

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摘要 植物三倍体具有生长迅速、叶片或花、果实硕大、新陈代谢旺盛、适应性强以及不孕等特点, 尤其适用于无性繁殖林木的遗传改良. 利用配子染色体加倍或四倍体与二倍体杂交等有性多倍化技术手段, 增加目标树种一套基因组的全部同源基因, 可以仅通过一轮次的育种过程实现多目标性状综合改良, 培育出生长快、纤维长、木素低、纤维含量高以及抗逆性增强的用材林新品种, 或者是生长迅速、叶片和果实等器官巨大、含胶量及药物成分含量显著提高的胶用、漆用、药用、叶用、果用新品种. 在人工诱导配子染色体加倍选育三倍体时, 实现有效处理时期的即时判别是提高三倍体育种效率的关键, 而选配高配合力亲本则是提高三倍体育种效果的保障. 随着林木三倍体育种理论和技术方法的突破及其不断向其他树种拓展, 三倍体育种必将在提高林产品产量、改善品质以及增强抗逆性等方面发挥更大的作用.

关键词 林木, 三倍体, 性状变异, 育种技术, 新品种

植物细胞核内染色体组加倍以后, 常常带来一些形态和生理上的变化, 表现为生长迅速, 细胞及部分器官巨大; 新陈代谢旺盛, 某些生化成分含量提高; 可孕性与结籽性降低, 甚至完全无籽; 生活力强, 对环境适应性增强等^[1,2]. 就主要利用营养器官的林木多倍体育种而言, 由于许多树种能够进行无性繁殖, 可以不必担心多倍体育性差而导致繁殖困难的问题, 而多年生习性又保证品种一旦选育成功就可以长期持续利用等^[2~4], 因此, 与多倍体品种较多的作物、果树^[1,2]一样, 林木多倍体具有巨大开发利用潜力. 而在林木多倍体中, 三倍体的直接价值要高得多, 这已经被长期的育

种实践所证明.

1 林木三倍体性状变异及其应用

林木三倍体表现最为突出的性状变异是植株高、径生长提速. 欧洲山杨(*Populus tremula*)天然三倍体的树高、胸径和材积生长分别超出二倍体11%, 10%和36%^[5]. 美洲山杨(*P. tremuloides*)与欧洲山杨杂种三倍体的树高、径生长至少比与同龄二倍体高出20%^[6,7]. 三倍体毛白杨(*P. tomentosa*)B301单株材积甚至比二倍体高出2.5倍^[8]. 在我国广泛栽培的“1-214”(P×canaden-

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sis “I-214”)、“中林46”(P.×*euramericana* “Zhonglin-46”)、“廊坊3号”(P.×*langfangensis*-3)、“银中杨”(P. *alba*×P. *berolinensis*)等均属于从成千上万杂交后代中选育出来的优良品种, 这些著名杨树品种相继被证明为三倍体^[9,10]的事实表明, 三倍体杨树具有更为速生的特性. 桑树(*Morus alba*)、橡胶树(*Hevea brasiliensis*)、漆树(*Toxicodendron vernicifluum*)、杜仲(*Eucommia ulmoides*)、桉树(*Eucalyptus urophylla*)、柳树(*Salix* spp.)、枣树(*Ziziphus jujuba*)等树种的三倍体也同样如此^[11~19]. 裸子植物三倍体大多生长缓慢、育性差^[20], 但银杏(*Ginkgo biloba*)三倍体却有较好的生长表现^[21]. 此外, 有人认为三倍体杨树存在后期生长慢的问题, 实际这正是三倍体速生性的体现. 一定林分密度下三倍体因生长快可以更早达到郁闭, 如不适时采收或间伐, 必然会因密度及水养资源限制而生长变缓. 在河北威县有2株1992年春定植的三倍体毛白杨孤立木, 迄今胸径分别达到71.7 cm和73.2 cm, 表明三倍体与二倍体一样, 在营养空间充裕时可一直保持较高的生长速率.

细胞及器官巨大性是林木三倍体性状变异又一突出表现. 如欧洲山杨×美洲山杨杂种三倍体的纤维长度比对照长18%^[6,7]. 5年生三倍体毛白杨纤维平均长度达1.28 mm, 比同龄普通毛白杨长52%^[3,4]. 三倍体柳树纤维素含量显著高于二倍体, 木质素含量显著低于二倍体^[16]. 三倍体细胞巨大性导致单位材积的纤维细胞数减少以及细胞表面积减小, 从而使分布于细胞壁的木质素等含量降低, 而纤维素含量则相应增加^[2,3], 表明林木三倍体是一种优良的制浆造纸等纤维用材原料. 而三倍体叶片、果实巨大性则是最为容易观察的性状^[14~19,22]. 三倍体枣树具有果实和叶片相对更大的特点^[13], 是大果品种选育的有效途径. 桑树、杜仲三倍体品种普遍比二倍体具有营养生长旺盛、叶形大且叶肉肥厚等特性, 叶产量比二倍体高30%以上^[11,12,18]. 三倍体巨大性叶片除了具有增加光合面积、促进营养生长作用外, 对于叶用以及以叶片为原料的林业产业发展亦具有重要价值.

林木三倍体还具有次生代谢产物显著提高和抗逆性增强的遗传特点, 更益于树种栽培区域扩大以及工业化生产和加工效益的提高. 如三倍体漆树的产漆量比二倍体高出1~2倍^[14]. 三倍体桑树的叶蛋白及可溶性糖含量比对照高出1.0%~3.0%^[11,12]. 橡胶树三倍体

品种“云研77-2”、“云研77-4”的4年平均产胶量分别是优良品种“GT1”的165.3%和143.1%^[23]; 且可溶性糖含量、SOD(superoxide dismutase)活性更高, 因此比GT1品种更抗寒^[24]. 三倍体杜仲的绿原酸、桃叶珊瑚苷、京尼平苷等药效成分含量分别比二倍体高出10.20%, 1.34%, 118.22%^[18]. 而环烯醚萜类、黄酮类等次生代谢产物被认为是植物防御物质, 其含量增加对于提高三倍体抗逆性具有重要作用. 研究表明, 三倍体青杨((P. *simonii*×P. *nigra*)×(P.×*beijingensis*))在干旱胁迫下, 相对电解质渗出率和丙二醛含量更低, 相对含水量和游离脯氨酸以及抗氧化能力更高, 证明三倍体青杨较二倍体具有更强的抗性^[25].

2 林木三倍体选育途径及技术关键

三倍体首先从自然界发现并逐渐得到重视. 其中, 欧洲山杨是最早发现的三倍体树木^[5]. 此后, 挪威云杉(*Picea abies*)、北美乔柏(*Thuja plicata*)、日本柳杉(*Cryptomeria japonica*)、非洲苏铁(*Encephalartos hildebrandtii*)、银杏、桑树、漆树、银荆(*Acacia dealbata*)、柳树、美洲山杨、银白杨(P. *alba*)、香脂杨(P. *balsamifera*)、毛白杨等天然多倍体相继检出^[1~4,14,20,21,26~28]. 大多是因其叶片或果实巨大、营养生长表现突出而引起关注甚至选择利用, 此后经染色体检查证明为三倍体. 形成原因多为自然四倍体与二倍体杂交, 以及未减数2n配子与正常异性配子杂交. 如在毛白杨种质资源库469株优树中检出28株三倍体^[27,28], 这些三倍体可能来源于天然2n花粉^[3,29]或天然2n雌配子^[30]. 美洲黑杨、欧洲山杨也存在可自然产生2n配子的雌株^[31,32]. 这种由天然2n配子生产的三倍体品种实际已经在生产中得到应用, 如我国云南广泛栽培的“云研77-2”、“云研77-4”橡胶树三倍体品种起源于母本“GT1”自然发生的2n雌配子^[33]. 而三倍体毛白杨系列品种则来源于天然2n花粉授粉杂交^[8]. 然而, 可产生高比率2n配子植株以及天然三倍体毕竟只占自然种群的极少数, 利用的限制性难题是如何将其从巨大的种群中分离出来^[3,4].

三倍体可以通过不同倍性体杂交途径获得. 最早人工选育欧洲山杨多倍体是通过三倍体与二倍体杂交获得的^[34]. 这是由于三倍体不均衡的减数分裂中可产生少量含有一套以上染色体的可育配子. 在杨树中以

三倍体做母本难以获得子代,而以三倍体为父本则可以获得一些染色体数等于或接近于三倍体、四倍体的植株^[3]。而获得的染色体数等于57的后代是否属于真正意义的三倍体仍有待验证,因为有产生非整倍体的可能。林木三倍体选育最为成功的树种当属桑树,其中大部分是通过四倍体与二倍体杂交途径获得的^[11,12,35]。杨树、柳树、桦树、相思树(*Acacia auriculiformis*)等树种中也有许多通过杂交获得的三倍体,并从中选育出新品种应用于生产^[4,36~38]。但该途径实现的前提是拥有可用于杂交的四倍体,而掌握合子或体细胞染色体加倍的有效处理时期是获得四倍体的关键^[39,40]。此外,获得的四倍体还必须具有较好的育性,相思树由于存在单倍性和二倍性配子受精结合后合子败育问题,只有采取胚挽救措施才能获得三倍体^[38,41]。

三倍体可以通过雌雄配子染色体加倍途径获得。通过理化处理人工诱导2n配子授粉杂交,可以节省四倍体诱导以及等待开花结实的时间,是林木三倍体育种中最快捷的途径。该途径在欧洲山杨、美洲山杨、美洲黑杨、香脂杨、银白杨、毛新杨(*P. tomentosa*×*P. bolleana*)、银腺杨(*P. alba*×*P. glandulosa*)、毛白杨、响叶杨(*P. adenopoda*)、银灰杨(*P. canescens*)、桑树、杜仲、桉树等树种中均有成功的报道^[2~4,19,42~54]。研究表明,掌握理化诱导2n配子的有效处理时期至关重要。其中,施加秋水仙碱溶液处理诱导花粉染色体加倍的有效处理时期为花粉母细胞减数分裂粗线期附近,高温诱导杨树2n花粉则以花粉母细胞减数分裂终变期-中期I时处理的效果最好,适时处理可以获得80%以上的2n花粉^[42,43]。由于未减数2n花粉存在因发育迟缓而导致的受精竞争能力差的问题^[44],可利用花粉倍性对辐射敏感性的差异,施加一定剂量的辐射抑制单倍体发育,从而提高2n花粉参与授精竞争获得三倍体的概率^[45]。而对于雌配子染色体加倍而言,由于大孢子发生和胚囊发育均包裹于子房内的胚珠中,通过石蜡组织切片难以做到即时观察^[2~4]。此时,可以选择利用大小孢子发生的时序相关性、雌花芽发育形态以及最佳授粉时期为参照,相对准确地解决大孢子、胚囊染色体加倍有效处理时期即时判别问题等,三倍体诱导率最高可达到15%~80%^[19,46~50]。

胚乳培养实际是一个细胞有性多倍化后三倍体植株再生的过程。目前有罗氏核实木(*Putranjiv aroxburghii*)、麻风树(*Jatropha pandurifolia*)、枸杞(*Lycium*

barbarum)、猕猴桃(*Actinidia chinensis*)、核桃(*Juglans regia*)、桑树、金合欢(*Acacia nilotica*)、印楝(*Azadirachta indica*)等树种通过未成熟或成熟胚乳培养获得了三倍体再生植株^[55,56]。其主要问题是愈伤组织继代培养中往往会产生非整倍体、混倍体变异,且每个胚乳外植体均需建立一套再生体系,诱导技术难度和工作量较大,而获得可供选择的三倍体群体相对较小等,具有育种应用的局限性。

3 林木三倍体性状表现影响因素

从不同白杨和青杨派杨树以及杜仲雌雄配子染色体加倍获得的杂种三倍体苗期生长和光合生理性状对比看,不同染色体加倍途径以及不同亲本组合获得的三倍体后代苗期生长存在较大差异,其中三倍体苗木平均地径和高生长以及光合速率、叶绿素含量、叶面积等均显著高于同亲本组合的二倍体;且不同三倍体群体内的苗木生长、光合速率、叶面积、叶绿素荧光等指标在群体内的变异大于群体间的变异^[3,18,25]。其原因在于林木育种目标性状多为数量遗传性状,受多基因控制的性状表现在群体中呈正态分布。表明三倍体的生长等性状表现既有赖于染色体组加倍的剂量效应,也取决于亲本间的杂合效应。而多倍体似乎更依靠杂合性^[57]。因此,开展林木三倍体育种时,应重视亲本的选择和选配,在有效利用染色体加倍带来的倍性优势的同时,更充分地发挥高配合力亲本组合产生的杂种优势。

在林木三倍体育种中要想在获得倍性效应的基础上更为充分地发挥杂合效应,除了亲本选配之外,有性多倍化诱导途径也有一定的影响。一般而言,基于不同倍性体以及自然和人工2n配子杂交选育三倍体的有性多倍化途径主要有3种,即FDR(first division restitution)型、SDR(second division restitution)型及PMR(post-meiotic restitution)型2n配子途径^[2,3]。因配子染色体加倍发生的时期不同,3条途径获得的2n配子传递亲本杂合性也不相同。就来源于同一青杨亲本组合的FDR型、SDR型以及PMR型2n配子而言,其传递亲本杂合性分别为74.8%, 39.6%, 35.9%^[58]。由此获得的3个三倍体群体苗木生长、光合生理等性状指标存在一定差异,其中FDR型2n配子来源的三倍体杂种后代群体生长相关性状平均值最大,但SDR及PMR型2n配子来源

的三倍体杂种后代群体中也有表现特别突出的基因型^[25]。这是因为这两种2n配子形成之前的减数分裂前期DNA发生同源重组,且转录本丰度越高的区域同源重组发生的频率越高^[59]。DNA单链同源重组以及染色体加倍使SDR型、PMR型2n配子具备了一定的杂合性,因此3种诱导途径都具有一定的利用价值。只是FDR型2n配子传递亲本杂和性相对较高,决定了其育种效率相对更高。

林木三倍体性状表现的影响机制研究尚不够深入。从群体水平分析青杨异源三倍体与二倍体群体苗期叶片基因表达、miRNA(microRNA)表达以及DNA甲基化变异发现,营养生长相关基因表达具剂量效应;miRNA表达不表现剂量效应;DNA去甲基化变异升高,而总甲基化水平降低等^[60-62]。显然,林木三倍体营养生长优势形成具有重要的遗传基础。而基因型与环境互作也会对三倍体性状表现产生重要影响。从三倍体杨树、柳树不同无性系的多地点栽培实验看,材积生长和纤维特性等在不同地点存在显著差异^[16,63]。因此,三倍体育种同样应该重视被选择群体的大小及其对育种效果的影响,坚持大群体、强选择,并为不同的栽培生态区选育不同的三倍体主栽品种。

4 问题与展望

综上,林木三倍体育种借助一定的技术手段增加其一套基因组的全部同源基因,从而通过一轮次的育种过程实现多目标性状综合改良,可培育出生长快、纤维长、木素低、纤维含量高以及抗逆性增强的短周期纤维工业用材林建设优良品种,或者是生长迅速、叶片和果实等器官巨大、含胶量及药物成分含量显著提高的胶用、漆用、药用、叶用、果用新品种。近年来,以杨树为代表的林木三倍体诱导技术取得了显著的进步,通过雌雄配子染色体加倍以及四倍体与二倍体杂交等有性多倍化过程,快速、高效地创制出了一批目标性状优良的三倍体新种质。尽管如此,相关研

究成果向其他树种拓展仍较为缓慢,其主要原因在于:(i)一些树种生殖生物学基础薄弱,尤其对于那些树体高大、进入生殖生长较晚且开花和结实周期长的树种,掌握其大小孢子发生发育规律就是一个非常艰难的过程;(ii)一些种子大、成熟期长的树种,其染色体加倍只能在树上非离体状态下进行,雌雄花受所处树冠位置和环境因素影响导致大小孢子发生发育进程存在差异,工作量大且存在一定安全问题,从而影响染色体加倍效率和效果;(iii)一些树种在三倍体育种技术方面虽有突破,但由于获得的可供选择的三倍体群体较小,或存在亲本选配不当等问题,导致育种效果欠佳;(iv)一部分树种存在自然产生或人工诱导获得的四倍体,但由于胚乳平衡数(endosperm balance number, EBN)障碍等原因,四倍体与二倍体杂交因合子败育往往导致育种失败;(v)裸子植物对理化诱变剂具有较高的耐受性,加之获得的多倍体大多存在生长缓慢等问题,多倍体育种并不被看好;(vi)三倍体优势目标性状形成机理尚未揭示,也在一定程度上影响了三倍体育种研究进程等。

由于林木树种本身生殖生长周期长等限制,难以仿效作物育种的研究思路,也就很难实现杂种优势最大限度的利用,而三倍体育种是一种可以助力林木良种选育跨越式发展的有效途径。值得注意的是,当前国内外从事植物多倍体育种的人员有逐渐增加的趋势,而投身于林木三倍体育种的队伍体量也开始变大。同时,综合SSR(simple sequence repeats)遗传距离和优良单株父本鉴定构建育种亲本群体,并与配子染色体加倍选育三倍体相结合的育种策略的成功应用^[64],也可以显著提高林木三倍体育种效率和效果。相信随着对三倍体应用价值认识的提高以及有关新技术方法的注入,在全体林木多倍体育种研究人员的共同努力下,一些理论和技术难题必将逐渐被攻克,林木三倍体育种将会焕发出新的生机与活力,在提高产量、改善品质以及增强抗逆性等新品种选育方面发挥更为突出的作用。

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Research progress and prospect of triploid breeding of forest trees

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Triploid plants characterized by fast growth, large leaves or flowers and fruits, high level of metabolism, good adaptability and sterility, triploid breeding is especially suitable for genetic improvement of tree species with good ability of vegetative propagation. Because triploids production by gametic chromosomal doubling or crossing tetraploid with diploid can make target tree species increase a set of homologous genes, new varieties characterized by fast growth, longer fiber, lower lignin, higher holocellulose and stress resistance for timber could be developed. Triploid new varieties with multiple use, such as gum, lacquer, medicine, leaves and fruits, which characterized by fast growth, large leaves and fruits, higher gum and pharmaceutical ingredient could also be achieved. Therefore, triploid breeding could improve multiple traits only by one round of breeding procedure. When triploid produced by induction of gametic chromosome doubling, determination of effective treating stages is the key factor to increase efficiency of triploid production. However, parental selection with higher combining ability was considered to be a guarantee for increasing effects on triploid production. As the breakthrough of theories and techniques in triploid breeding of tree species and its application to other species going on, it is believed that triploid breeding will play an important role in increasing yield of forest production, improving wood quality and stress resistance.

forest trees, triploid, trait variation, breeding techniques, new variety

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