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梨(*Pyrus communis* L.)多倍体新种质表型变异多样性研究

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摘　要:【目的】为选育梨(*Pyrus communis* L.)突破性品种提供新种质。**【方法】**以二倍体梨品种'丰产'及其衍生的系列多倍体无性系为研究试材,对其在试管内及移出试管外在田间生长的自根苗植株的表型变异进行了观测。**【结果】**多倍体植株比二倍体茎粗、节间短;四倍体的叶色比二倍体深,厚度比二倍体大;多倍体和二倍体的叶形指数差异显著,叶缘差异明显。不同倍性无性系的叶缘变异表现多样性,有全缘变异,也有锐锯齿变异。试管繁殖条件下,混倍体叶片形状不一,有大量畸形叶片产生,而二倍体、三倍体和四倍体很少观察到畸形叶片产生。移栽入田间生长后,相对于二倍体,多倍体植株的株高显著变矮、节间长显著变短、地面以上30 cm处的树干直径显著变小。混倍体对自然环境条件适应性差,没有获得在试管外正常生长的植株。**【结论】**相同环境条件下,梨体细胞染色体加倍衍生的多倍体植株,比二倍体对照生长慢,且株高明显矮于二倍体。

关键词: 梨; 二倍体; 多倍体; 新种质; 表型变异

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Diversity of phenotypic variation of polyploid plants new germplasm in pear (*Pyrus communis* L.)

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Abstract:【Objective**】**Pear (*Pyrus communis* L.) is a nutrient-dense fruit with strong consumer demand and high commercial value. However, most cultivated pear varieties are often susceptible to diseases and fruit quality is decreasing with the increase of planting time. Therefore, genetic improvement of existing pear cultivars or breeding new cultivars is a continuous target for pear breeding. Polyploid breeding of fruit crops has an important value in new cultivar selection and fertility improvement. In order to provide polyploid germplasm for selecting innovative cultivar in pear, new polyploid plants were obtained from diploid pear cultivar 'Fertility' (*Pyrus communis* L.) by *in vitro* colchicine treatment of leaf explants. The phenotypic variations of polyploid plants and their diploid control were compared and evaluated. **【**Methods**】**The *in vitro* plantlets and field plants of the diploid pear cultivar 'Fertility' and polyploid clones derived from it were selected as materials. For *in vitro* plantlets, the leaf traits, including leaf length, leaf width, leaf thickness, petiole length and petiole width, were measured. Leaf index were calculated by the ratio of leaf length to leaf width. The variations of leaf colour and leaf margin were observed and photographed. For plants grown in the field, the plant height above the ground was measured, ten plants were random selected and measured for each ploidy clone. The length and the number of internodes of current year's new shoots were measured and counted, three new shoots were selected from each plant, and total

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thirty new shoots were measured for each ploidy clone. The average internode length was calculated by new shoot length/the number of internode. The base diameter of current year's new shoots were measured. All collected data were analysed using DPS v3.01 statistical software. 【Results】The phenotypic characteristics of *in vitro* polyploid plantlets had a wide range of variation. Polyploid plantlets had evidently thicker shoots and shorter stem internodes than diploid plantlets. The leaf shapes of polyploids showed a variety of variations, including oval, ovate, and oblong. Leaf shapes of mixploid were irregular, including abnormal or deformed leaves. Leaf base of most polyploids became more broaded than that of the diploid control. The variation of leaf bases of polyploids included obtuse, oblique and cuneate. The variation of leaf tips of polyploids included obtuse, acuminate and spike. The leaf thickness of all polyploid clones was significantly thicker than diploid control. Leaf colour of tetraploid plants was darker than diploid control. All polyploid clones had significantly shorter petiole length than diploid control. All polyploid clones had significantly longer leaf length than diploid control. The ratio of leaf length and leaf width was significant different between polyploid clone and diploid control. Two triploid clones had significantly higher leaf index than diploid control, and all other polyploids had significantly lower leaf index than diploid control. The traits of leaf margins between polyploid plants and diploid plant were markedly different. The leaf margins of different ploidy plants showed a wide range of variations, including margin entire and margin sharply pointed teeth. In the *in vitro* proliferation, many deformed leaves were observed in mixploids, and few deformed leaves were observed in diploid, triploids and tetraploids. For the plants growing in the field, compared to the diploid plants, polyploid plants grew slower and had significantly dwarfer plant height, significantly shorter internode length, and significantly smaller trunk diameter at 30 cm above the ground. Generally, in the current year, polyploid plants only had one time growth, namely growth in the spring, but diploid plants had two times growth, namely growth in the spring and in the autumn, respectively. The mixploids showed poor endurance to natural environment, mixploid plants were not obtained in the field. 【Conclusion】 Pear polyploids derived from somatic cells by *in vitro* colchicine treatment showed a wide range of variation in morphological characteristics. Generally, compared to the diploid control, polyploids had thicker leaf thickness and shorter petiole length. Leaf index was significantly different between polyploids and diploid. Leaf thickness, petiole length and leaf index can be used as effective indicator for early selecting polyploid variation from diploid cultivar. Under the same environment, polyploid plants had slower growth and shorter plant height than its diploid control. Mixploids could grow well in tube, but they were difficult to survive in the field.

Key words: Pear; Diploid; Polyploid; New germplasm; Phenotype variation

在基因组(genome)和表型组(phenome)水平上研究植物的多样性对其保存和植物育种都具有重要意义。染色体重复增加基因表达的多样性[1-2],诱导表型的变异[3],丰富植物群体的遗传多样性[4]。植物由二倍体变为多倍体后还常常伴随其他性状(如叶、茎、花等)的表型变异[5-7]、气孔大小[8]及育性的变异[9]。多倍化被认为是植物适应性进化和物种形成的主要机制[10]。多倍体可用于新品种的选育,也可用于特定性状的遗传研究。但植物多倍体自然发生的机率很低,采用人工诱变技术创造人们所期望的多倍体已成为现代遗传学家和育种学家普遍应用的一项技术,并在很多种植物上获得了成功[11-22]。但对诱变获得的多倍体,除1 a生植物有比较深入的田间植株表型变异(包括营养性状和经济性状)的研究报道外[23-24],对于果树这种多年生木本植物所获得的多倍体在田间表型变异的表现鲜有报道,大多报道集中在通过倍性鉴定证明获得的新材料是多倍体,或观察表明多倍体的气孔保卫细胞增大或叶片变厚、叶色变深等性状的变异。本实验室从2000年开始,采用离体叶片体细胞不定梢再生技术和化学药剂秋水仙碱诱导染色体加倍技术相结合的方法诱导西洋梨品种'Fertility'(*P. communis* L.)[25]获得了一批形态变异似多倍体的无性系,对这些变异无性系经过染色体计数和细胞流式仪分析鉴定方法证明这些变异

包括三倍体、四倍体和混倍体[12],并且这些多倍体变异无性系的离体生根能力较二倍体亲本明显下降[13]。本文在试管内和田间条件下,分别观测本实验室经人工诱变获得的9个不同倍性的多倍体无性系植株及其二倍体亲本的表型变异多样性,并验证这些表型变异在试管内和田间条件下的一致性和稳定性,为梨新种质的创制提供研究技术基础,丰富梨的遗传种质库,为将来选育梨突破性品种提供新的种质材料。

1　材料和方法

1.1　材料

以二倍体品种'丰产'(*P. communis* L. 'Fertility')和用秋水仙碱处理离体叶片获得的体细胞染色体加倍的多倍体无性系(包括三倍体、四倍体和混倍体)[12-13]的试管苗及移栽到田间生长的自根苗植株为试材。

1.2　方法

试管苗叶片性状测量:从生根试管苗上选取充分展开和成熟的叶片(茎中部叶片)(预试验观察表明生根试管苗叶片性状比较稳定),每个无性系取30个植株,每株选取2个叶片,每个无性系共计测量60个叶片。用游标卡尺(精度0.01 mm)测量每个叶片的叶长度、叶宽度(最大叶宽度)、叶厚度、叶柄长度和叶柄宽度(叶柄中部)。计算叶形指数(叶长度/叶宽度)。观察叶色、叶缘变异并拍照。观察记载方法参照《果树种质资源描述符—记载项目及评价标准》[26]。

田间植株性状:对在田间网室内生长4 a的多倍体株系及二倍体对照进行了植株生长性状的测量。每个无性系测量10株,每株测量3个新梢,共计30个新梢。测量指标包括:株高(地面上部植株的最大高度)、新梢长度(包括春梢和秋梢,即在当年生新梢停止生长、落叶前,测量当年生新梢的长度)、新梢节数、新梢粗度(新梢基部直径)、节间长(梢的长度/总节数)。叶片性状的测量:在每个测量梢的中部选取有代表性的5个叶片,每植株选2个梢,每个无性系10株,共计100个叶片。叶片性状的测量指标与测量方法同试管苗叶片。

1.3　统计分析

试验结果采用DPS v3.01软件进行统计分析,不同处理平均值用Duncan法进行多重比较分析。

2　结果与分析

2.1　梨不同倍性试管苗的生长形态特征

形态特征如茎的粗度、节间长度、叶片厚度、叶色和叶形指数(叶长度/叶宽度)等通常被用作鉴定植物倍性水平的指示标记[20,27-28]。本研究中,试管苗的多倍体变异无性系和二倍体对照在植物形态特征上表现出明显差异(表1,图1)。

2.1.1　茎的变异　与亲本二倍体(图1-A和E)相比,多倍体表现为茎变粗(图1- B~D、F~H),节间变短(图1- C、D、G、H)。

2.1.2　叶的变异　不同倍性多倍体无性系叶片表型性状差异明显,表现出了表型变异的多样性。与二倍体亲本对照相比,多数多倍体的叶基变得更阔。二倍体为楔形(图2-A),三倍体有宽楔形(图2-B)和楔形(图2-C),四倍体有宽楔形(图2-D)和近圆

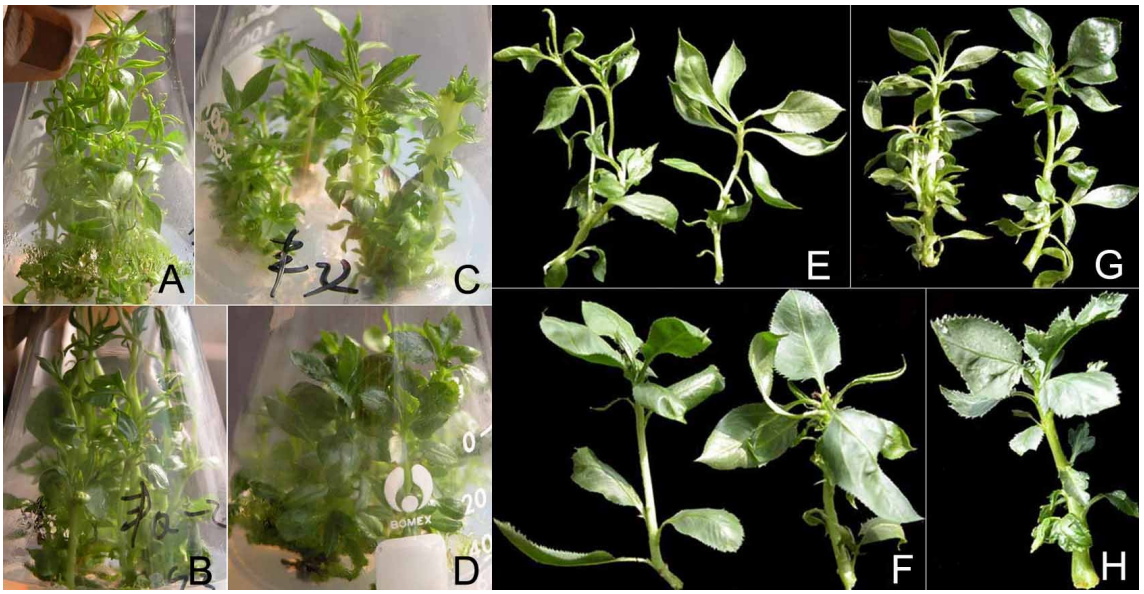
表1　梨试管苗不同倍性无性系叶片形态特征比较

Table 1 Comparison of leaf shape among different ploidy clones of pear *in vitro*

株系 Plant clone	叶长度 Leaf length/mm	叶宽度 Leaf width/mm	叶长度/叶宽度 Leaf length/Leaf width	叶片厚度 Leaf thickness/mm	叶柄长度 Petiole length/mm	叶柄宽度 Petiole width/mm
Mix-1	16.76 ± 0.96 d	10.94 ± 0.80 de	1.54 ± 0.06 e	0.14 ± 0.01 ab	4.98 ± 0.38 e	0.61 ± 0.06 c
Mix-2	18.79 ± 1.37 c	12.52 ± 0.58 ab	1.50 ± 1.50 e	0.15 ± 0.01 a	5.12 ± 0.38 de	0.71 ± 0.02 a
4x-1	20.08 ± 1.83 bc	13.12 ± 0.68 a	1.52 ± 0.05 e	0.13 ± 0.01 bcd	6.56 ± 0.65 bc	0.65 ± 0.02 b
4x-2	19.02 ± 1.78 bc	11.37 ± 0.58 cd	1.60 ± 0.05 de	0.10 ± 0.00 e	5.14 ± 0.61 de	0.59 ± 0.02 cd
4x-3	20.09 ± 0.82 bc	12.09 ± 0.79 bc	1.67 ± 0.04 cd	0.13 ± 0.01 cd	6.78 ± 0.60 b	0.53 ± 0.04 ef
4x-4	19.51 ± 1.08 bc	11.00 ± 1.05 d	1.72 ± 0.16 c	0.12 ± 0.01 d	5.91 ± 0.41 cd	0.65 ± 0.02 b
3x-1	18.34 ± 1.57 cd	11.48 ± 0.85 cd	1.58 ± 0.05 de	0.12 ± 0.01de	5.0 ±0.86 e	0.56 ± 0.03 de
3x-2	21.01 ± 0.57 b	9.96 ± 0.36 e	2.09 ± 0.08 a	0.13 ± 0.01 cd	6.94 ± 0.53 b	0.56 ± 0.02 de
3x-3	16.76 ± 0.96 d	7.74 ± 0.60 f	2.13 ± 0.06 a	0.14 ± 0.01 abc	5.74 ± 0.34 de	0.49 ± 0.02 g
2x	27.08 ± 2.08 a	12.03 ± 0.40 bc	1.88 ± 0.05 b	0.08 ± 0.01 f	7.92 ± 1.05 a	0.51 ± 0.03 fg

注:同一列中标有不同小写字母表示差异显著。下同。

Note: Values within the same columns followed by the different small letters were significantly different according to Duncan's multiple range test ($P < 0.05$). The same below.

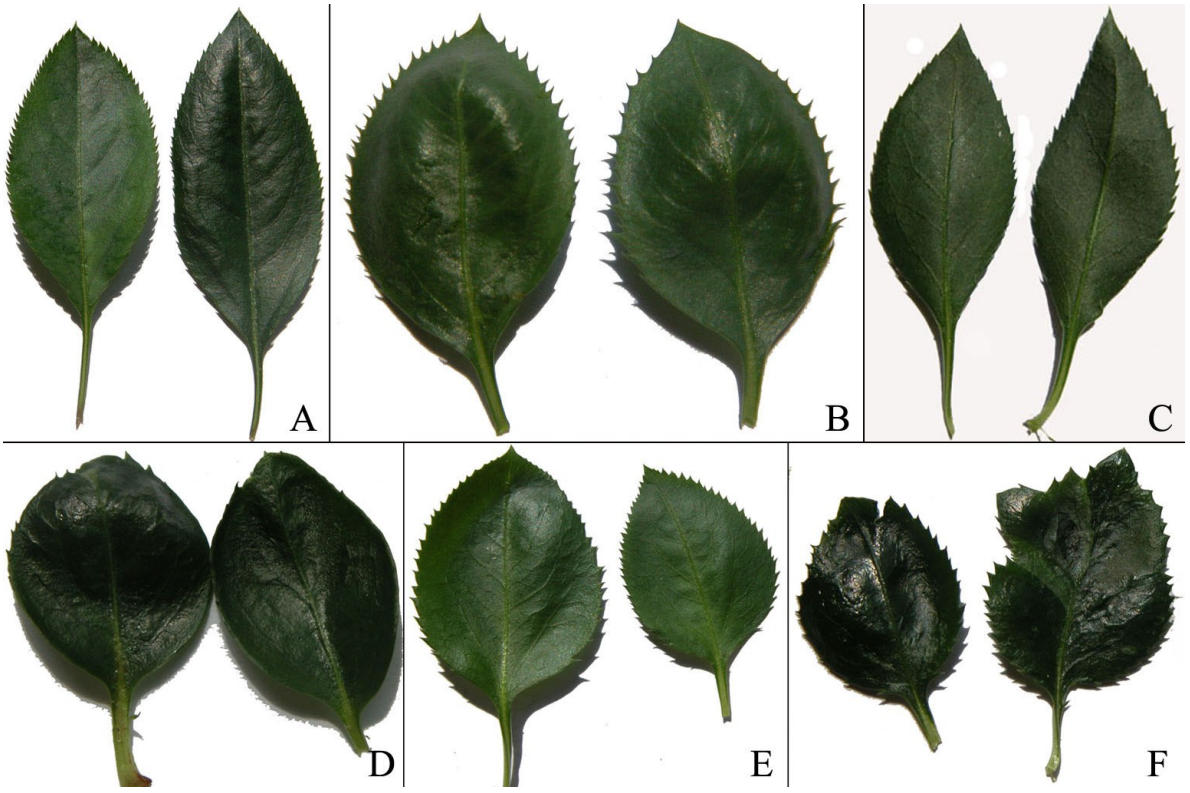


A和E. 二倍体对照；B和F. 三倍体‘3x-1’； C和G. 四倍体‘4x－2’；D. 四倍体‘4x－1’；H. 混倍体

A and E. Diploid control; B and F. Triploid ‘3x-1’; C and G. Tetraploid ‘4x-2’; D. Tetraploid ‘4x-1’; H. Mixploid

图1 多倍体变异无性系和二倍体对照试管苗生长形态比较

Fig. 1 The comparison of shoots morphological characteristics between diploid and polyploid clones *in vitro*



A. 二倍体对照；B. 三倍体 ‘3x-1’；C. 三倍体 ‘3x-2’；D. 四倍体 ‘4x-4’；E. 四倍体 ‘4x-1’；F. 混倍体‘Mix-1’

A. Diploid control; B. Triploid ‘3x-1’; C. Triploid ‘3x-2’; D. Tetraploid ‘4x-4’; E. Tetraploid ‘4x-1’; F. Mixploid ‘Mix-1’

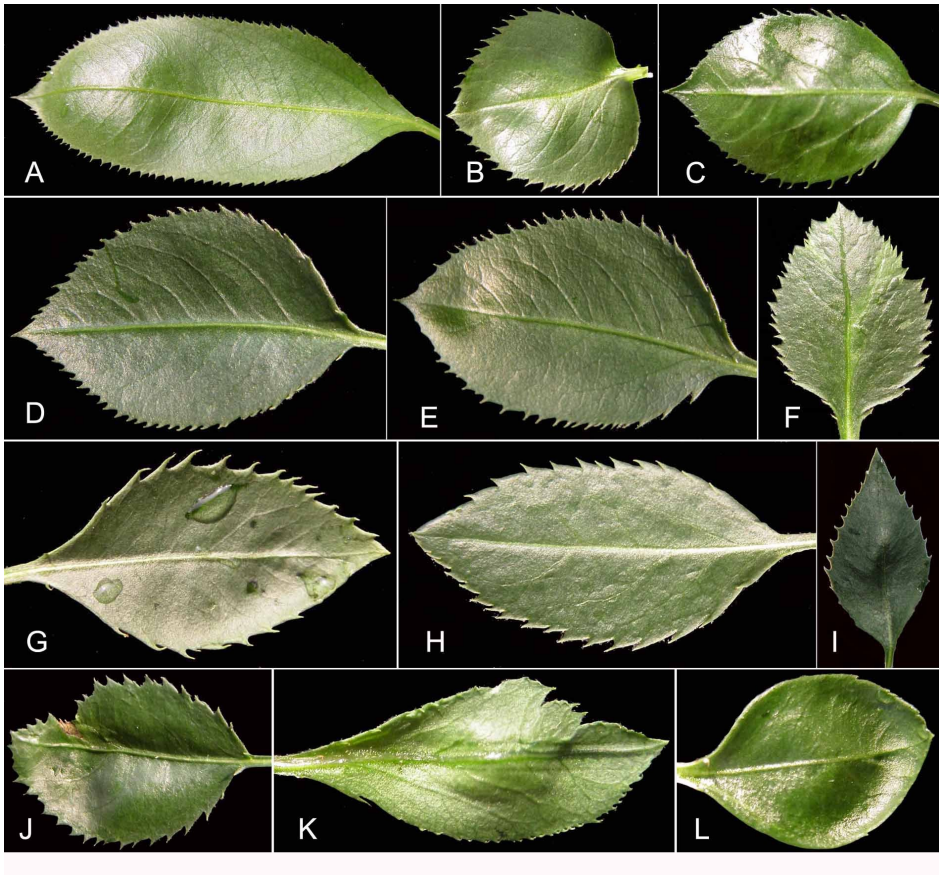
图2 梨不同倍性无性系试管苗叶片表型性状变异多样性

Fig. 2 The diversity of leaf phenotypic traits variation of different ploidy clones of pear *in vitro*

形（图2-E），混倍体为近圆形（图2-F）。多倍体的叶尖有突尖（图2-B、E、F）、渐尖（图2-C）和钝尖（图2-D）；多倍体的叶片形状由二倍体的狭椭圆形（图2-A）变为椭圆形（图2-B）或纺锤形（图2-C）或广椭圆形（图2-D~F）。整倍体的叶片形状表现比较规则（图2-A~E），而混倍体的叶片形状不一，有畸形叶片产生（图2-F）。多倍体的叶柄（图2-B、D~F）比二倍体（图2-A）短、宽（三倍体‘3x－2’除外）。多倍体的叶色一般变深。四倍体‘4x－2’有很强的光泽度（肉眼观察叶片发亮）（图2-D）。

与二倍体相比，多倍体的叶长度显著变短（表1），叶厚度显著变厚，叶柄长度显著变短，叶柄宽度显著变大（除‘4x-3’和‘3x-3’与二倍体差异不显著外）。叶宽度的变异，不同倍性无性系表现不一致。混倍体‘Mix-1’、四倍体‘4x-4’、三倍体‘3x-2’和‘3x-3’比二倍体显著变小；四倍体‘4x-1’比二倍体显著变大；而混倍体‘Mix-2’、四倍体‘4x-2’、‘4x-3’及三倍体‘3x-1’和二倍体差异不显著。叶形指数在多倍体和二倍体之间表现差异显著。三倍体‘3x-2’和‘3x-3’的叶形指数比二倍体显著变大，表明叶片变得窄长（图2-C）；其余多倍体无性系的叶形指数比二倍体显著变小，表明这些多倍体的叶片变得阔圆（图2-B、D、E）。这一结果表明叶形指数、叶片厚度及叶柄长度都可用做试管苗无性系多倍体变异早期选择的有效指示标记。

2.1.3　叶缘变异　不同倍性无性系叶缘锯齿的变异也表现出多样性（图3）。与二倍体对照相比（图3-A），叶缘锯齿变异多种多样（图3-B~E、G、I），有复锯齿（图3-F、H、J）变异，有全缘变异（图3-L），也有叶缘锯齿变深（图3-B~H）或变浅变异（图3-K）。这一结果表明多倍体变异其叶缘也表现出与其亲本二倍体明显不同的变异特征。



A. 二倍体；B. 四倍体‘4x-1’；C. 三倍体‘3x-1’；D. 四倍体‘4x-2’；E. 四倍体‘4x-3’；F. 混倍体‘Mix-2’；G. 三倍体‘3x-4’；H. 三倍体‘3x-2’；I. 三倍体‘3x-3’；J. 混倍体‘Mix-2’；K. 混倍体‘Mix-1’；L. 四倍体‘4x-4’

A. Diploid; B. Tetraploid ‘4x-1’; C. Triploid ‘3x-1’, D. Tetraploid ‘4x-2’; E. Tetraploid ‘4x-3’; F. Mixploid ‘Mix-2’; G. Triploid ‘3x-4’; H. Triploid ‘3x-2’; I. Triploid ‘3x-3’; J. Mixploid ‘Mix-2’; K. Mixploid ‘Mix-1’, L. Tetraploid ‘4x-4’

图3　梨不同倍性多倍体叶缘变异多样性

Fig. 3 The diversity of leaf margins variations of different ploidy polyploids in pear

2.2 梨多倍体无性系在田间表型变异多样性

混倍体没有获得移栽到田间成活的植株。对在田间生长良好的三倍体和四倍体的观察和数据测量分析表明:与二倍体相比,多倍体比二倍体生长缓慢,主要体现在多倍体的株高比二倍体显著变矮(表2),但三倍体和四倍体及四倍体不同株系之间无显著差异;地面以上30 cm处树干直径也表现为多倍体显著小于二倍体(表2),三倍体'3x-1'与3个四倍体'4x-1'、'4x-2'、'4x-3'之间也表现为差异显著,但3个四倍体株系之间差异不显著。

当年生梢(春梢和秋梢的和)的长度,多倍体显著小于二倍体,但不同多倍体无性间差异不显著(表2)。当年生梢的生长,二倍体有明显的春梢和秋梢生长,而多倍体大多只有春梢生长,只有个别梢有秋梢生长,这也可能是多倍体当年生梢长度显著短于二倍体及多倍体比二倍体生长较缓慢的原因。

表2 梨不同倍性无性系植株营养生长特性比较

Table 2 Comparison of vegetative growth characteristics of pear neopolyploids growing in the field

株系 Plant clone	株高 Plant height/cm	新梢长度 Length of current year's shoot/cm	春梢数 No. of spring shoot	秋梢数 No. of autumn shoot	节间长度 Internode length/cm	干径 Trunk diameter/cm
丰产 Fertility (2x)	217.1 ± 28.1 a	126.2 ± 37.0 a	30	30	3.08 ± 0.16 a	18.20±2.26 a
3x-1	105.4 ± 14.2 b	60.4 ± 12.6 b	30	10	2.64 ± 0.23 c	10.44±1.23 c
4x-1	119.6 ± 18.8 b	46.0 ± 8.8 b	30	0	2.82 ± 0.10 b	12.18 ± 0.73 b
4x-2	113.5 ± 12.9 b	46.5 ± 5.2 b	30	0	2.87 ± 0.17 b	13.25±1.17 b
4x-3	112.6 ± 13.3 b	47.9 ± 5.2 b	30	6	2.90 ± 0.18 b	13.00 ± 1.98 b

3 讨 论

随着倍性的增加易导致细胞变大,因此多倍体植物常具有较大和较厚的叶片、较大的花和果、短节间和较粗的茎及其他性状的变异。倍性的变化会引起各种各样的表型变异,但不同种或不同基因型的多倍体其表型变异也不同[29],表明不同基因型对倍性变异的反应不同。本文对离体秋水仙碱诱变获得的梨多倍体试管苗无性系和产生这些多倍体的二倍体亲本的生长形态特征进行了比较,结果发现,多倍体试管苗不仅有茎明显变粗、节间变短、叶形变阔、叶片变厚等这些一般多倍体变异所具备的特征,而且还有其他性状的变异,如三倍体'3x-2'和'3x-3'的叶形不是比二倍体变得更阔,而是变得更窄长,表现为叶形指数增大(表1)。多倍体叶缘的变异也表现出多样性,有全缘变异,也有不同类型的锯齿变异。多倍体新种质移栽入田间后,比二倍体具有较小的株高和树干直径,这和'金诺橘'的四倍体比二倍体具有较小的株高和较小的干周[30]及杂交兰的染色体加倍后比二倍体生长变缓[16]的研究结果相一致。多倍体的叶片厚度比二倍体变大,这和Laere等[31]报道的*Spathiphyllum wallisii*的四倍体比二倍体具有更厚叶片的研究结果一致。

体细胞衍生的同源多倍体表型变异多样性的分子机制可能是由于基因的重复增加了基因表达的多样性[1-2],或是染色体加倍后引起染色体结构的变化或表观遗传的修饰[32],最终引起了表现变异。

混倍体有畸形叶片产生,没有得到移栽到田间成活的植株,其表型性状的畸形或缺陷可能是由于混倍体存在非整倍的细胞或染色体的不平衡所致[33-34],因为倍性的鉴定是通过细胞流式仪检测获得,这一检测技术对于区分整倍变异是有效的,但不能区分非整倍变异,所以混倍体畸形叶片的发生不能否定有非整倍体存在的可能。

在细胞水平上,染色体组的增加,导致细胞的增大,这主要是根据染色体组的大小和气孔保卫细胞的大小及表皮细胞面积具有显著正相关关系而获得,而在表型性状这个尺度上并不是都表现为正相关,染色体组的大小和植株的最大高度呈负相关[35]。本研究中的多倍体的植株高度也显著小于二倍体,这与前人研究结果相一致[34]。本研究中多倍体比二倍体生长较缓,也可能是由于二倍体在1 a内有春梢和秋梢2次生长,而多倍体主要有春梢1次生长的缘故。

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