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‘艳丽’草莓微繁殖苗与普通苗的表型差异及转录组分析

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摘 要:【目的】分析草莓微繁殖苗与普通苗表型差异,通过转录组测序揭示草莓微繁殖苗与普通苗差异表达的基因,探索草莓微繁殖苗与普通苗表型差异的调控机制。**【方法】**以草莓品种‘艳丽’为试材,在繁苗期和栽培期调查微繁殖苗与普通苗之间的表型差异,并以茎尖生长点为试材进行转录组测序分析。**【结果】**在繁苗期,微繁殖原种苗的匍匐茎数、新茎数、叶片数均明显高于普通苗,而株高、冠径、叶面积差异不明显。在栽培期,微繁殖一代苗比普通苗开花结果略早,但是两者在果实大小、产量、可溶性固形物含量上没有明显差异。微繁殖原种苗与普通苗的茎尖生长点里有1 803个基因显著差异表达,相对于普通苗,微繁殖原种苗中上调表达基因1 469个,下调表达基因334个。差异表达的基因涉及光合作用、植物激素合成及信号转导、植物病原菌互作等。**【结论】**‘艳丽’草莓微繁殖苗的繁苗能力明显高于普通苗,这一表型差异与植物激素合成及信号转导通路上基因的差异表达有关。

关键词:草莓;微繁殖;转录组测序

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Phenotypic variation between micro-propagated and conventional plants of ‘Yanli’ strawberry and its mechanism revealed by transcriptome analysis

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Abstract:【Objective】Strawberry, the perennial herb of the family Rosaceae, is widely cultivated in the world due to high economic and nutritional value. Currently, the area of cultivation of strawberry is increasing rapidly in China. Strawberry plants are mainly propagated by runners which would lead to spreading of some diseases. Therefore, micro-propagation technology has been used to generate disease-free plants to improve the quality of products. Some studies have reported that micro-propagated strawberry plants have more runners, leaves, and even high net photosynthetic rate. However, the molecular mechanism of the differences is rarely reported. In this study, we investigated the differentially expressed genes between the micro-propagated and the conventional plants of strawberry cultivar ‘Yanli’ through transcriptomics analysis.【Methods】The micro-propagated stock plants of cultivated strawberry (*Fragaria*×*ananassa* Duch.) cultivar ‘Yanli’ were transplanted and were compared with the conventional plants propagated by runner mode in the field. Some of the investigations and analysis were as follows: (1) Investigation of morphological indexes during the vegetative propagation stage. The micro-propagated stock plants and the conventional plants were planted in the pots in open field, and the number of leaves, branch crowns, runners, etc. were recorded every 14 days. (2) Phenological observation. The first generation of the micro-propagated stock plants and the conventional plants were cultivated in

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solar greenhouse, and the growth and development status were investigated every 3 days and the first flowering date, flower blooming date, and the fruit ripening date were recorded. (3) Investigation of morphological indexes during the flowering and fruiting stage. Plant height, plant diameter, number of leaves and leaf area were measured from 30 plants in the period of the beginning of the flowering, fruit ripening, and the end of the first fruit harvest. (4) Fruit yield and quality. The hardness and soluble solid content of the first fruit were measured through a hardness tester and a refractometer, besides, the weight of single fruit was measured through an electronic scale. (5) Transcriptomics analysis. The shoot tips of the micro-propagated ‘Yanli’ and the conventional plants cultivated in the open field for 30 days, were used for the RNA-seq. The library was constructed and clean reads were obtained by removing reads with 3’-end adapters, poly-N (N indicates that the base information cannot be determined) (≥ 10%), and low-quality sequences (Q≤10 bases account for more than 50% of the total sequence bases). A short sequence assembly software Trinity was used to perform *de novo* assembly and the longest transcript as unigene was selected for subsequent annotation, quantification, and differential expression analysis. Here, differential expression genes (DEGs) were screened by threshold $|\log_2\text{Fold Change}| > 1$ and corrected p-value < 0.05. To explore the gene function, multiple protein databases (BLAST+/Uniprot, protein domain Recognition (HMMER/PFAM), pathway function (GO/KEGG databases), homologous protein clustering (eggNOG) were used to annotate unigene CDS, and NR database, Rfam database, plant-related *Arabidopsis* database were used to annotate unigene sequences of unpredicted CDS. 【Results】Among morphological indicator difference between the micro-propagated and the conventional plants, the number of runners of the micro-propagated plants increased by 3-4 times, leaves increased by 1.8 times but new stems decreased during the vegetative propagation stage compared with those of the conventional plants. By transcriptome sequencing, a total of 236 717 178 clean reads were obtained then the 55.6% predicted unigenes and 63.2% unpredicted unigenes were annotated according to the above databases. Among the 1 803 DEGs, 1 469 DEGs were up-regulated, while 334 were down-regulated in the shoot apical meristems of the micro-propagated plants. According to GO analysis, the DGEs were annotated by molecular function, biological process and cellular component. The top 20 groups with the most differences were selected to analyze. Here, sucrose synthase, sucrose metabolism pathway, hormone metabolism, alcohol dehydrogenation enzymes, etc. were the most significantly down-regulated, while chitinase, organic nitrogen compounds, jasmonic acid, etc. were the most significantly up-regulated. According to KEGG analysis, the top 15 paths with a large number of annotation genes were classified into four groups: metabolism, genetic information processing, cellular processes, and environmental information processing. The most significant pathways were photosynthesis-antenna, plant hormone signal transduction, isoquinoline alkaloid biosynthesis, terpenoid backbone biosynthesis, linoleic acid metabolism. Among the 27 DEGs of plant hormone signal transduction, 5 DEGs of auxin-responsive protein, four TIFYs, and the two jasmonate related genes *JAZ10* and *JAZ1* were up-regulated in the micro-propagated plants. The expressions of arginine biosynthesis, zeatin biosynthesis, tyrosine metabolism genes were all down-regulated in the micro-propagated plants. Also, some specific genes were analyzed, such as the *GA20x*, the *WRKY33* which were up-regulated. Strigolactone synthesis gene *CCD7* was also up-regulated in the micro-propagated plants. Those differential expression genes might cause the difference between the micro-propagated plants and the conventional plants. 【Conclusion】In this study, the number of runners and leaves of the micro-propagated plants were significantly higher than those of the conventional plants, and the number of branch crowns was significantly lower. Transcriptome sequencing analysis showed that the genes related to plant hormones were significantly and

differentially expressed. It showed that hormone signaling played a key role in the morphological development of the micro-propagated plants.

Key words: Strawberry; Micropropagation; Transcriptome sequencing

草莓(*Fragaria* × *ananassa* Duch.)是蔷薇科多年生草本植物,因具有特殊的香气、漂亮的果型、富含维生素C等特性而受到广大消费者的青睐[1]。作为一种经济作物,其栽培面积和产量均居浆果类水果前列[2]。我国草莓产量占全球总产量的42%,每年平均增加8.1%[3]。为保证产量和品质,在生产上普遍采用一年一栽制,每年春季至秋季采用匍匐茎繁殖方式繁殖生产用苗。然而,匍匐茎繁殖过程中可能感染病毒,也可能传递母株初期的病害从而导致减产[4]。微繁殖技术可以获得无病毒种苗还可有效地防治大部分土传病害[5],这对农业生产和新品种推广有着重要作用并且这种技术已广泛应用于草莓产业[6-7]。

尽管草莓微繁殖可以获得无病毒材料,但微繁殖苗及其后代中变异情况也十分普遍,例如形态学、细胞学的变异[8],极少数再生植株还会出现遗传物质的改变[9-10]。近年来,有关草莓微繁殖过程中变异稳定性的研究越来越受到关注,研究发现,微繁殖苗的酚类、类黄酮和抗氧化活性有所增加[11]。这些变异因草莓品种和组培时间的不同而有所差异,并且不能稳定遗传。研究发现草莓的再生子代在育苗期和结果期都出现变异情况,比如叶柄增长、匍匐茎抽生数量增加、花期改变、果实着色不同[12]。有研究表明微繁殖苗的匍匐茎数量、冠径、开花时间和果实产量高于普通苗[13-14]。由于匍匐茎繁殖是草莓生产上的最主要繁苗方式,因此草莓微繁殖苗产生较多匍匐茎这一现象引起人们的广泛关注。

不同品种草莓匍匐茎的抽生时间和数量对环境的要求不同。对于短日照草莓,高温长日照条件利于匍匐茎的抽生,即光照时间大于14 h,温度高于17 ℃。对于四季性草莓,匍匐茎的抽生需要同样的温度条件,但光照条件随着品种不同而有所变化[15]。总体而言,四季草莓匍匐茎抽生数量少于一季性草莓,甚至会出现极端的无匍匐茎情况。外源施加赤霉素是促进匍匐茎抽生的常规手段,赤霉素的信号转导过程同样依赖于环境的作用。短日照条件下,外源施加赤霉素可以促使一季性、四季性的栽培草莓和森林草莓匍匐茎的抽生。甚至对不产生匍匐茎的草莓品种,可以使用赤霉素诱导匍匐茎的抽生[16]。

尽管国内外关于草莓微繁殖苗的变异报道很多,但对其变异机制的研究较少。'艳丽'草莓是沈阳农业大学选育的优质、抗病草莓新品种。笔者以'艳丽'草莓为试材,在繁苗期和栽培期调查微繁殖苗与普通苗的表型差异,利用转录组测序分析探究微繁殖苗与普通苗表型差异的分子机制。

1 材料和方法

1.1 植物材料

草莓(*Fragaria*×*ananassaa* Duch.)品种'艳丽'的微繁殖原种苗(试管苗驯化移栽成活的苗)和普通苗(在田间通过匍匐茎繁苗方式繁育出的苗)于2018年夏季在沈阳农业大学教学科研基地露地繁育子苗。2018年秋季,将微繁殖一代苗(微繁殖原种苗通过田间匍匐茎繁苗方式繁育出的子苗)和普通苗定植到日光温室进行促成栽培。温室内采用大垄双行栽培模式,垄长6.9 m,垄高35 cm,垄面宽30 cm。微繁殖苗一代苗和普通苗各定植一垄,株距15 cm,每垄定植80株。采用常规栽培技术进行管理。

1.2 繁苗期形态指标调查

2018年5月22日选取长势相同的健壮的微繁殖原种苗和普通苗各30株(每10株为1个重复),每14 d观察并记录植株的叶片数、新茎数、匍匐茎抽生数量、冠径、株高等形态指标。

1.3 栽培期的物候期观测

2018年9月8日将长势相同大小一致的'艳丽'微繁殖一代苗和普通苗定植于日光温室中。选择生长环境相同的微繁殖一代苗和普通苗各30株挂牌编号,每3 d观察植株的生长发育情况,分别记录植株的始花期、盛花期和果实始熟期。

1.4 栽培期的形态指标调查

从植株的始花期开始,选择生长环境相同的微繁殖一代苗和普通苗各30株(每10株为1个重复),分别在植株的始花期、果实始熟期和第一茬果采收结束期测量株高、冠径、叶片数、叶面积。

1.5 果实产量及品质调查

在第一茬果成熟时,以每个调查形态指标的植

株为对象，随机分组，将一级序果采摘称重，记录一级序果平均单果质量。使用硬度计和折射仪分别测量一级序果的硬度和可溶性固形物含量。

从果实始熟期开始，以每个调查形态指标的植株为对象，采收成熟果实称重，每10株为1个重复，计算平均单株产量。

1.6　微繁殖苗和普通苗转录组测序

2018年6月22日，分别从微繁殖苗和普通苗取茎尖生长点，液氮速冻后交由武汉康测科技有限公司进行RNA-seq文库构建和转录组测序。基于Illumina测序平台，对文库进行双端测序并对测序数据进行如下步骤的质控：去除3’端的接头污染和至少10 bp overlap；去除含N（N表示无法确定碱基信息）较多（≥10%）的reads；去除低质量的序列（Q≤10的碱基占整体序列碱基数目的50%以上）。使用短序列组装软件Trinity进行*de novo*组装拼接并选取最长的转录本作为Unigene，以此进行后续的注释、定量、差异表达分析。

1.7　差异表达基因的筛选和功能注释

以logFC的绝对值>1且$p < 0.05$作为阈值进行差异基因筛选。对*de novo*拼接转录组的注释，分为两部分注释，一部分是对Unigene进行编码区（CDS）预测，将得到的蛋白序列注释到多个蛋白数据库，包括同源性搜索已知测序数据（BLAST+/Uniprot）、蛋白域识别（HMMER/PFAM）、通路作用（GO/Kegg databases）、同源蛋白聚类（eggNOG）等，另一部分对未预测出CDS的Unigene序列也进行相应的注释，包括NR数据库、Rfam数据库、植物相关的拟南芥数据库等。

1.8　数据统计分析

数据的整理和分析采用SPSS软件，利用*t*检验进行差异性分析（$p < 0.05$），利用WPS中的Excel进行图表的制作。

2　结果与分析

2.1　‘艳丽’微繁殖苗与普通苗表型比较

草莓栽培基本上都是一年一栽，每年春季至秋季采用匍匐茎繁殖方式繁殖生产用苗。以‘艳丽’草莓微繁殖原种苗和普通苗作为种苗，比较了两者在匍匐茎抽生数量、叶片数、新茎数、株高、叶面积、冠径等形态指标上的差异。如图1所示，微繁殖原种苗和普通苗在繁苗期间存在明显的表型差异。

A. 微繁殖原种苗俯视图；B. 普通苗俯视图；比例尺=5 cm。

A. Top view of micro-propagated stock plants; B. Top view of conventional plants; scale bars = 5 cm.

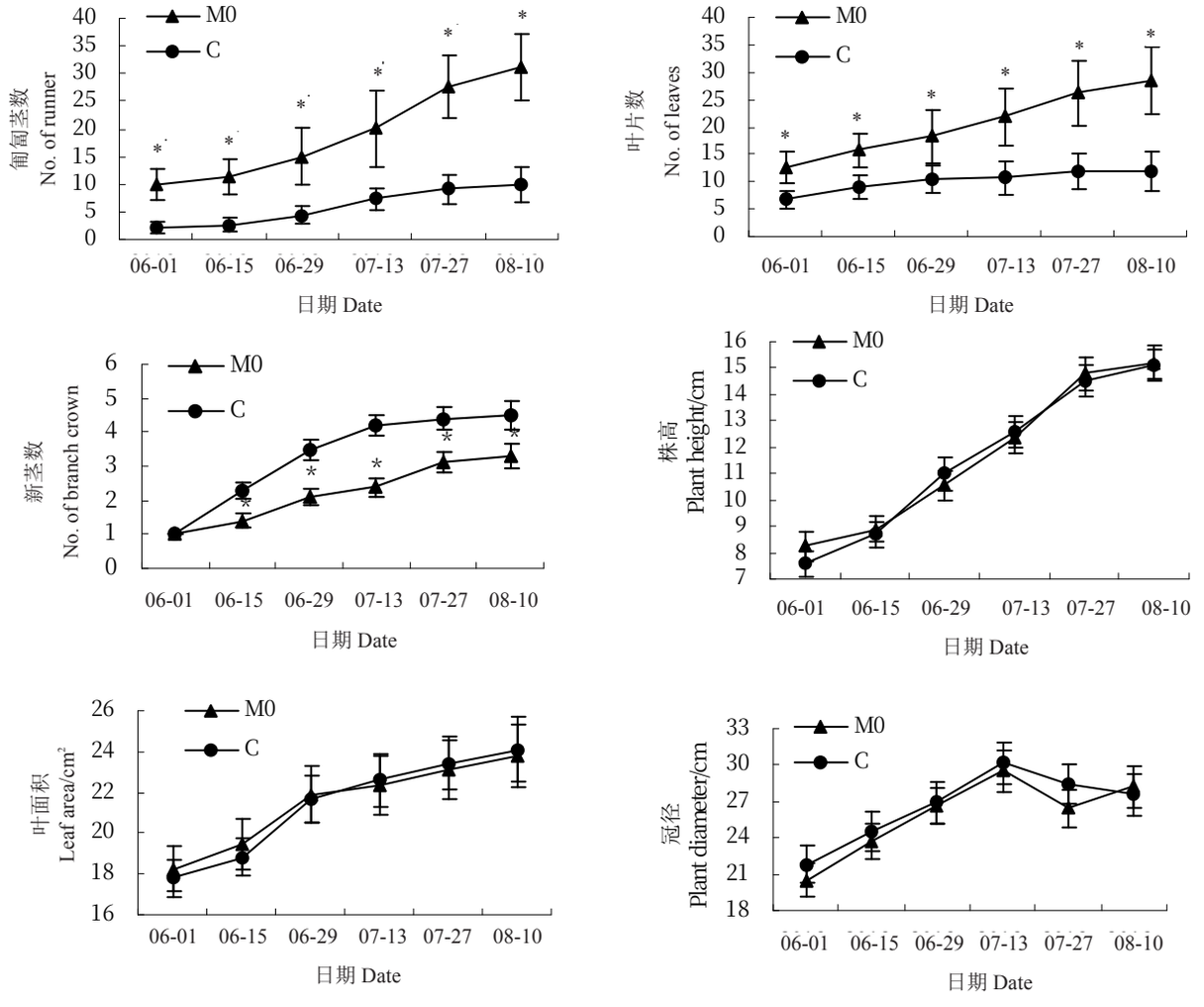
图1　微繁殖原种苗与普通苗在繁苗期的表型

Fig. 1　Phenotype between micro-propagated stock plants and conventional plants in the vegetative propagation stage

如图2所示，繁苗期微繁殖原种苗匍匐茎抽生数量显著高于普通苗，是普通苗的3~4倍，如在7月27日，普通苗平均每株抽生9.11条匍匐茎，而微繁殖原种苗平均每株抽生匍匐茎数量为27.68条，后者是前者的3.04倍；在叶片数上，微繁殖苗原种苗高于普通苗，前者约是后者的1.80倍，且在调查后期差异更加明显；在新茎数上，普通苗大于微繁殖原种苗，在整个繁苗期，普通苗的新茎数大约是微繁殖苗的1.47倍；在株高、叶面积、冠径三项指标中，微繁殖原种苗与普通苗没有显著差异。

2.2　微繁殖一代苗与普通苗在栽培期的表型比较

2.2.1　*微繁殖一代苗与普通苗物候期的比较*　物候期是反映草莓植株生长情况的重要指标，也是指导田间管理的重要依据。表1反映了微繁殖一代苗和普通苗的物候期。结果表明，微繁殖一代苗在整个生长季的三个物候期都早于普通苗。



M0. 微繁殖原种苗；C. 普通苗。

M0. Micro-propagated stock plants; C. Conventional plants.

图 2 微繁殖苗原种苗与普通苗的形态指标比较

Fig. 2 Comparison of morphological indexes between micro-propagated stock and conventional plants

表 1 微繁殖一代苗和普通苗物候期的比较

Table 1 Comparison of first generation of micro-propagated and conventional plants on the phenophase

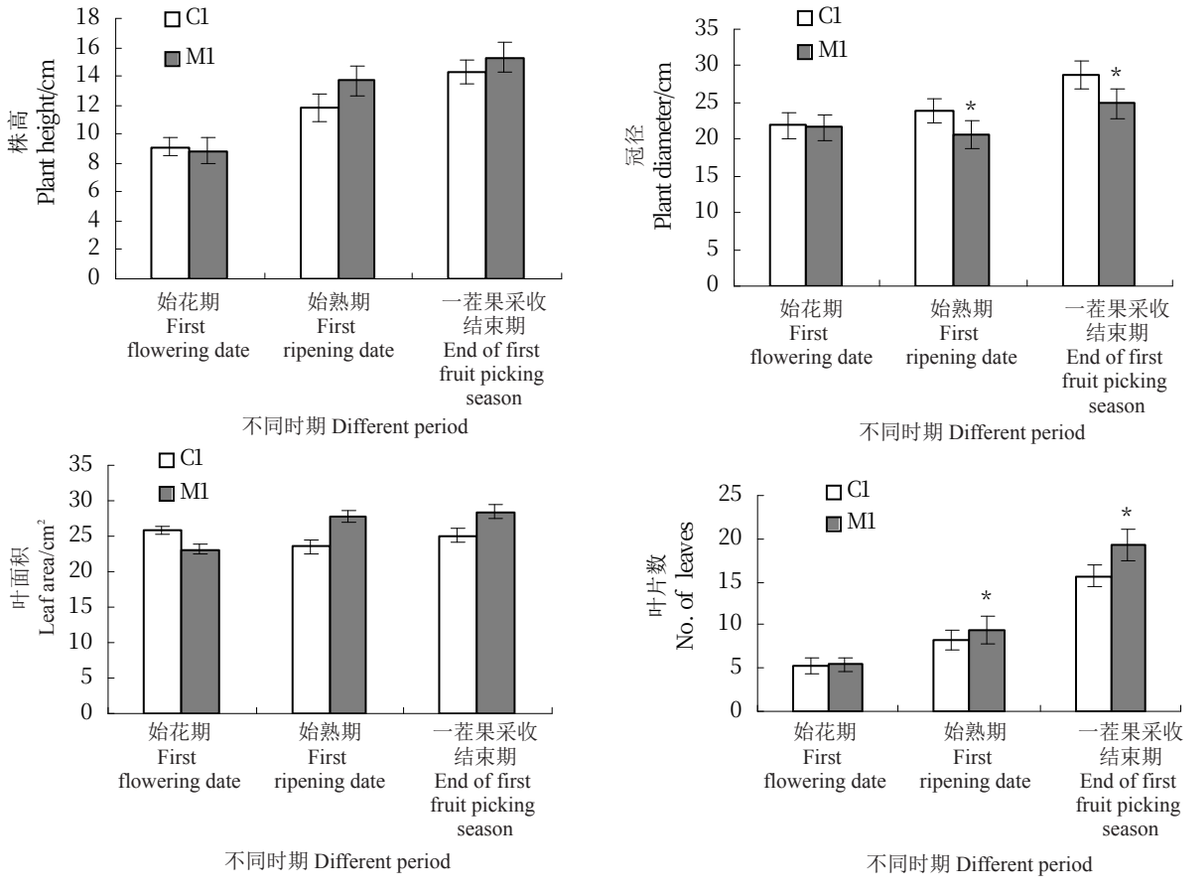
材料 Material	始花期 First flowering date	盛花期 Flower blooming date	果实始熟期 Fruit ripening date
微繁殖一代苗 First generation of micro-propagated plants	11-02	12-08	12-14
普通苗 Conventional plants	11-08	12-11	12-20

2.2.2 *微繁殖一代苗与普通苗在开花结果期的形态指标比较* 图3中展示了开花结果期的微繁殖一代苗和普通苗的表型差异，在始花期时，二者的各项指标无显著性差异，微繁殖一代苗在叶片数上略高于普通苗，其他表型数据略低于普通苗；在果实始熟期和第一茬果采收结束期时，二者仅在叶片数和冠径上有显著差异，微繁殖一代苗的叶片数显著高于普通苗，但冠径显著小于普通苗，其他指标如株高、叶面积，微繁殖一代苗均略高于普通苗，但是无显著性差异。

2.2.3 *微繁殖一代苗与普通苗的果实产量和品质的比较* 表2反映了微繁殖一代苗与普通苗的果实产量和果实品质数据，从表2可以看出，微繁殖一代苗的平均单株产量和一级序果的可溶性固形物含量略高于普通苗，但差异不显著。

2.3 ‘艳丽’微繁殖原种苗与普通苗茎生长点转录组分析

2.3.1 *测序数据质控与分析* 测序数据去掉接头、低质量的reads后获得clean data，两组共获得clean reads为236 717 178，总clean bases为34 583 365 537。



M1. 微繁殖一代苗；C1. 普通苗。

M1. First generation of micro-propagated plants; C1. Conventional plants.

图 3　微繁殖苗一代苗与普通苗的形态指标比较

Fig. 3　Comparison of morphological indexes between first generation of micro-propagated and conventional plants

表 2　微繁殖一代苗与普通苗果实产量及品质的比较

Table 2　Comparison of first generation of micro-propagated and conventional plants on quality and yield

材料 Material	一级序果平均单果质量 Average weight of first fruit/g	平均单株产量 Average plant yield/g	*w*（可溶性固形物） Soluble solid content/%	硬度 Hardness/(kg·cm^{-2})
微繁殖一代苗M1 First generation of micro-propagated plants	33.2±1.6	213.8±17.0	13.1±0.6	2.54±0.22
普通苗C Conventional plants	33.3±1.0	202.3±11.6	12.8±0.4	2.56±0.27

两组间的Q20大于99%，Q30平均大于94%，说明测序结果可靠，可用于下一步转录组数据分析。

2.3.2　差异表达基因注释及分析　以logFC的绝对值>1且$p < 0.05$作为标准进行差异基因的筛选。两组共获得差异基因1 803个，相对于普通苗，微繁殖原种苗中上调表达基因为1 469个，下调表达基因334个。为了更好地了解基因功能，使用同源搜索已知序列数据（BLAST/Uniprot）、蛋白域识别（HMMER/PFAM）、路径作用（GO/KEGG databases）、同源蛋白聚类（eggNOG）、NR数据库、Rfam数据库、植物相关的拟南芥数据库对Unigene进行注释，预测得到CDS的Unigene中，被注释的占55.6%，没有预测得到CDS的Unigene中，被注释的占63.2%（图4）。

2.3.3　差异表达基因GO富集分析　对微繁殖苗和普通苗差异基因进行GO的富集，按照分子功能（molecular function）、生物过程（biological process）和细胞组分（cellular component）进行GO分类。选取了差异性最大的前20个组对上下调差异基因GO富集进行分析（图5），其中下调差异基因富集最显著的有蔗糖合成酶活性、蔗糖代谢途径、激素代谢、醇脱氢酶等。上调差异基因富集最显著有几丁质酶、有机氮化合物、茉莉酸等。

2.3.4　差异表达基因KEEG功能注释　对微繁殖苗

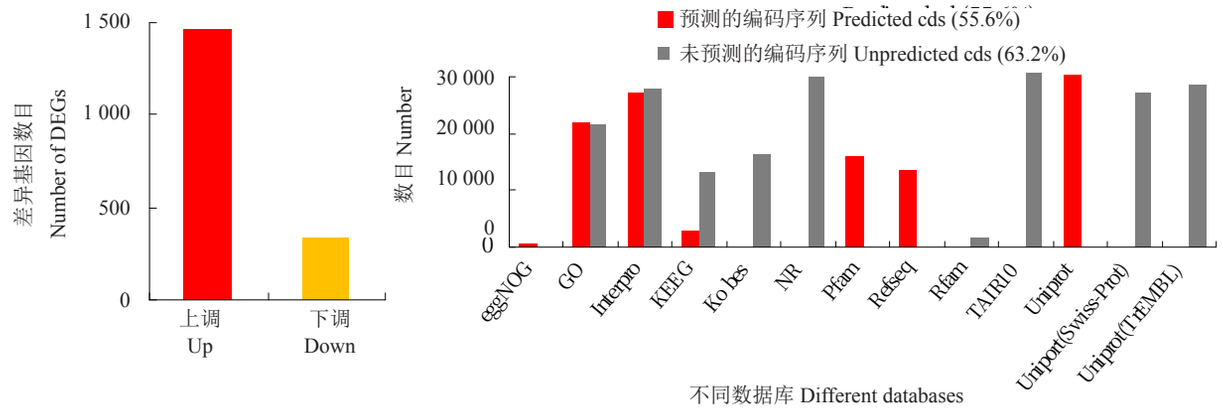


图 4　差异表达基因和不同数据库注释分布

Fig.4　Differentially expressed genes and annotation distribution in different databases

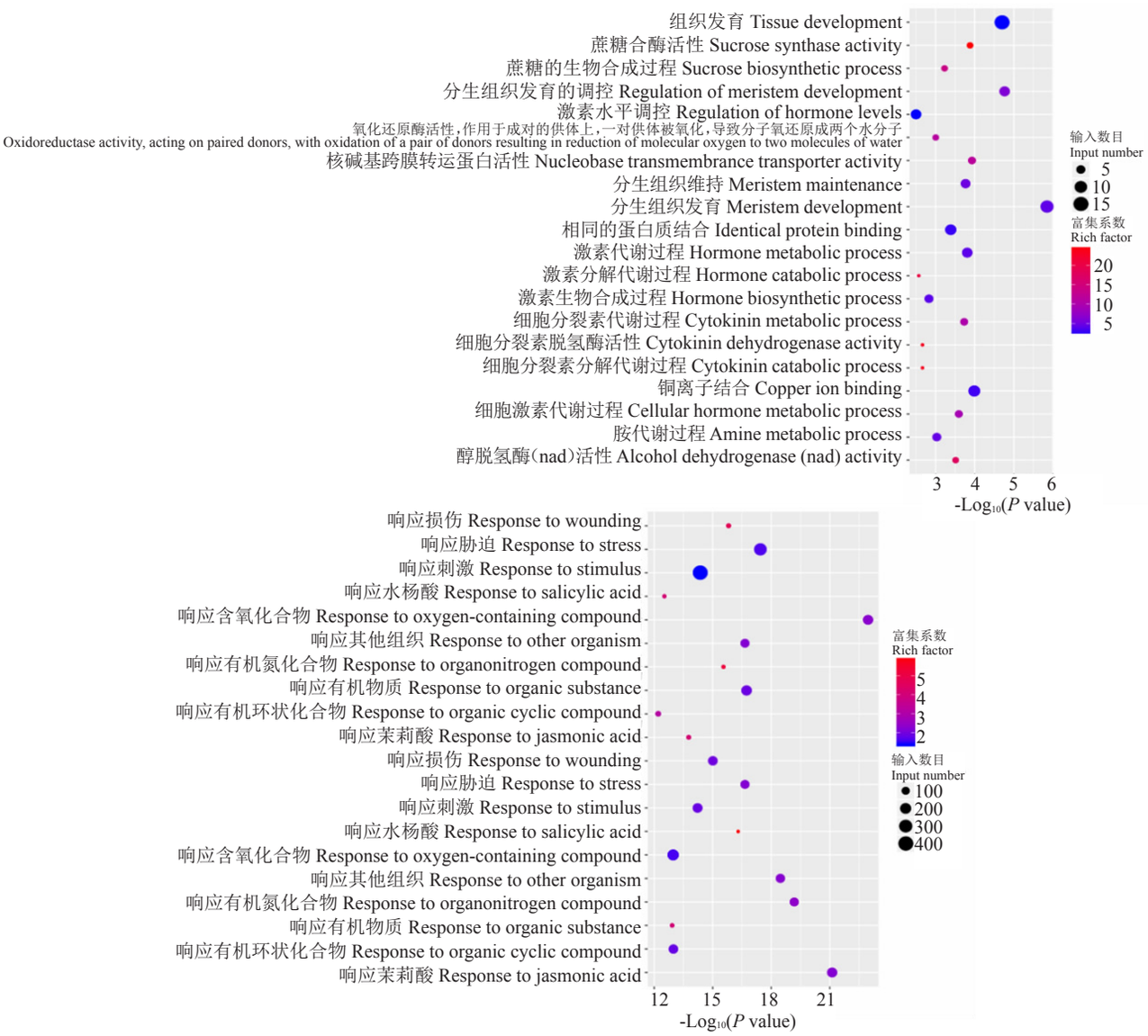


图 5　微繁殖苗与普通苗茎尖差异基因的 GO 富集分析

Fig. 5　GO enrichment analysis of differentially expressed genes in shoot tip of micro-propagated stock plants and conventional plants

与普通苗茎尖差异基因进行KEEG分析，分别从代谢过程、遗传信息处理、细胞过程以及环境信息处理这四组注释基因数最多的前15个通路（pathway）进行归类分析，进一步深入了解差异基因所在通路情况。

KEEG通路主要有氨基酸生物合成、碳代谢、蔗糖淀粉代谢、剪接体、植物病原菌互作、胞饮作用和植物激素信号转导等。本研究中，植物能量代谢、次级代谢生物合成、氨基糖和核苷酸糖代谢、植物病原菌互作、激素合成及信号转导相关途径的差异基因数量最多。而显著富集的有17条不同的途径，下调基因富集的途径有3条，上调基因富集有14条（图6）。在这些富集通路中，最显著途径有光合作用天线蛋白和植物激素信号转导，前者包含8个预测的叶绿素ab结合蛋白基因，后者包括27个差异基因，

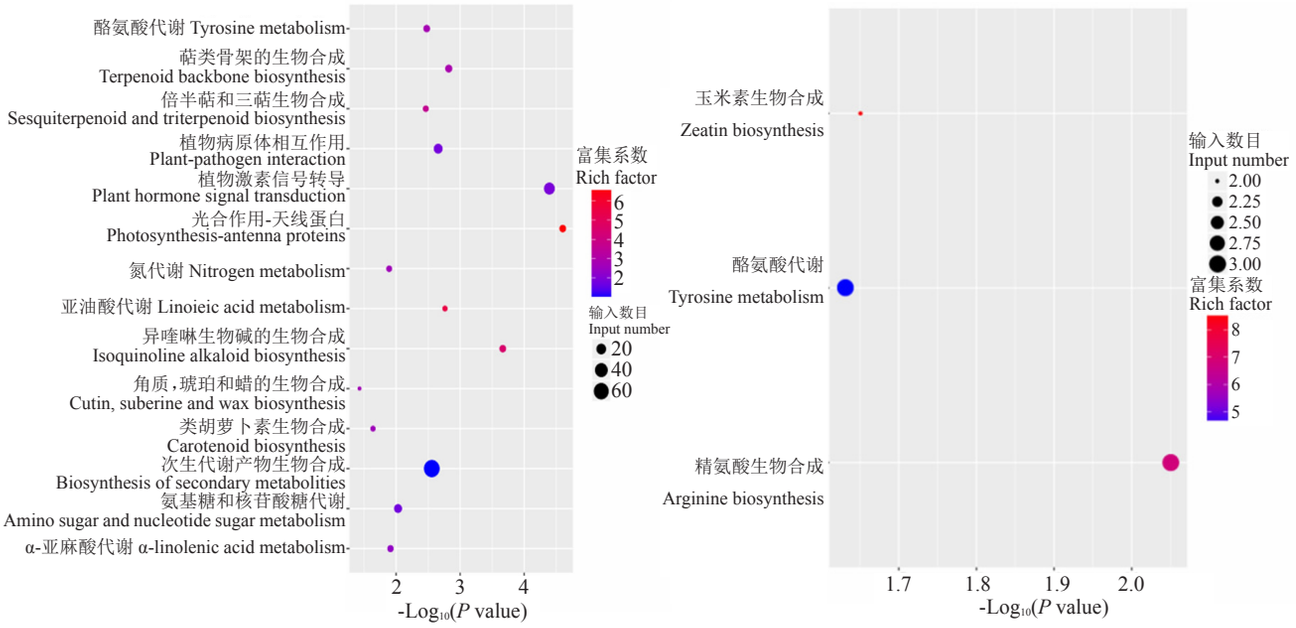


图6　微繁殖苗与普通苗茎尖差异基因 KEEG 气泡图

Fig. 6　Transcriptome differentially expressed genes KEGG bubble diagram shoot tip of micro-propagated stock plants and conventional plants

部分注释到蛋白数据库的激素相关基因有13个，具体基因表达水平见表3。因此可以推测微繁殖苗光合效率高于普通苗。

3　讨　论

草莓的微繁殖苗与普通苗相比，在原种苗阶段会表现出生长势旺盛、繁殖繁殖系数高等特点，在匍匐茎的抽生能力上差异尤其明显，这些表型差异有利于草莓苗的繁殖。草莓微繁殖作为培育脱毒苗的一种方法在生产中得到广大应用和认可。笔者发现‘艳丽’微繁殖苗的长势普遍强于普通苗，尤其是匍匐茎的抽生数量显著高于普通苗，这与前人研究结果一致[2,17-18]。除此之外，微繁殖苗的叶片数目显著多于普通苗，但叶片面积并没有显著差异。叶片的发生与顶端分生组织有关，且叶片的发生过程受激素和特定基因的调控，因此分析了激素信号转导途径的差异基因（表3），通过uniprot蛋白数据库注释，发现6个生长素响应基因、5个*TIFY*基因、2个茉莉酸相关基因和其他未注释到的基因。生长素作为植物器官启动的调节器，其在顶端分生组织的运输对叶片的发生起重要调控作用[19]。因此推测，这些激素信号通路相关的基因与微繁殖苗叶片数显著增多的特征密切相关。

独脚金内酯、细胞分裂素、生长素参与植物分枝调控[20]，三者之间存在协同以及反馈作用[21]。本研究表明，微繁殖苗的新茎数显著低于普通苗，笔者推测与独脚金内酯调控相关。差异表达基因分析发现一个胡萝卜素裂解双加氧酶7（carotenoid cleavage dioxygenase 7，CCD7）基因表达水平上调（logFC为1.58）（表3），这说明了微繁殖苗体内独脚金内酯含量过高可能导致植株分蘖减少。另外，研究表明生长素表达会促进独脚金内酯合成基因 *CCD7*、*CCD8*、*D27*的表达进而抑制细胞分裂素合成，调控侧芽生长[21]。KEEG和GO富集发现，生长素响应基因

表3 光合作用蛋白、植物激素信号转导途径和其他特定差异

Table 3 Photosynthesis proteins, plant hormone signal transduction pathways and other specific genes

通路 Pathway	基因ID Gene ID	基因表达量 Gene expression		差异倍数对数值 logFC	*P*值 *P* value	错误值 FDR	Uniprot 数据库 Uniprot database
		微繁殖苗 Micro-propagated plants	普通苗 Conventional plants				
光合作用-天线蛋白 Photosynthesis-antenna proteins	TRINITY_DN21798_c1_g3	22.72	8.78	1.26	0.00	0.00	叶绿素a/b结合蛋白 Chlorophyll a-b binding protein
	TRINITY_DN22850_c0_g2	0.43	0.18	1.25	0.01	0.15	叶绿素a/b结合蛋白 CP29.3 Chlorophyll a-b binding protein CP29.3
	TRINITY_DN25567_c1_g3	25.94	9.70	1.39	0.00	0.00	叶绿素a/b结合蛋白3 Chlorophyll a-b binding protein 3
	TRINITY_DN27001_c0_g1	6.83	3.47	1.05	0.00	0.00	叶绿素a/b结合蛋白 Chlorophyll a-b binding protein
	TRINITY_DN28268_c1_g1	22.17	10.73	1.06	0.00	0.00	叶绿素a/b结合蛋白 Chlorophyll a-b binding protein
	TRINITY_DN28268_c1_g2	189.16	91.24	1.01	0.00	0.00	叶绿素a/b结合蛋白 Chlorophyll a-b binding protein
	TRINITY_DN29844_c0_g2	15.73	7.25	1.11	0.00	0.00	叶绿素a/b结合蛋白 Chlorophyll a-b binding protein
	TRINITY_DN38943_c0_g1	5.69	2.63	1.04	0.00	0.03	叶绿素a/b结合蛋白 Chlorophyll a-b binding protein
植物激素信号转导 Plant hormone signal transduction	TRINITY_DN22012_c0_g2	9.09	2.84	1.58	0.00	0.00	生长素响应蛋白 Auxin-responsive protein
	TRINITY_DN22209_c0_g1	1.91	0.90	1.11	0.00	0.04	生长素响应蛋白 Auxin-responsive protein
	TRINITY_DN23303_c0_g2	6.66	2.58	1.63	0.00	0.00	生长素响应蛋白 Auxin-responsive protein
	TRINITY_DN26371_c0_g2	115.43	50.18	1.21	0.00	0.00	生长素响应蛋白 Auxin-responsive protein
	TRINITY_DN29211_c1_g2	22.61	11.10	1.03	0.00	0.00	生长素响应蛋白 Auxin-responsive protein
	TRINITY_DN29919_c0_g1	9.59	2.31	2.12	0.00	0.00	TIFY转录因子10B TIFY 10B
	TRINITY_DN32058_c0_g1	116.18	52.06	1.16	0.00	0.00	TIFY转录因子10A TIFY 10A
	TRINITY_DN32058_c0_g4	68.59	30.13	1.17	0.00	0.00	TIFY转录因子10A TIFY 10A
	TRINITY_DN33849_c2_g1	6.91	2.78	1.18	0.00	0.03	TIFY转录因子6B TIFY 6B
	TRINITY_DN25672_c0_g1	46.85	14.38	1.79	0.00	0.00	茉莉酸ZIM结构域蛋白10 Jasmonate ZIM-domain protein 10
	TRINITY_DN32058_c0_g2	36.48	15.70	1.07	0.00	0.00	茉莉酸ZIM结构域蛋白1 Jasmonate ZIM-domain protein 1
	TRINITY_DN22271_c2_g2	3.12	1.34	1.19	0.01	0.17	赤霉素20氧化酶1 GA20OX1
	TRINITY_DN33193_c3_g1	3.48	2.39	1.58	0.00	0.00	类胡萝卜素双加氧酶7 Carotenoid cleavage dioxygenase 7
其他基因 Other genes	TRINITY_DN19717_c0_g1	2.57	0.71	1.81	0.00	0.00	几丁质酶4 Class IV chitinase
	TRINITY_DN25384_c1_g2	4.97	1.81	1.36	0.00	0.00	几丁质酶5 Class V chitinase
	TRINITY_DN25390_c0_g1	22.26	3.65	2.45	0.00	0.00	几丁质酶 Chitinase
	TRINITY_DN27816_c1_g1	4.85	1.76	1.56	0.00	0.00	WRKY转录因子33 WRKY transcription factor 33
	TRINITY_DN27816_c1_g2	22.48	10.75	1.03	0.00	0.01	WRKY转录因子33 WRKY transcription factor 33

上调，玉米素生物合成基因表达水平显著下调，表明这类激素可能参与了微繁殖苗的新茎分枝发生。

草莓匍匐茎的发生受光周期、温度、GA的影响，如前人研究发现DELLA蛋白调控GA进而影响匍匐茎发生[22]。*GA20x*是GA生物合成过程中的一个重要基因，通过差异基因筛选，笔者发现一个预测编码GA20x类蛋白（TRINITY_DN26800_c0_g1，logFC为1.32）的基因在微繁殖苗中上调表达。因此，可以推测微繁殖苗匍匐茎数量的增多可能是*GA20x*上调导致。赤霉素与其他激素之间也存在互作关系，这种激素间的复杂网络调控关系与植物发育密切关联[23]。

本研究中，KEEG通路中最为显著的捕光天线蛋白包括8个叶绿素a/b结合蛋白，属于光系统Ⅱ（PSⅡ），主要是促进光合作用的正常进行，参与植物光合与生长发育[24]。因此推测‘艳丽’微繁殖苗光合作用强于普通苗，这一结果与‘全明星’和‘丰香’草莓微繁殖苗净光合速率高于普通苗相一致[25]。抗病是草莓生产中非常重要的性状，通过分析病原菌互作相关基因，发现*WRKY33*基因在微繁殖苗中表

达显著上调，而拟南芥 *WRKY33* 过表达具有抵抗真菌作用[26]。几丁质酶在植物防卫的作用早有研究，笔者也发现3个几丁质酶基因表达上调。因此，推测‘艳丽’草莓微繁殖苗的抗病性高于普通苗。

4　结　论

对草莓微繁殖苗与普通苗形态指标调查发现，微繁殖苗匍匐茎数、叶片数显著高于普通苗，新茎数显著低于普通苗，而其他形态指标无明显差异。转录组测序结果表明，植物激素相关基因差异表达，这说明激素信号在微繁殖苗形态发育中发挥了重要作用。另外，光合作用、抗病相关基因的差异表达表明草莓微繁殖苗可能有较强的抗病性。

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