

基于叶片微观结构变化的番茄磷水平检测方法^{*}

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摘要: 以温室番茄为研究对象,借助扫描电镜技术(SEM)和透射电镜技术(TEM)研究了不同磷养分水平下的叶片表面和断面显微结构以及叶肉细胞超微结构变化。结果表明:正常磷水平下的番茄叶片厚度为(124±2.14) μm,表面气孔和绒毛密度分别为(233.0±5.5)个/mm²和(34.2±1.33)根/mm²,绒毛高度为(97.0±2.83) μm,气孔大小为(13.91±0.85) μm。缺磷会造成叶片厚度明显变薄,引起叶片表面气孔密度、绒毛密度和绒毛高度明显降低,并且会破坏叶绿体结构的完整性,但对气孔大小的影响不明显;磷过量,会使气孔大小、绒毛密度和维管束数量显著增加,并引起细胞壁厚度增加,但对气孔密度、绒毛高度和叶绿体结构影响不明显。

关键词: 番茄 微结构 磷水平 快速检测

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引言

现阶段我国设施作物养分实时、无损检测多以光谱检测为主,现有光谱技术虽能精确测量氮营养水平^[1-2],但在磷营养检测方面应用却很少。Milton等研究结果表明,植物磷营养元素对光谱特性有较大影响,但其影响不像氮营养水平那样一致^[3]。韩小平等利用光谱技术对不同施磷水平下番茄叶片光谱信息的变化规律进行了研究,结果表明检测精度较低^[4]。这是因为缺磷植物叶片细胞伸长受影响的程度超过叶绿素所受的影响,因此其单位叶面积中叶绿素含量反而较高^[5-6];又因为缺磷的植株,体内碳水化合物代谢受阻,有糖分积累,易形成花青素^[7-8]。所以,缺磷植株叶片的光谱反射率受叶绿素和花青素这2个色素的影响较大^[9-11],从而影响叶片光谱分析的可靠性^[12-13]。因此需要探索新的方法来提高磷养分水平的检测精度。

植物营养学家研究表明,磷营养水平的变化不仅能引起叶片颜色、大小等宏观特征的变化,叶片结构以及叶肉细胞内部各细胞器结构和形态也会发生显著的变化^[14-17]。Singh等研究了不同磷养分条件下棉花叶片参数的变化情况,结果表明,缺磷会引起叶面积变小^[18];Bosu等研究结果表明,水分差异会引起作物内部磷浓度变化,进而影响叶片绒毛密度

变化^[19];Heskel等研究了不同磷水平条件下叶片气体交换、细胞超微结构及其它特征变化,结果表明,叶绿体和线粒体数量受磷水平变化影响很大^[20]。

扫描电镜技术能提供叶片样本表面微结构高分辨形貌图像^[21-23],透射电镜技术能提供叶肉内部超微结构信息^[24-25]。本文研究不同磷水平条件下番茄叶片的微观结构和超微结构变化,从微观尺度分析磷水平对叶片表面形态、叶片厚度、叶肉细胞结构等的影响。

1 材料与方法

1.1 材料

供试材料由江苏大学玻璃温室提供,试验于2013年4—6月份进行。供试品种为粉霞,穴盘育苗,第2片真叶完全展开后,采用无机基质盆栽营养液栽培方式,正常营养液浇灌。

1.2 养分处理

40 d后,划分不同磷水平试验区,在各试验区定时定量浇灌不同磷离子浓度的营养液。营养液配方采用山崎配方,设置0.25、1.00、1.50 3个施磷水平,磷离子浓度分别为0.167 5、0.67、1.005 mol/L,形成缺磷胁迫、适量、过量3个不同营养水平的作物样本,每个处理5个重复。

对每个营养水平相同节位(倒七叶)的叶片进

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行取样,每个养分水平 5 片,取样时间相同(上午 10:00)。避开叶脉取小块叶肉组织(3 cm × 4 cm)放入事先配好的 4% 戊二醛固定液中,带回实验室进行处理。

1.3 样品处理

1.3.1 扫描电镜样品制备与观察

样品经阶梯脱水后在临界点干燥仪(Quorum, K850 型,英国)中进行干燥处理,将制备好的样品粘在导电胶上,放入离子溅射仪(SHINKKU VD 公司, MSP-1S 型,日本)中进行镀膜,溅射金属厚度 20 nm,然后将其粘贴在扫描电镜(FEI, Quanta 200 型,美国)样品台上,在 15 kV 加速电压下进行观察,拍照。

1.3.2 透射电镜样品制备与观察

将经过初固定处理的材料再依次进行如下处理:①洗涤:用磷酸缓冲液(pH 值 7.0)洗涤 3 次,每次 30 min。②固定:将材料转移到 1% 钨酸中,4℃ 下过夜进行后固定。③洗涤:用磷酸缓冲液(pH 值 7.0)洗涤 3 次,每次 30 min。④脱水:用 30%、50%、70%、90% 和 100% 的乙醇分别进行脱水,每次 15 min。⑤浸透:分别用不同比例环氧丙烷与包埋剂(先后依次为 3:1、1:1、1:3)进行浸透,浸透时间 2、4、4 h,最后在纯 Epon812 包埋剂中过夜。⑥包埋:用包埋剂进行包埋,并在恒温箱内进行加温聚合:35℃ (过夜)—45℃ (过夜)—60℃ (过夜)。⑦切片、染色、电镜观察:在超薄切片机(Leica EM UC7 型,德国)上进行切片,经过双染色后在透射电镜(日本电子,JEM1010 型,日本)下观察拍照。

1.4 微观结构观察方法

1.4.1 叶片表面微形态

在扫描电子显微镜下观察各处理叶片表面气孔、绒毛、维管束等微结构形态变化,测量气孔密度、气孔大小、绒毛密度和绒毛高度等。选取几个有代表性的视野拍照。每个样本电镜观察统计至少 10 个视野,取平均值。

1.4.2 叶片断面微结构观察

在扫描电镜下观察各处理叶片断面,测量表皮、栅栏组织、海绵组织和整个叶片厚度以及各组织细胞大小。选取典型视野进行拍照。每个样本电镜观察统计至少 10 个视野,取平均值。

1.4.3 叶肉细胞超微结构观察

在透射电镜下观察各处理叶片细胞间隙、细胞形态以及细胞内部各主要细胞器的变化情况,选取有代表性的视野拍照。

1.5 统计方法

本文的数据是每个处理的均值 ± 标准差,缺磷

组、磷过量组、对照组的显著性分析由 SPSS19.0 软件进行单因素方差分析得到, $P < 0.05$ 认为差异显著。

2 结果与分析

2.1 磷水平对叶片表面微形态的影响

对叶片表面的显微结构观察结果(图 1)表明,正常磷水平下,叶片表面 1 mm² 的范围内气孔平均数量为 (233.0 ± 5.5) 个,气孔大小为 (13.09 ± 1.14) μm;表面分布着锥形和蘑菇形绒毛,但以锥形为主,绒毛密度为 (34.2 ± 1.33) 个/mm²,绒毛高度为 (97.0 ± 2.83) μm;另外,看到突起在叶片表面的维管束(图 1a、1d)。缺磷会引起叶片表面气孔数量减少,但气孔大小变化不明显,气孔密度为 (190.3 ± 7.1) 个/mm²,气孔大小为 (13.91 ± 1.61) μm;表面绒毛数量较少,绒毛基本以锥形为主,绒毛密度为 (20.6 ± 1.02) 根/mm²,绒毛长度 (70.4 ± 3.72) μm;突起维管束数量减少,叶面出现凹凸不平现象(图 1b、1e)。磷过量时,表面气孔明显增大,但密度变化不明显,气孔大小为 (19.04 ± 1.27) μm,气孔密度为 (235.8 ± 4.1) 个/mm²,绒毛密度增加明显,为 (78.8 ± 2.14) 根/mm²,但高度减小不明显,为 (92.2 ± 4.40) μm;表面维管束数量增加(图 1c、1f)。图中 S 代表气孔,Tr 代表绒毛,Vb 代表维管束。

磷对表面微观结构影响的显著性分析如表 1 所示。缺磷能引起叶片表面气孔密度、绒毛密度和绒毛高度显著降低,但对气孔大小影响不显著;磷过量会引起气孔大小、绒毛密度和维管束数量显著增加,对气孔密度和绒毛高度影响不显著。

2.2 磷水平对叶肉组织的影响

正常水平,番茄叶片厚度为 (124.12 ± 2.14) μm,其中海绵组织厚度为 (70.31 ± 1.78) μm,栅栏组织厚度为 (51.42 ± 1.41) μm,上下表皮厚度分别为 (8.71 ± 0.28) μm 和 (8.82 ± 0.12) μm;缺磷会引起番茄叶片变薄,叶片厚度为 (95.72 ± 1.77) μm,组织内的海绵组织和栅栏组织厚度都变薄,海绵组织厚度为 (33.72 ± 2.57) μm,栅栏组织厚度为 (45.77 ± 2.36) μm,细胞普遍较小,上、下表皮厚度变化不明显,分别为 (8.59 ± 0.25) μm 和 (8.80 ± 0.41) μm。磷过量时,则会引起叶片组织厚度明显增加,叶片厚度为 (152.26 ± 4.48) μm,海绵组织厚度 (87.94 ± 2.38) μm,栅栏组织厚度为 (52.32 ± 0.98) μm(图 2)。图中 PP 为栅栏组织,SP 为海绵组织。

磷对叶片厚度以及叶片各组织厚度影响的显著

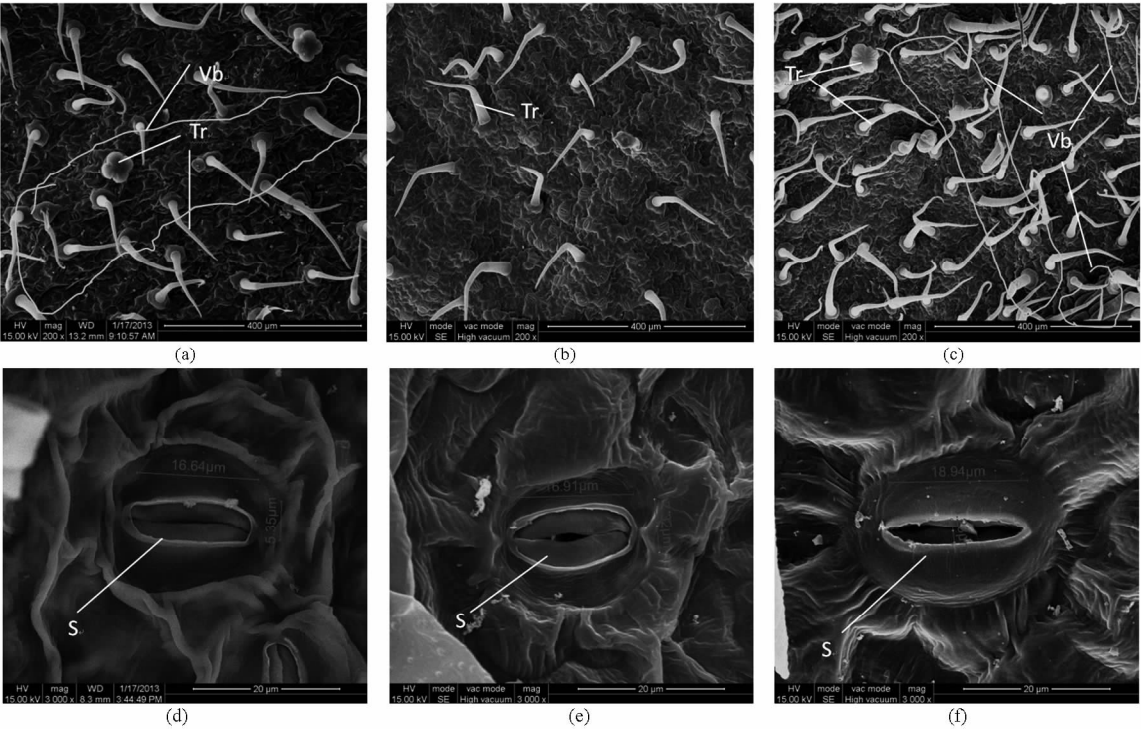


图 1 不同磷水平对番茄叶片表面微形态影响

Fig. 1 Effects of phosphorus on surface micro-structure of leaf

(a) 叶表面形态 (×200) 正常 (b) 叶表面形态 (×200) 缺磷 (c) 叶片表面形态 (×200) 高磷
(d) 气孔 (×3 000) 正常 (e) 气孔 (×3 000) 缺磷 (f) 气孔 (×3 000) 高磷

表 1 磷对叶片表面微结构的影响

Tab. 1 Effects of phosphorus on surface micro-structure of leaf

磷处理水平	气孔密度/(个·mm ⁻²)	气孔大小/μm	绒毛密度/(根·mm ⁻²)	绒毛高度/μm
0. 25	190. 3 ± 7. 1 ^b	13. 91 ± 1. 61 ^b	20. 6 ± 1. 02 ^c	70. 4 ± 3. 72 ^b
1. 00	233. 0 ± 5. 5 ^a	13. 09 ± 1. 14 ^b	34. 2 ± 1. 33 ^b	97. 0 ± 2. 83 ^a
1. 50	235. 8 ± 4. 1 ^a	19. 04 ± 1. 27 ^a	78. 8 ± 2. 14 ^a	92. 2 ± 4. 40 ^a

注:表中数值为 10 个独立样本的均值 ± 标准差,不同的字母代表不同处理的显著性差异 ($P < 0.05$)。

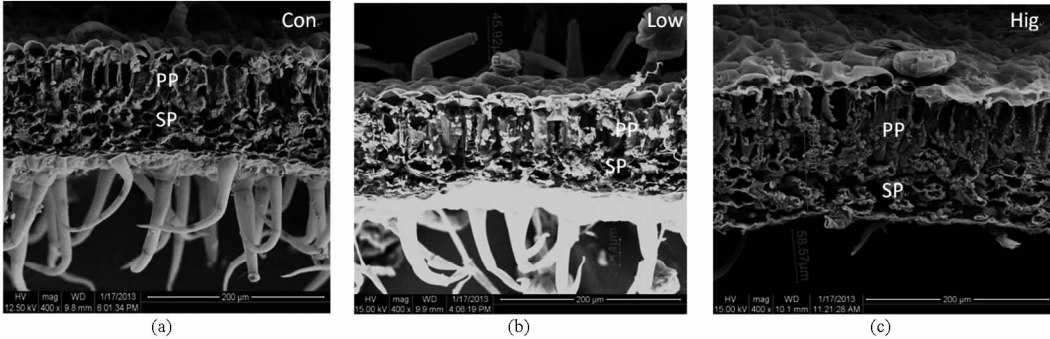


图 2 不同磷水平对番茄叶肉组织影响 (×400)

Fig. 2 Effects of phosphorus on palisade tissue and spongy tissue

(a) 正常 (b) 低磷 (c) 高磷

性分析如表 2 所示。缺磷会引起叶片明显变薄,海绵组织和栅栏组织也显著变薄,但对上下表皮组织厚度的影响不明显。磷过量时,会引起叶片厚度显著变厚,其中海绵组织明显变厚,栅栏组织、上下表皮厚度变化不明显。

2.3 磷水平对细胞超微结构的影响

磷水平对叶肉细胞超微结构影响如图 3 所示,图中为一个叶肉细胞的部分结构。对照组叶片中栅栏组织细胞排列紧密无间隙,断面形状呈矩形,长约为 $(50.75 \pm 1.23) \mu\text{m}$,宽约 $(16.34 \pm 0.62) \mu\text{m}$;海

表 2 磷对叶片厚度的影响

Tab.2 Effects of phosphorus on thickness of leaf

磷处理水平	叶片厚度/ μm	海绵组织厚度/ μm	栅栏组织厚度/ μm	上表皮厚度/ μm	下表皮厚度/ μm
0.25	95.72 ± 1.77^c	33.72 ± 2.57^c	45.77 ± 2.36^b	8.59 ± 0.25^{ns}	8.80 ± 0.41^{ns}
1.00	124.12 ± 2.14^b	70.31 ± 1.78^b	51.42 ± 1.41^a	8.71 ± 0.28^{ns}	8.82 ± 0.12^{ns}
1.50	152.26 ± 4.48^a	87.94 ± 2.38^a	52.32 ± 0.98^a	8.94 ± 0.41^{ns}	8.97 ± 0.18^{ns}

注：表中数值为 10 个独立样本的均值 $\pm$ 标准差，不同的字母代表不同处理的显著性差异，ns 表示差异不显著 ($P < 0.05$)。

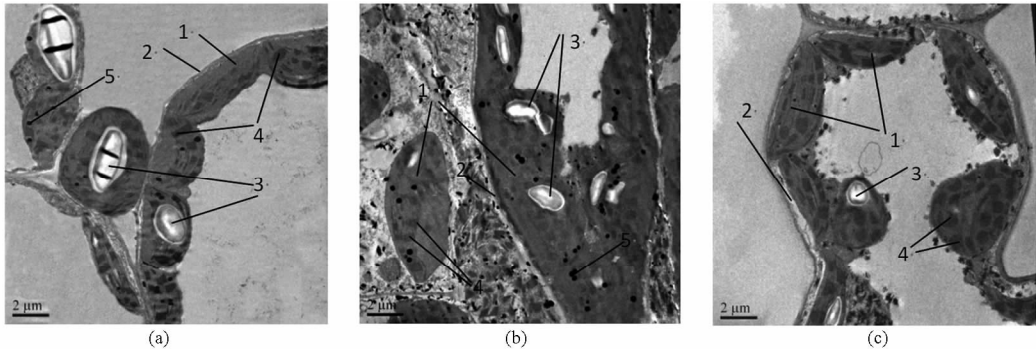


图 3 不同磷素水平对番茄叶肉细胞超微结构的影响

Fig.3 Effect of phosphorus on ultra-structure of cell as compared with control

(a) 正常 (b) 低磷 (c) 高磷

1. 叶绿体 2. 细胞壁 3. 淀粉粒 4. 基粒 5. 嗜铁颗粒

绵组织细胞之间存在间隙,间隙面积约占海绵组织整体的 15.4% 左右,海绵细胞中约 2/3 断面呈圆形,直径约为 $(23.72 \pm 0.96) \mu\text{m}$,约 1/3 断面呈矩形,长约为 $(37.15 \pm 1.15) \mu\text{m}$,宽约为 $(11.95 \pm 0.58) \mu\text{m}$;细胞内叶绿体外形规则,呈梭形,紧贴细胞壁分布,叶绿体之间紧密相贴或空隙很小,基粒片层和基质片层清晰可见,基粒片层沿叶绿体长轴方向排列,叶绿体基质中有少量淀粉粒和极少量嗜铁颗粒(图 3a)。缺磷时,栅栏组织细胞排列紧密无间隙,细胞断面形状呈矩形,长约 $(44.56 \pm 1.14) \mu\text{m}$,宽约 $(17.78 \pm 0.85) \mu\text{m}$;海绵组织细胞之间存在间隙,其间隙相比正常叶片海绵组织间隙大,间隙面积约占海绵组织整体的 21.8% 左右,海绵组织中细胞基本呈圆形,细胞直径约 $(15.67 \pm 0.68) \mu\text{m}$ 。叶绿体外形不规则,部分叶绿体解体,叶绿体内部基粒片层变模糊、松散,叶绿体内部存在少量淀粉粒和部分嗜铁颗粒(图 3b)。磷过量时,栅栏组织细胞排列紧密无间隙,细胞断面形状呈矩形,长约 $(51.14 \pm 1.33) \mu\text{m}$,宽约 $(16.68 \pm 0.35) \mu\text{m}$,海绵组织之间

存在间隙,间隙面积占总体的 16.5% 左右。海绵细胞断面以圆形为主,有小部分细胞断面呈矩形,圆形细胞直径约为 $(18.27 \pm 0.75) \mu\text{m}$ 。细胞壁变厚,叶绿体外形规则,呈梭形,内部基粒片层清晰,内部存在少量淀粉颗粒,并存在少量嗜铁颗粒,有大量黑色物质分布在叶绿体周围(图 3c)。

3 结论

- (1)不同磷水平条件下,番茄叶片微观结构信息各特征量存在明显差异。
- (2)叶片变薄,表面气孔密度、绒毛密度和高度降低,海绵组织细胞断面呈圆形且细胞间隙变大,叶绿体结构变松散等指标可以作为缺磷的检测依据。
- (3)气孔尺寸、绒毛密度和维管束数量的增加,以及细胞壁增厚等指标可以作为磷过量的检测依据。
- (4)作为快速检测手段,偏振图像技术能够有效地识别目标表面微观结构和内部结构的细微变化,可以用于作物磷水平信息测量。

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A Detection Method of Tomato Phosphorus Level Based on Micro-structure of Leaf

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Abstract: The object of this study was to seek a new way to detect the phosphorus levels of tomato rapidly. Taking the tomato grown under different phosphorus levels as the object of the research, SEM (Scanning Electron Microscope) and TEM(Transmission Electron Microscope) were adopted to evaluate the effects of phosphorus levels on micro-structure and ultra-structure of tomato leaf. The results showed that for the control treatment plant ,the thickness of leaf was (124 ± 2.14) μm, the stomatal and trichome density was (233.0 ± 5.5) mm⁻² and (34.2 ± 1.33) mm⁻² respectively, the height of trichomes was (97.0 ± 2.83) μm, and the size of the stoma was 13.91 ± 0.85 μm. In comparison with the control treatment, the thickness of leave, the density of stomata, the density and the height of trichomes decreased significantly in response to low phosphorus levels, while the changes in stomata size were not significant. The size of stomata, the density of trichomes, the number of vascular bundle were increased in high phosphorus level plants, while the density of stomata, the length of trichomes did not change markedly. At the ultra-structural level, low phosphorus supplied cell contained chloroplast so that it had begun to lose their structural integrity, and high phosphorus supplied cell had thicker cell wall, but the structure did not change observably. Based on the results, a new method to detect phosphorus levels rapidly was proposed.

Key words: Tomato Micro-structure Phosphorus levels Rapid detection

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Experimental Investigation of Biomass Gasification in a Pilot-scale Fluidized Bed Gasifier

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Abstract: The effects of equivalence ratio (R_{ER}) and steam/biomass (S/B) ratio on gasification performances such as temperature distribution, gas composition and gasification stability were investigated in a pilot-scale fluidized bed gasifier with different feedstocks (sawdust, rice husk, wood pellet and straw pellet). The results showed that typical product gas percentage components from gasification were as follows: H_2 27.1% ~ 30.4% , CO 29.7% ~ 32.6% , CO_2 25.3% ~ 27.9% , CH_4 4.9% ~ 5.8% . The uniformity of temperature distribution increased with R_{ER} and S/B ratio. More uniform temperature distribution was obtained in the case of sawdust and rice husk gasification. In the downstream direction of the dense phase zone, H_2 and CO increased significantly whereas CO_2 and O_2 decreased considerably. In the dilute phase zone, the components of product gas varted slightly. Increasing the moisture content of sawdust will deteriorate the gasification stability. An abrupt increase of temperature in the bottom of gasifier was observed during the rice husk gasification. A consistent increase of temperature drop and pressure drop was observed in the dense phase zone in the case of wood pellet and straw pellet gasification.

Key words: Biomass Fluidized bed Gasification Pilot plant