水分胁迫对柑橘果皮细胞壁结构与代谢的影响

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摘要：研究水分胁迫下，盆栽‘暗柳橙（*Citrus Sinensis* Osbeck cv. Anliu）’的果实成熟期果皮细胞壁超微结构、细胞壁物质成分、细胞壁代谢相关酶活性的变化规律及其之间关系。结果表明，在果实发育成熟期，果皮细胞壁代谢相关水解酶果胶酶、纤维素酶、果胶甲酯酶的活性随着水分胁迫的加强而增加，多酚氧化酶活性与果胶酶活性变化趋势相反，果皮细胞壁代谢相关成分离子结合型果胶、共价结合型果胶、半纤维素、纤维素的含量随着水分胁迫的加强而降低，水溶性果胶含量随着水分胁迫的加强而增加，果皮细胞壁超微结构随着水分胁迫的加强而加速解体。

关键词：水分胁迫；柑橘；果皮；细胞壁；代谢
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Effect of soil moisture on cell-wall metalbolism of pericarp in citrus

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Abstract: Based on the potted sweet orange ‘An liu (*Citrus Sinensis* Osbeck cv. Anliu)’, we did some researches of ultrastructural changes in the mesocarp cell wall. Cell wall composition, and related enzymes in cell wall metabolism were done during ripening phase in under water stress condition. Results showed that the activities of polygalacturonase (PG), cellulose (CX) and pectinesterase (PME) were more increased, water stress was more severe in potted plant, and the changes of polyphenoloxidase (PPO) activities was on the contrary. The weights of ionically associated pectin, covalently bound pectin, hemicellulose and cellulose about the composition cell wall was induced with more severe water stress. In addition, the decomposion of ultrastructure in the mesocarp cell wall was accelerated by the more severe water stress.

Key Words: water stress; citrus; mesocarp; cell wass; metalbolism

水分胁迫是影响作物生产力的最普遍的环境胁迫[1]，南方雨水较多，但是雨季分布不均匀，干旱过后降雨常加重柑橘皱皮果和裂果的发生[2]。不同程度控水对果树的果实形态和生理代谢方面的影响有很多报道。国内外学者就水分胁迫从叶片、根系的形态和显微结构的变化[3~5]以及气孔反应[6]、光合作用[4,6]、碳水化合物代谢[7~12]等生理生化指标的反应等方面进行了广泛研究；但是，不同程度的水分胁迫对果皮细胞壁代谢的影响研究较少。生产中观察得知，大雾与雨水的变化与柑橘成熟期皱皮现象密切相关[13]。因此，在成熟

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期研究不同程度的水分胁迫对柑橘果皮细胞壁代谢的影响,深入揭示水分胁迫下柑橘果实发育过程中皱皮果形成的生理机制,为生产上防止柑橘皱皮果形成提供科学理论指导。

1 材料与方法

以3年生结果数约35个果的盆栽红橘砧木暗柳橙(*Citrus Sinensis* Osbeck cv. Anliu)15株为试材,3个处理;在果实成熟转色期11月5日~11月10日、11月15日~11月20日、12月5日~12月10日分别进行3次间歇水份胁迫,处理1为轻度水分胁迫,土壤体积含水量保持在14%~18%;处理2为重度水分胁迫,极少量灌水,土壤体积含水量保持在13%以下;余下为对照,正常灌溉处理,土壤体积含水量保持在19%~25%。土壤水分的测定用艾格瑞生态技术有限公司生产的快速便携式测墒计(MPKIT)监测水分变化,测定土壤深度为15cm,每24h测1次,根据前期预备试验的经验灌水,约30min后,水分在植株根系四周分布均匀,在植株的东、南、西、北四个方向测定土壤体积含水量,取平均值,使其保持在3种水分条件下:

$$土壤体积含水量\ v\% = 土壤水容积/土壤总容积 \times 100$$

柑橘果皮细胞壁超微结构的观察,参照常规制样,每个处理观察10个片子;果皮细胞壁物质(CWM)的提取和测定参照茅林春等[14],食品分析[15],Siddiqui 等[16]的方法;果皮细胞壁代谢相关酶多聚半乳糖醛酸酶(PG)活性的测定参照谭兴杰等[17]、Pathak 和 Sanwal[18]的方法,果胶甲酯酶活性(PME)的测定参照 Hagerman 和 Austin 等[19]的方法,纤维素酶(CX)、过氧化物酶(POD)及多酚氧化酶(PPO)活性的测定参照赵亚华等[20]的方法;果皮细胞壁成分和代谢相关酶测定分别重复3次。

2 结果与分析

2.1 水分胁迫对‘暗柳橙’成熟期果皮超微结构的影响

不同程度水分胁迫对盆栽‘暗柳橙’成熟期中果皮超微结构影响很大。对照的细胞壁结构基本完整,胞间层、初生壁完整存在(图版1,2)。处理1胞间层(中间电子密度大的暗层)、初生壁部分开始瓦解,不是太明显;但是,出现了大量泡囊,其中有大量嗜锇颗粒(图版3、4、5)。处理2条件下,胞间层(中间电子密度大的暗层)几近消失(图版6),不连续分布(图版7),初生壁降解,细胞壁逐步分解,断续分布,这期间也有大量嗜锇颗粒的泡囊存在(图版6、7、8),较处理1更多。3个处理整体来看,水分胁迫越严重,细胞壁降解程度越大。

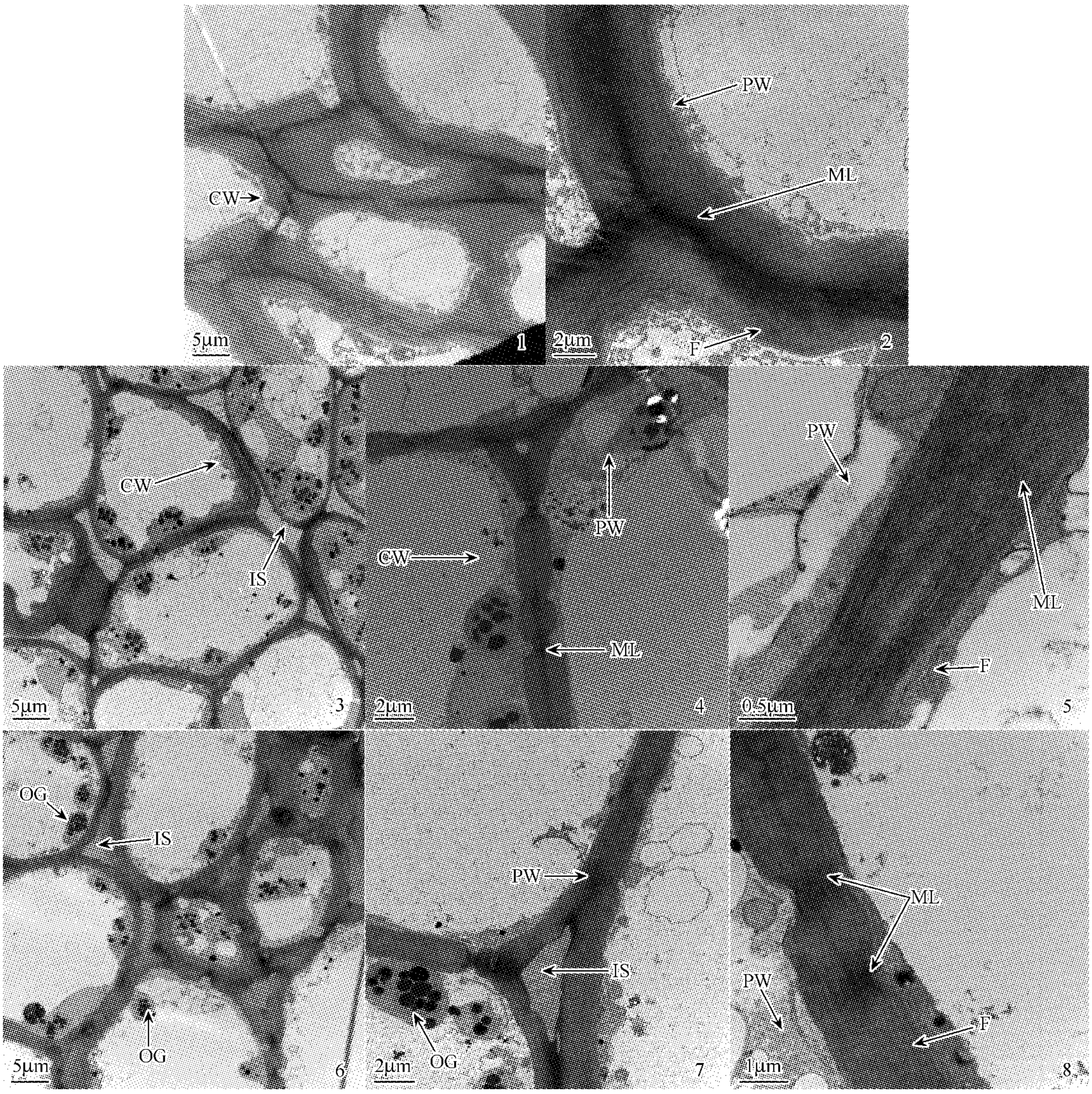
表1 水分胁迫‘暗柳橙’果皮成熟期细胞壁超微结构的比较观察

Table 1 Ultrastructural changes in the mesocarp cell wall during ripening period of potted plant ‘Anliu’ sweet orange under water stress

项目 Item	对照 Control (CK)	处理1(轻度水分胁迫) Moderate water stress (MS)	处理2(重度水分胁迫) Severe water stress (SS)
细胞壁整体结构 Ultrastructure in the mesocarp Cell wall	完整 Intact-structure	部分解体 Decomposition partially	松散紊乱 Decomposition
胞间层 Middle lamella	完整 Intact-structure	部分瓦解 Disruption partially	解体、不连续 Disruption
初生壁 Primary wall	完整 Intact-structure	部分解体、不连续 Degradation partially	解体 Degradation
电子密度 Electronical density	均匀 Well-proportioned	部分均匀 Proportioned partially	不均匀 Asymmetrical
嗜锇颗粒 Osmiophilic globule	无 Nothing	少量 A few	很多 A large number

2.2 水分胁迫对‘暗柳橙’成熟期果皮细胞壁成分的影响

盆栽‘暗柳橙’成熟期果实发育过程中,3个处理水溶性果胶质含量增加,处理2>处理1>对照($P<0.05$)(图1);3个处理的离子结合果胶质含量在后期都降低,处理2的降低幅度最大,处理1和对照差异不显著(图2);处理2和处理1的共价结合果胶质含量处于下降趋势,以处理2降幅最大,对照先增后降(图版3);三者半纤维素含量都降低,处理2的半纤维素含量降幅最小($P<0.01$)(图4);处理1和处理2的纤维素含量下降,对照的纤维素含量在果实发育后期有较大幅度增加(图5),可见随着水分胁迫的加强,处理2胞壁成分降解程度最大,处理1次之,对照降解最少。表明3个处理果实成熟速度不同,处理2>处理1>对照。



图版 Explanation of plate

CW. 细胞壁 ML. 胞间层 IS. 胞间隙 PW. 初生壁 F. 微纤丝 OG. 嗜锇颗粒

1 ~2:盆栽'暗柳橙'正常灌水成熟期果皮细胞壁超微结构;3 ~5:盆栽'暗柳橙'处理1 成熟期果皮细胞壁超微结构;6 ~8:盆栽'暗柳橙'处理2 成熟期果皮细胞壁超微结构

CW:cell wall; ML:middle lamella; IS:intercellular space; PW:primary wall; F: microfibril; OG:osmiophilic globule

1 —2: Ultrastructural changes in the mesocarp cell wall during ripening period of potted plant 'Anliu' sweet orange under control condition; 3 —5: Ultrastructural changes in the mesocarp cell wall during ripening period of potted plant 'Anliu' sweet orange under moderate stress condition; 6 —8: Ultrastructural changes in the mesocarp cell wall during ripening period of potted plant 'Anliu' sweet orange under severe water stress condition

2.3 水分胁迫对'暗柳橙'成熟期果皮细胞壁代谢相关酶活性的影响

盆栽'暗柳橙'成熟期 3 个处理的果皮果胶酶活性是前期下降,后期增加;纤维素酶活性和果胶甲酯酶活性整个成熟期都增加,果胶酶、纤维素酶、果胶甲酯酶活性增幅大小依次为:处理2 >处理1 >对照(图6、7、8);3 个处理的过氧化物酶活性前期没有差异,后期以不同幅度增加,增幅大小为:对照 >处理1 >处理2($P<0.01$)(图9);果实成熟后期(12 月05 日 ~12 月25 日)的处理1、处理2 多酚氧化酶活性增加,对照降低,三者之间差异极显著($P<0.01$)(图10)。

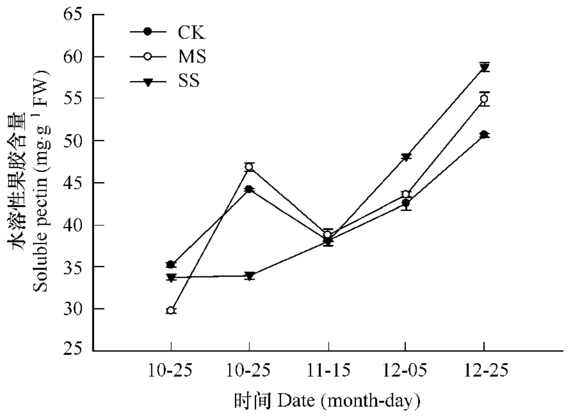


图 1　水分胁迫对'暗柳橙'果皮水溶性果胶质含量的影响

Fig. 1　Effect of water stress on the soluble pectin content in pericarp of potted 'Anliu' sweet orange during fruit maturation

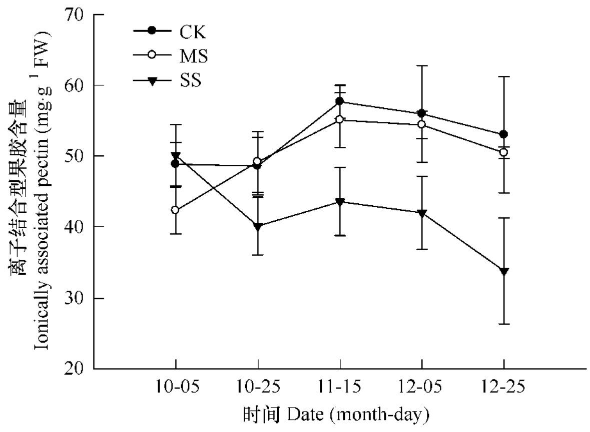


图 2　水分胁迫对'暗柳橙'果皮离子结合型果胶含量的影响

Fig. 2　Effect of water stress on the ionically associated pectin content in pericarp of potted 'Anliu' sweet orange during fruit maturation

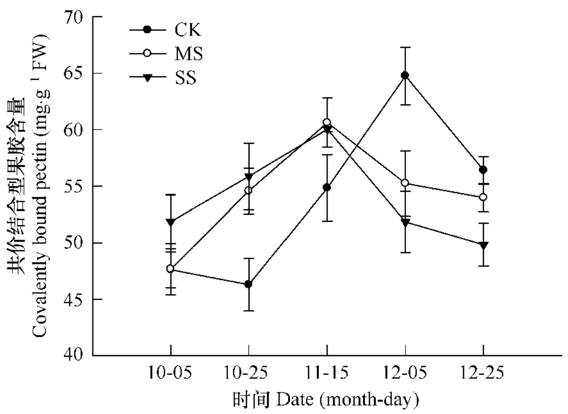


图 3　水分胁迫对'暗柳橙'果皮共价结合果胶质含量的影响

Fig. 3　Effect of water stress on the covalently bound pectin content in pericarp of potted 'Anliu' sweet orange during fruit maturation

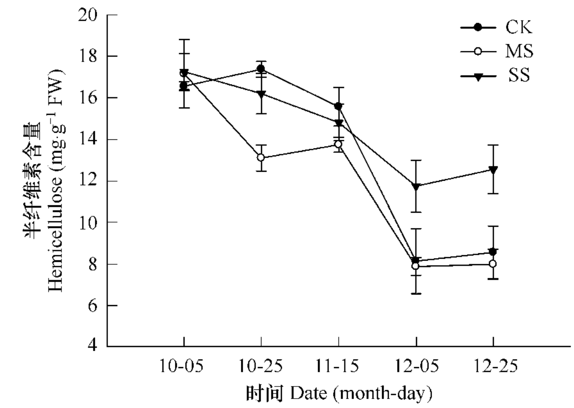


图 4　水分胁迫对'暗柳橙'果皮半纤维素含量的影响

Fig. 4　Effect of water stress on the hemicellulose content in pericarp of potted 'Anliu' sweet orange during fruit maturation

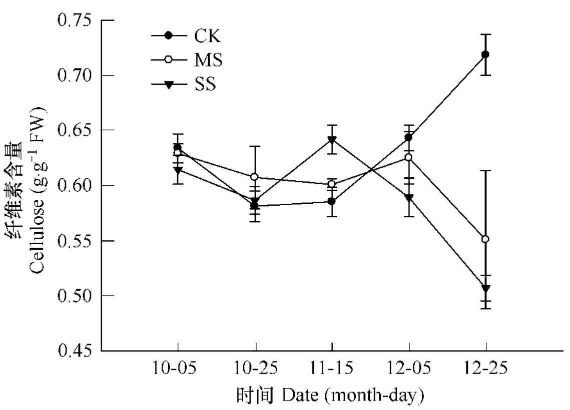


图 5　水分胁迫对'暗柳橙'果皮纤维素含量的影响

Fig. 5　Effect of water stress on the cellulose content in pericarp of potted 'Anliu' sweet orange during fruit maturation

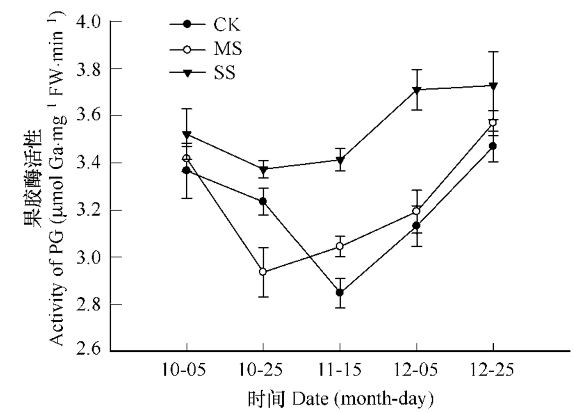


图 6　水分胁迫对'暗柳橙'果皮果胶酶(PG)的影响

Fig. 6　Effect of water stress on the activities of PG in pericarp of potted 'Anliu' sweet orange during fruit maturation

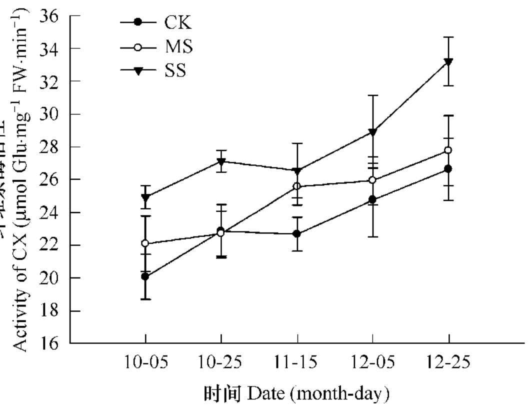


图 7 水分胁迫对‘暗柳橙’果皮纤维素酶(CX)活性的影响

Fig. 7 Effect of water stress on the activities of CX in pericarp of potted ‘Anliu’ sweet orange during fruit maturation

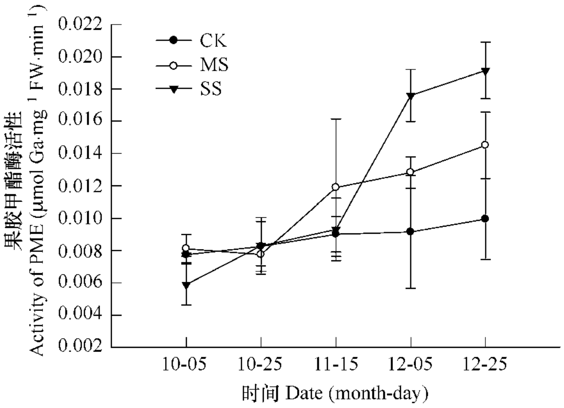


图 8 水分胁迫对‘暗柳橙’果皮果胶甲酯酶(PME)活性的影响

Fig. 8 Effect of water stress on the activities of PME in pericarp of potted ‘Anliu’ sweet orange during fruit maturation

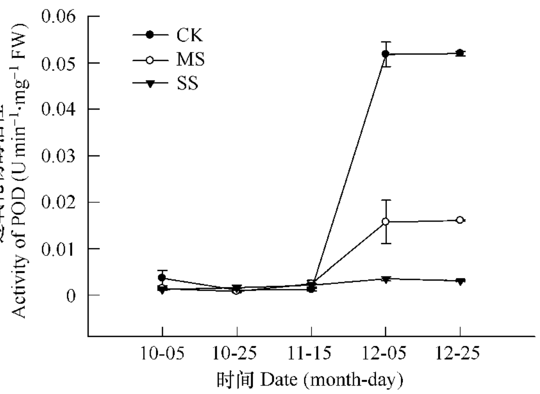


图 9 水分胁迫对‘暗柳橙’果皮过氧化物酶(POD)活性的影响

Fig. 9 Effect of water stress on the activities of POD in pericarp of potted ‘Anliu’ sweet orange during fruit maturation

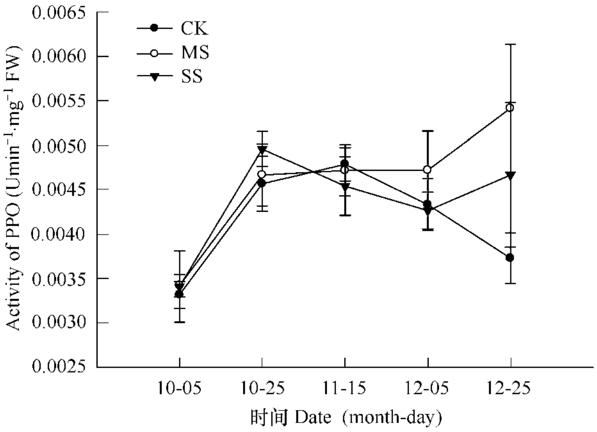


图 10 水分胁迫对‘暗柳橙’果皮多酚氧化酶(POD)活性的影响

Fig. 10 Effect of water stress on the activities of PPO in pericarp of potted ‘Anliu’ sweet orange during fruit maturation

3 讨论

果实正常成熟过程中,伴随着水溶性果胶质的不断增加,共价结合和离子结合果胶质的含量相应减少[14],果实组织中原果胶分解为可溶性果胶[21]。纤维素的降解意味着细胞壁的解体和果实的软化[22]。半纤维素(主要成分为木葡聚糖)分子在细胞壁的“经纬模型”中起着“闩锁”的作用,其分子的降解将导致细胞壁结构的松懈[23]。水分胁迫会引起细胞脱水,使合成酶活性降低或失活而使分解酶活性加强,抑制合成代谢而加强分解代谢,加快植物的衰老过程。本研究中,果实发育后期细胞壁代谢相关水解酶中,果胶酶活性、纤维素酶活性、果胶甲酯酶活性随着水分胁迫的加强而增加,多酚氧化酶活性与果胶酶活性变化趋势相反,因为其参与木质素的合成有关[24],酶活性的变化导致果皮细胞壁代谢相关成分离子结合型果胶、共价结合型果胶、半纤维素、纤维素的含量随着水分胁迫的加强而降低,水溶性果胶含量随着水分胁迫的加强而增加,进而引发果皮细胞壁超微结构的变化,使细胞壁发生过度松弛,细胞壁一旦发生过度松弛,在膨压作用下,细胞壁就发生溃裂。严重缺水时因果实的水势比叶片的水势高,因而,叶片从果实中夺取水分,使果实体积缩小,遇骤雨或大雾果实吸收过快常造成裂果,李建国等[25,26]报道台风雨来临时遮雨生长棚内甜橙果实突发性猛长,大气中的蒸气压亏的减小势必使叶的蒸腾减弱,从根吸收上来的土壤水分大量、急速涌入果实,使果实突发性

猛长,导致了裂果的增多。生产上也观察到类似的现象:在果实发育后期,来一场大雾或暴雨将会使细胞壁来不及松弛,减弱壁的强度而被果肉撑破,导致皱皮果现象、甚至裂果的发生[13,26]。但是重度水分胁迫过于提高了壁中水解酶的活性,处理1、2的植株要比对照的植株早些进入衰老过程,并且有嗜锇颗粒的出现,表明它们已经进入了衰老状态;并且随着水分胁迫的加强,衰老过程加速。因此,在暗柳橙成熟期,不同程度水分胁迫对果皮的细胞壁代谢影响很大,可能会引发生产上的一系列问题,如裂果、皱皮果等农业灾害。

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