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6个杧果品种耐寒性的研究

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摘 要:【**目的**】杧果易受冬季低温寒害,造成产量下降甚至绝收,严重影响杧果产业的发展。通过对杧果不同品种耐寒性的研究,为杧果耐寒品种选育及栽培布局提供理论依据。【**方法**】以'贵妃''凯特''红玉''金煌''热品6号'和'台农2号'为试材,测定了低温胁迫下其叶片相对电导率(REC)、丙二醛(MDA)含量和超氧化物酶(SOD)、过氧化物酶(POD)、过氧化氢酶(CAT)活性的变化。【**结果**】低温胁迫下,杧果叶片各指标均高于对照,且品种间存在极显著差异。随着温度下降及时间延长,6个杧果品种REC和MDA含量不断升高,CAT活性先升后降;不同品种SOD和POD活性变化不同,'凯特''红玉'和'金煌'3者活性先升后降,'贵妃'和'台农2号'SOD活性呈2种不同变化,'热品6号'SOD活性先升后降,'贵妃'和'台农2号'POD活性呈降-升-降变化,'热品6号'呈3种不同变化。【**结论**】低温胁迫过程中杧果叶片REC、MDA含量升高,SOD、POD和CAT活性先升后降,6个品种的耐寒性由弱到强依次为'台农2号'(-0.97 ℃)<'贵妃'(-1.68 ℃)<'热品6号'(-2.11 ℃)<'凯特'(-2.32 ℃)<'金煌'(-4.04 ℃)<'红玉'(-5.14 ℃)。

关键词:杧果;低温;耐寒性;半致死温度;保护酶

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A study on cold resistance in six mango cultivars

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Abstract:【Objective】Mango is susceptible to chilling injury in winter. Chilling injure causes yield reduction and even total crop failure, which is disastrous for farmers and seriously affects the development of mango production. Previous studies focused mainly on frozen injury in mango in winter, but there have been few reports about the mechanisms of response to cold temperatures. In this study, the semi-lethal temperature (LT_{50}) and changes in activities of protective enzymes in mangos under chilling stress were studied in order to provide a theoretical basis for cultivar introduction, cold resistance breeding and cultivation.【Methods】Two-year-old grafted seedlings of six mango cultivars including 'Guifei' 'Keitt' 'Hongyu' 'Chiin Hwang' 'Repin No.6' and 'Tainoung No.2' were placed in incubators for low temperature treatments. The experiment set six temperature treatments at 6, 3, 0, -3, -6 and -9 ℃ under darkness for 3, 6 and 9 h, and therefore there were totally nineteen treatment groups including the control group, each composed of three replicates (seedlings). Mature leaves were collected immediately after 3, 6 or 9 h of treatments and divided into two parts. One was used to determine the relative electric conductivity (REC); the other was frozen in liquid nitrogen then stored at -80 ℃ until analyses of malondialdehyde (MDA) and the enzymatic activities of superoxide dismutase (SOD), catalase (CAT) and peroxidase activity (POD). The method of REC measurement was modified from that of Yang Huageng's, and the semi-lethal temperature (LT_{50}) was determined according to Zhu Genhai. Activities of MDA, SOD, CAT, POD and the content of MDA were determined following the Assay Kit Protocols.【Results】The REC value of the six mango cultivars increased following an S curve with the decrease of temperature. The rapid increase in REC in different cultivars occurred at different temperatures. The REC values of 'Keitt' 'Repin No.6' and 'Tainoung

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No.2' increased significantly when temperature was below 0 ℃; those of 'Guifei' and 'Chiin Hwang' under chilling stress for 6 and 9 h were similar to those of 'Keitt' and others. When temperature was lower than −3 ℃, the REC values of 'Guifei' and 'Chiin Hwang' exposed to low temperature for 3 h rose significantly. Regressed logistic equations between temperature and REC values in the 6 cultivars exposed to the temperatures for 9 h were established. LT_{50} value was calculated from the established equations, which can be used to measure the cold resistance of mango. The cold resistance based on LT_{50} in six mango cultivars was in the order of 'Hongyu' (−5.14 ℃)> 'Chiin Hwang' (−4.04 ℃)> 'Keitt' (−2.32 ℃)> 'Repin No.6' (−2.11 ℃)> 'Guifei' (−1.68 ℃)> 'Tainoung No.2' (−0.97 ℃). MDA content in all cultivars under cold stress was higher than the control. MDA content in all the cultivars gradually increased with the decrease of temperature, and reached the highest value at −9 ℃. Highest value of MDA was found in 'Tainoung No.2', and the lowest value was in 'Hongyu'. MDA in 'Tainoung No.2' was higher than in the other cultivars. MDA contents in 13 treatments were significantly were higher than the other cultivars at the same condition. 'Hongyu' had the lowest MDA content among all the cultivars, its peak value being 61.22% that of 'Tainoung No.2'. The range of increase in MDA was the largest in 'Guifei' and lowest in 'Hongyu' and 'Chiin Hwang'. With drop in temperature, SOD and POD activities showed different change patterns in different cultivars. SOD activity in 'Keitt' 'Hongyu' 'Chiin Hwang' and 'Repin No.6' rose first and then declined. There were differences in trend in 'Guifei' and 'Tainoung No.2' with time of treatment. The curve of 'Guifei' under cold stress for 3 h was in an M pattern similar to that of 'Keitt' under cold stress for 6 and 9 h. SOD activity in 'Tainoung No.2' showed an N pattern in the cases of cold exposure for 3 and 9 h, while it increased continuously in the case of cold exposure for 6 h. 'Guifei' maintained a significantly higher SOD activity than other cultivars at all the low temperatures, followed by 'Chiin Hwang' and 'Keitt'. Low temperature stress increased SOD activity. Compared to the control group, the rising range of 'Repin No.6' was larger, while those of 'Hongyu' and 'Guifei' were smaller than the others'. POD activity 'Keitt' 'Hongyu' and 'Chiin Hwang' increased in the early period and then decreased. The enzyme activity 'Guifei' and 'Tainoung No.2' displayed a trend of decreasing− increasing−decreasing, but its change was slight. 'Repin No.6' showed different trends at different durations of cold exposure, W shape for 3 h, N shape for 6 h and decreasing−increasing−decreasing for 9 h. POD activity in 'Hongyu' was significantly higher than that in the other cultivars. In all the treatments in 'Tainoung No.2', POD activities were lower than in the other cultivars. POD activity in the six mango cultivars exposed to low temperatures was higher than in the control. POD activity rose significantly when temperature dropped below 3 ℃, and the rising range was the largest in 'Hongyu' and smallest in 'Tainoung No.2'. CAT activity was higher in chilling stressed groups than in the control. CAT activity in the six mango cultivars increased and then decreased with temperature drop but its peak values were different. 'Guifei' 'Keitt' 'Repin No.6' and 'Tainoung No.2' had their peak CAT activities at 0 ℃, and 'Hongyu' and 'Chiin Hwang' at −6 ℃. CAT activities in 'Hongyu' in 9 cold treatments were all significantly higher than in the other cultivars. Fourteen treatments in 'Tainoung No.2' were lowest among all the cultivars. Same to POD, the largest and lowest increasing range was found in 'Hongyu' and 'Tainoung No.2', respectively. 【Conclusion】REC, MDA content and SOD, POD and CAT activities were higher under chilling stresses compared with the control. With temperature drop, REC and MDA content in the six mango cultivars increased; CAT activity rose first and then decreased; the change patterns of SOD and POD activities differed among the six mango cultivars. The LT_{50} and the changes in protective enzyme activities showed that cold resistance in the six mango cultivars was in the order of 'Hongyu' > 'Chiin Hwang'> 'Keitt'> 'Repin No.6' > 'Guifei' > 'Tainoung No.2'.

Key words: Mango; Low temperature; Cold resistance; Semi−lethal temperature (LT_{50}); Protective enzyme

杧果（*Mangifera indica* L.）是漆树科杧果属热带果树，性喜长日照高温天气，我国种植的适宜区和次适宜的区划指标年极端最低气温为 0 ℃和−2.5 ℃[1−2]。杧果作为经济价值高的果树种类，受到政府重视与果农青睐，在海南、广东、广西、云南、台湾、福建南部及川贵南端河谷地带均有分布[3]。但近年来我国气候变化异常，冬季低温寒害在我国杧果主要产地时有发生[1−2,4−9]，给杧果生产造成不同程度的经济损失，福建南部作为杧果种植北缘地，安全越冬尤为重要。引进、筛选、培育和推广耐寒性强的品种可有效地减轻或避免杧果寒害的不利影响，保障我国杧果产业健康发展。

杧果寒害调查及灾后处理报道较多[4−9]，温度对杧果授粉[10]、果实形态形成[11−12]等方面的研究较为深入，有关杧果品种耐寒性鉴定的研究报道少。在耐寒性鉴定指标上，冯美利[13]指出叶片组织结构的紧密度可作为杧果耐寒性鉴定指标；在品种筛选和品种间耐寒性对比上，Lao等[14]利用叶绿素荧光反应测定杧果叶片对低温的敏感性，得出‘金煌’‘海顿’‘爱文’比‘Mahachanok’耐寒的结论；黄馨莹[15]通过测定不同温度梯度处理后的10个杧果品种叶片的电解质渗透率、乙烯生成量及呼吸作用，筛选出‘金煌’‘爱文’等5个耐低温品种；唐力生等[16]的研究表明‘台农1号’1 a(年)生嫁接苗在极端最低气温低于−1 ℃时死亡，极端最低气温高于3 ℃时能正常生长，极端最低气温在3~5 ℃持续9 d，叶片无受害症状。为进一步了解杧果品种的耐寒性及其生理生化变化情况，笔者选用在杧果北缘栽培区——福建种植较广的‘金煌’‘贵妃’以及其他4个优良品种为试材，利用人工气候箱模拟低温，对2 a生嫁接苗叶片的相对电导率（REC）、丙二醛（MDA）含量和超氧化物酶（SOD）、过氧化物酶（POD）和过氧化氢酶（CAT）活性的变化进行测定，探讨品种耐寒性，为耐寒品种的筛选应用及耐寒生理基础研究提供理论依据。

1　材料和方法

1.1　材料

供试材料为‘贵妃’‘凯特’‘红玉’‘金煌’‘热品6号’和‘台农2号’6个品种的2 a生营养袋嫁接苗。供试材料以2 a生本地土杧营养袋苗为砧木，于2013年4月采用切接法嫁接以上6个品种，每个品种20株，嫁接后定植于福建省农业科学院果树研究所基地内，常规管理。

1.2　方法

2014年12月，每个品种选取生长一致、健康的营养袋苗18株移入室内，48 h后置于GXZ-0288光照培养箱（宁波江南仪器厂）内进行低温处理，温度波动±1 ℃。在暗环境下，试验以3 ℃为降阶，设6、3、0、−3、−6、−9 ℃共6个温度降阶；设3、6、9 h 3个处理时间，即每2株苗为1个重复，6株苗为3次重复，以处理时间相同温度每次降3 ℃，直至降到−9 ℃为一组试验。每个品种另取3株于相同时间移入室内，置于室温（25 ℃）下48 h后开始取样，作为对照。每品种均为19个处理，处理序号见表1。

低温处理时间满足要求后，每株取无病虫害、无机械损伤的老熟叶片3~5枚为测定样品。叶片用自来水洗净，后用纯水冲洗2遍，用干净的纱布吸干水分，用剪刀剪去叶脉及叶片边缘，留下中间部分叶片用于试验。样品分为2份，一份叶段剪成0.5 cm²样叶混匀后测定相对电导率（REC），参照杨华庚等[17]的方法，LT_{50}计算参照朱根海等[18]的方法；另一份用液氮冻存后置于−80 ℃保存，测定丙二醛（MDA）含量、超氧化物酶（SOD）活性、过氧化物酶（POD）活性、过氧化氢酶（CAT）活性，测定方法均参照试剂盒说明（南京建成生物研究所）。

表1　试验处理

Table 1 The treatments in the experiment

	N1	N2	N3	N4	N5	N6	N7	N8	N9	N10	N11	N12	N13	N14	N15	N16	N17	N18	N19
时间Time/h	3	3	3	3	3	3	3	6	6	6	6	6	6	9	9	9	9	9	9
温度Temperature/℃	25	6	3	0	−3	−6	−9	6	3	0	−3	−6	−9	6	3	0	−3	−6	−9

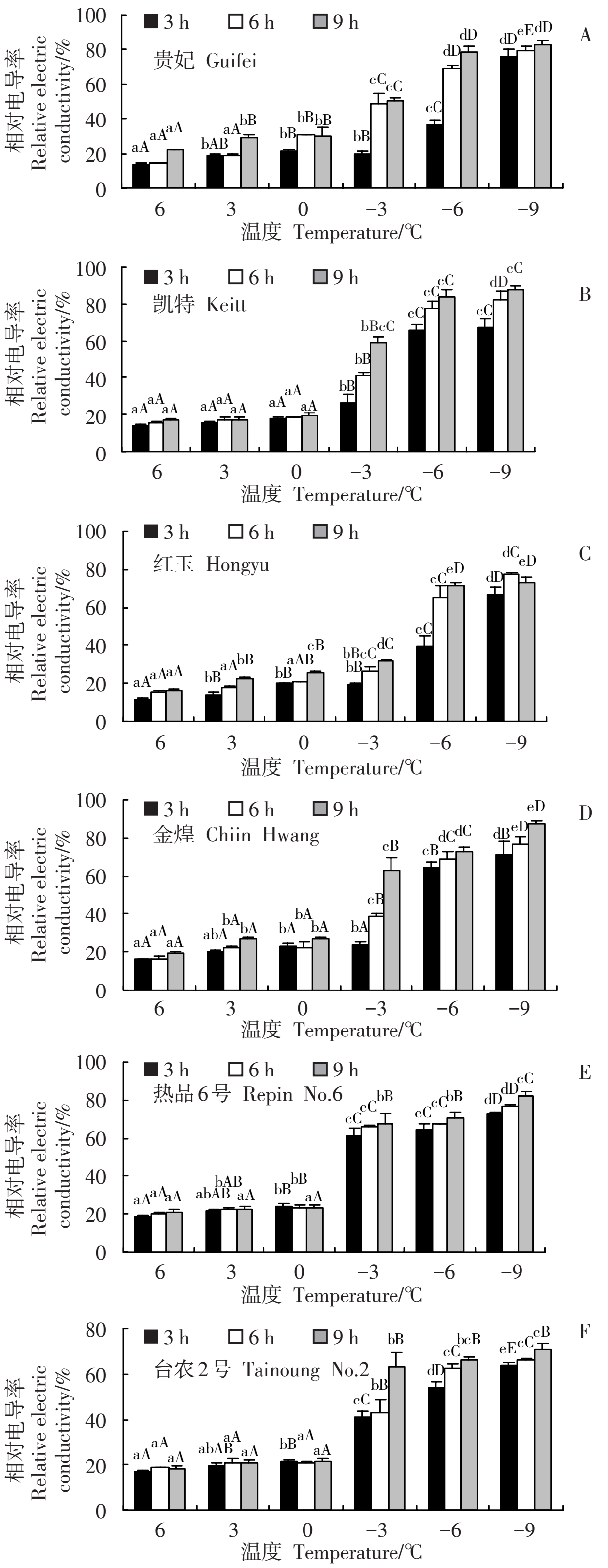
1.3　数据分析

数据采用SPSS软件进行Duncan’s分析、非线性回归方程拟合及检验，用Excel软件进行绘图。

2　结果与分析

2.1　低温对REC的影响

由图1可看出，6个杧果品种的叶片REC均随温度的下降而上升，总体呈现S型。各品种叶片REC在低温胁迫6 ℃下与−9 ℃下差异极显著，分别从11.53%~22.05%升高至63.86%~87.74%。不同杧果品种REC



小写字母和大写字母分别表示同一时间处理下不同低温叶片REC的差异水平为显著 $P<0.05$ 和极显著 $P<0.01$。下同。

Lowercase and capital letters indicate the difference at the level of $P<0.05$ and $P<0.01$ respectively for REC among different temperature treatments at the same time. The same below.

图1　低温胁迫对6个杧果品种叶片REC的影响

Fig. 1 Effect of low temperature on REC in leaves of six mango cultivars

出现急剧上升的温度不同。当温度低于0 ℃时，‘凯特’‘热品6号’‘台农2号’以及低温处理6 h和9 h的‘贵妃’和‘金煌’REC极显著升高；当温度低于−3 ℃时，‘红玉’和低温处理3 h的‘贵妃’和‘金煌’REC极显著升高。

2.2　不同品种的 LT_{50}

根据材料在不同低温胁迫9 h下RCE拟合不同品种的logistic方程、相关系数及 LT_{50}（表2），结果显示REC与logistic方程拟合效果较好，LT_{50} 为−5.14~−0.97。其中‘红玉’LT_{50} 最低，为−5.14 ℃，说明其最耐寒；‘台农2号’LT_{50} 最高，仅为−0.97 ℃，耐寒性最差；其他品种耐寒性由强到弱依次为‘金煌’（−4.04 ℃）>‘凯特’（−2.32 ℃）>‘热品6号’（−2.11 ℃）>‘贵妃’（−1.68 ℃）。

表2　6个杧果品种REC回归方程及半致死温度

Table 2 The REC regression equations and semi-lethal temperatures of the six mango cultivars

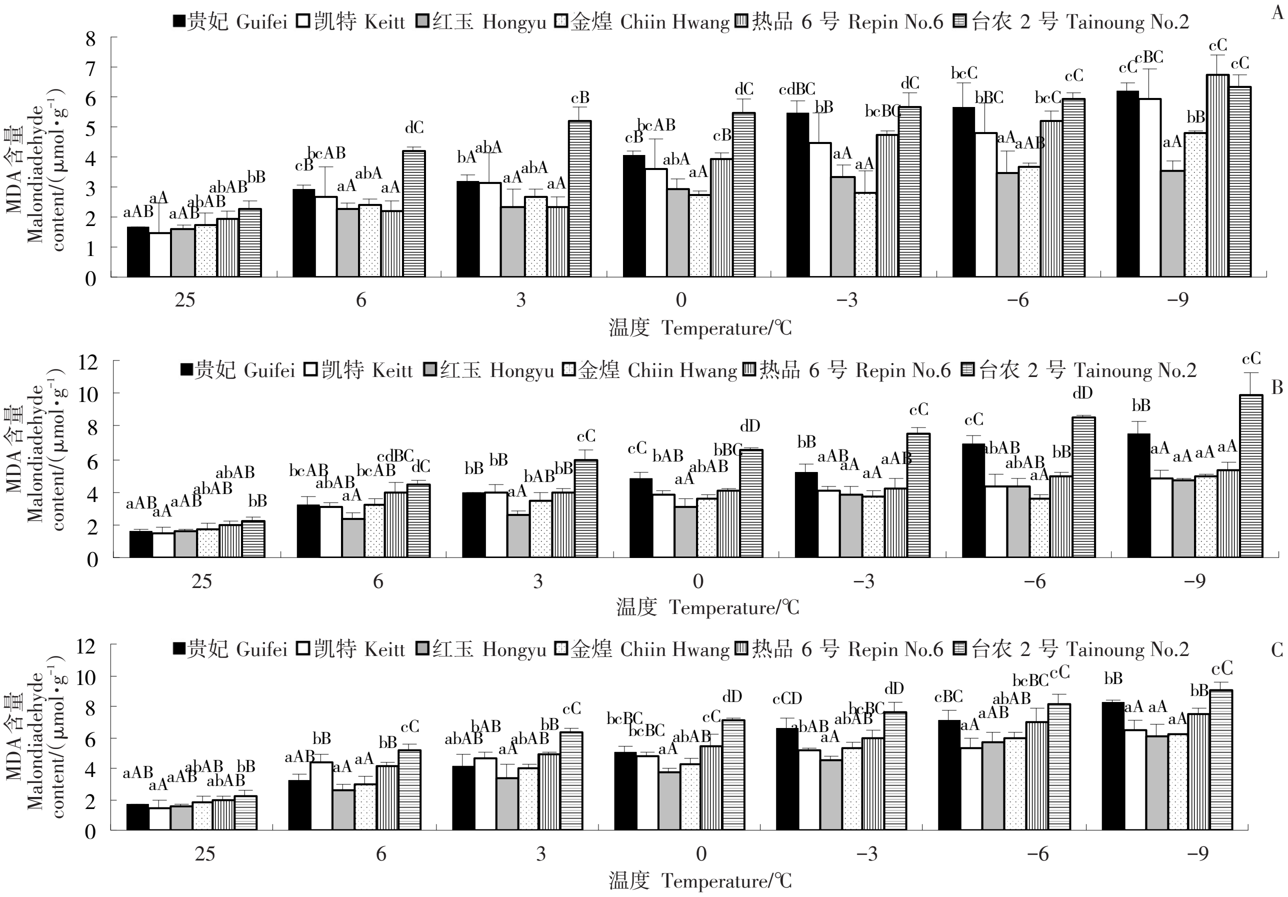
品种 Variaties	回归方程 Logistic equation	相关系数 R	半致死温度 LT_{50}/℃
贵妃 Guifei	$y=89.869\,3/(1+1.668\,1e^{0.303\,7x})$	0.945 5	−1.68
凯特 Keitt	$y=100.006\,8/(1+2.267\,2e^{0.352\,7x})$	0.970 1	−2.32
红玉 Hongyu	$y=153.878\,7/(1+2.309\,5e^{0.163\,1x})$	0.947 6	−5.14
金煌 Chiin Hwang	$y=122.995\,3/(1+2.182\,6e^{0.192\,9x})$	0.973 1	−4.04
热品6号 Repin No.6	$y=100.362\,2/(1+1.652\,6e^{0.237\,9x})$	0.946 0	−2.11
台农2号 Tainoung No.2	$y=81.448\,5/(1+1.316\,3e^{0.284\,1x})$	0.939 6	−0.97

2.3　低温对MDA含量的影响

由图2可知，6个品种所有低温处理的叶片MDA含量均高于对照，除‘红玉’和‘热品6号’N2和N3处理不显著外，其他处理均显著或极显著高于对照。MDA含量随温度下降逐渐升高，各品种均在−9 ℃时达到峰值，说明随着温度降低，细胞膜脂过氧化加强。从MDA含量来看，‘台农2号’高于其他品种，N2~3、9~13、14~17、19等13个处理MDA含量显著或极显著高于同等条件下的其他品种；‘红玉’MDA含量最低，N3、6~10、13、14~17、19等12个处理的MDA含量最低。到达峰值时，MDA含量上升幅度最大的品种为‘贵妃’，最小的为‘红玉’，温度降到−9 ℃时，它们分别是对照的4.47倍和2.96倍。

2.4　低温对SOD活性的影响

除‘红玉’与‘台农2号’N8处理时叶片的SOD活性稍低于对照（无显著差异），6个杧果品种所有处理均高于对照，说明低温下杧果叶片SOD酶活性提高了，但不同品种叶片SOD活性变化不同（图3）。



A~C. 低温处理 3、6、9 h。差异性为相同处理下不同品种间的比较。下同。

A–C. Low temperature treatment for 3, 6 and 9 h respectively. Differences among six mango cultivars at the same treatment. The same below.

图 2 低温胁迫对 6 个杧果品种叶片 MDA 含量的影响

Fig. 2 Effect of low temperature on MDA content in leaves of six mango cultivars

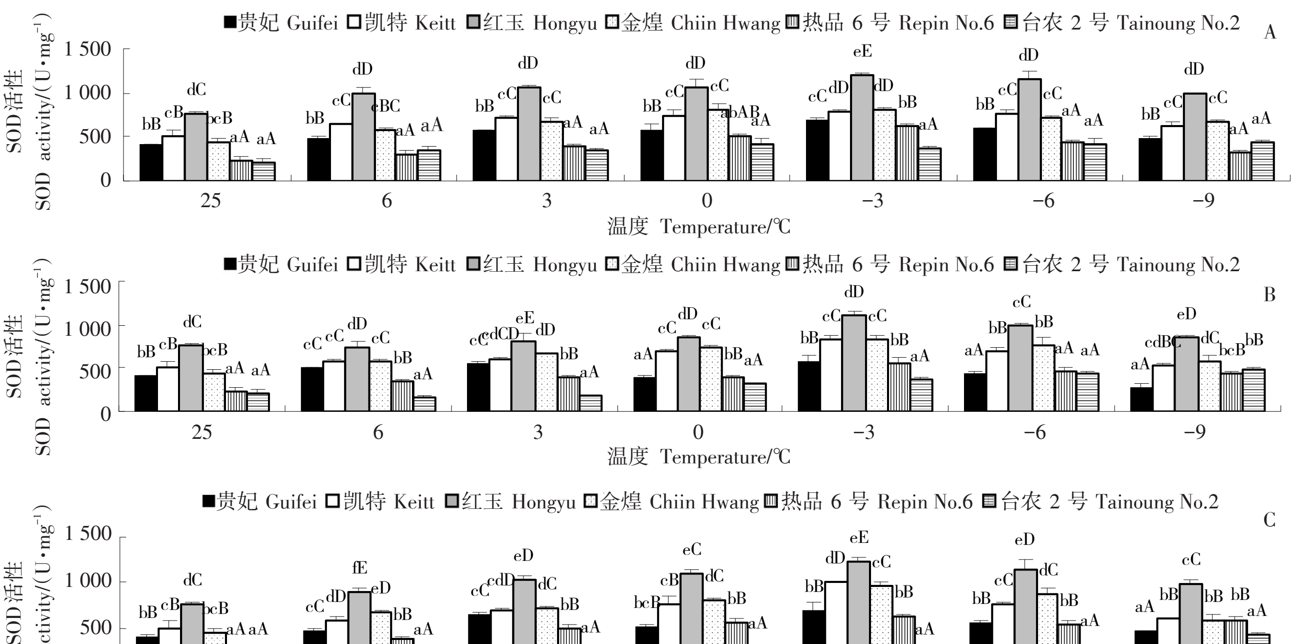


图 3 低温胁迫对 6 个杧果品种叶片 SOD 活性的影响

Fig. 3 Effect of low temperature on SOD activity in leaves of six mango cultivars

‘凯特’‘红玉’‘金煌’和‘热品6号’先上升后降低,在-3 ℃时达到最高峰;‘贵妃’总体呈双峰变化,分别在3 ℃和-3 ℃达到峰值;‘台农2号’3 h和9 h处理均呈升-降-升变化,6 h处理SOD活性不断上升,均在-9 ℃达到峰值。到达峰值时,升幅最大的品种为‘热品6号’,最小的是‘红玉’。在整个低温胁迫过程中,‘红玉’一直保持较高活性,所有处理的活性均极显著高于其他品种;‘金煌’和‘凯特’次之。

2.5 低温对POD活性的影响

6个杧果品种所有低温处理叶片POD活性均高于对照,其中‘金煌’和‘热品6号’所有处理极显著高于对照。不同品种叶片POD活性随温度下降变化有所不同(图4)。‘凯特’‘红玉’和‘金煌’呈先上升后下降趋势,其中‘凯特’和‘金煌’在-3 ℃达到峰值,而‘红玉’峰值出现于-6 ℃;‘贵妃’和‘台农2号’变化平缓,呈降-升-降变化;‘热品6号’呈3种变化趋势,3 h处理呈W形变化,6 h处理为升-降-升,9 h处理为降-升-降,变化趋势虽不同,但达到峰值温度相同,为-3 ℃。与SOD缓慢上升不同,当温度降到3 ℃以下时,POD活性上升明显,到达峰值后下降。其中,‘红玉’升幅最大,‘台农2号’最小。低温期间,‘红玉’12个处理的活性极显著高于其他品种,而‘台农2号’所有处理均低于其他品种,其中7个处理的活性显著低于其他品种。

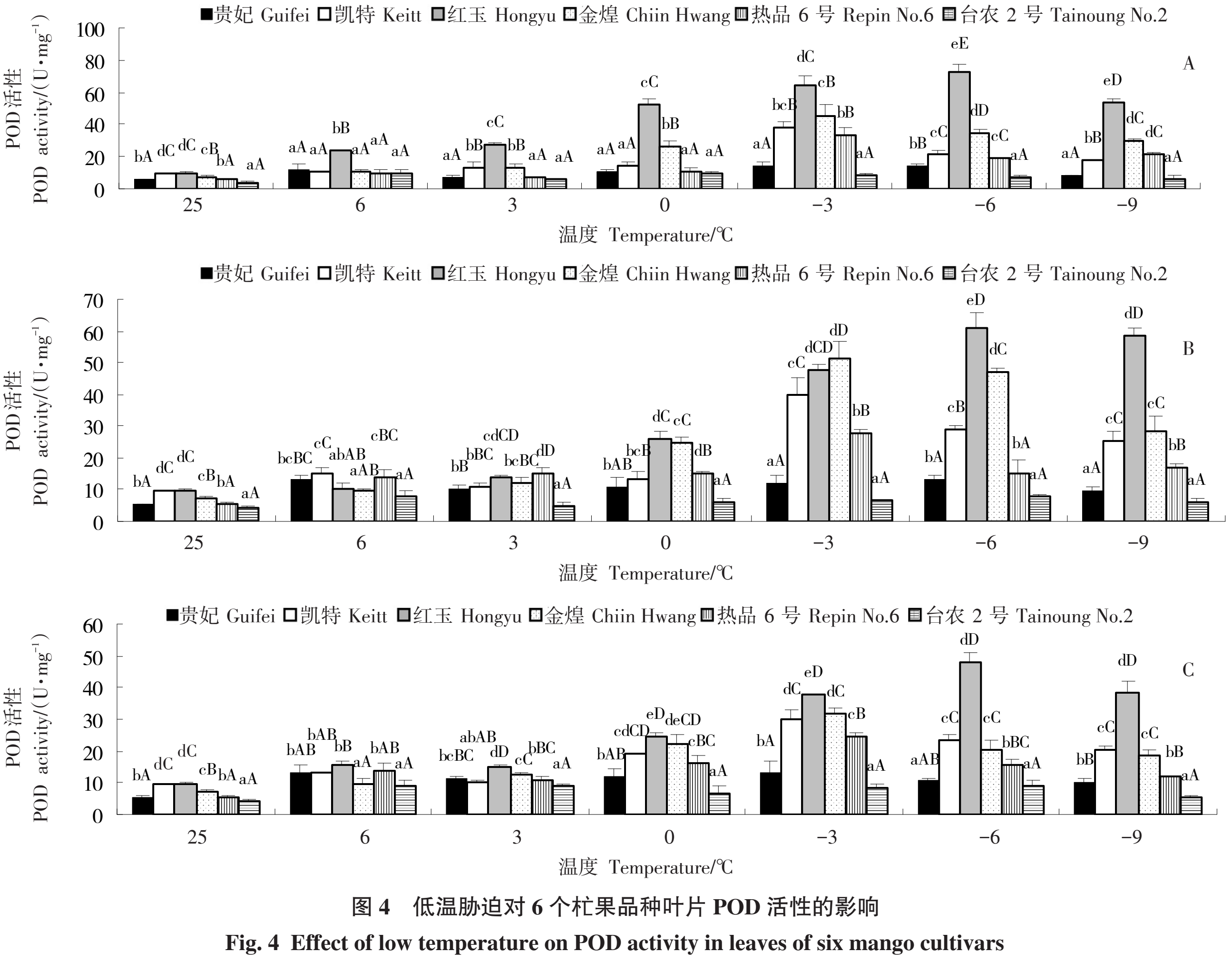


图4 低温胁迫对6个杧果品种叶片POD活性的影响

Fig. 4 Effect of low temperature on POD activity in leaves of six mango cultivars

2.6 低温对CAT活性的影响

经低温胁迫后,6个杧果品种叶片CAT活性均高于对照(图5)。随着温度的下降,杧果各品种叶片CAT活性呈先升高后降低的趋势,但不同品种CAT活性达到峰值的温度有所不同。‘贵妃’‘凯特’‘热品6号’和‘台农2号’均在0 ℃达到最高峰,而‘红玉’和‘金煌’则在温度降至-6 ℃时出现峰值。与POD相似,升幅最大与最小的品种分别‘红玉’和‘台农2号’,前者所有处理均显著高于对照。低温过程中品种间CAT活性有差异,‘红玉’9个处理的活性极显著高于其他品种,而‘台农2号’14个处理的活性为最低。

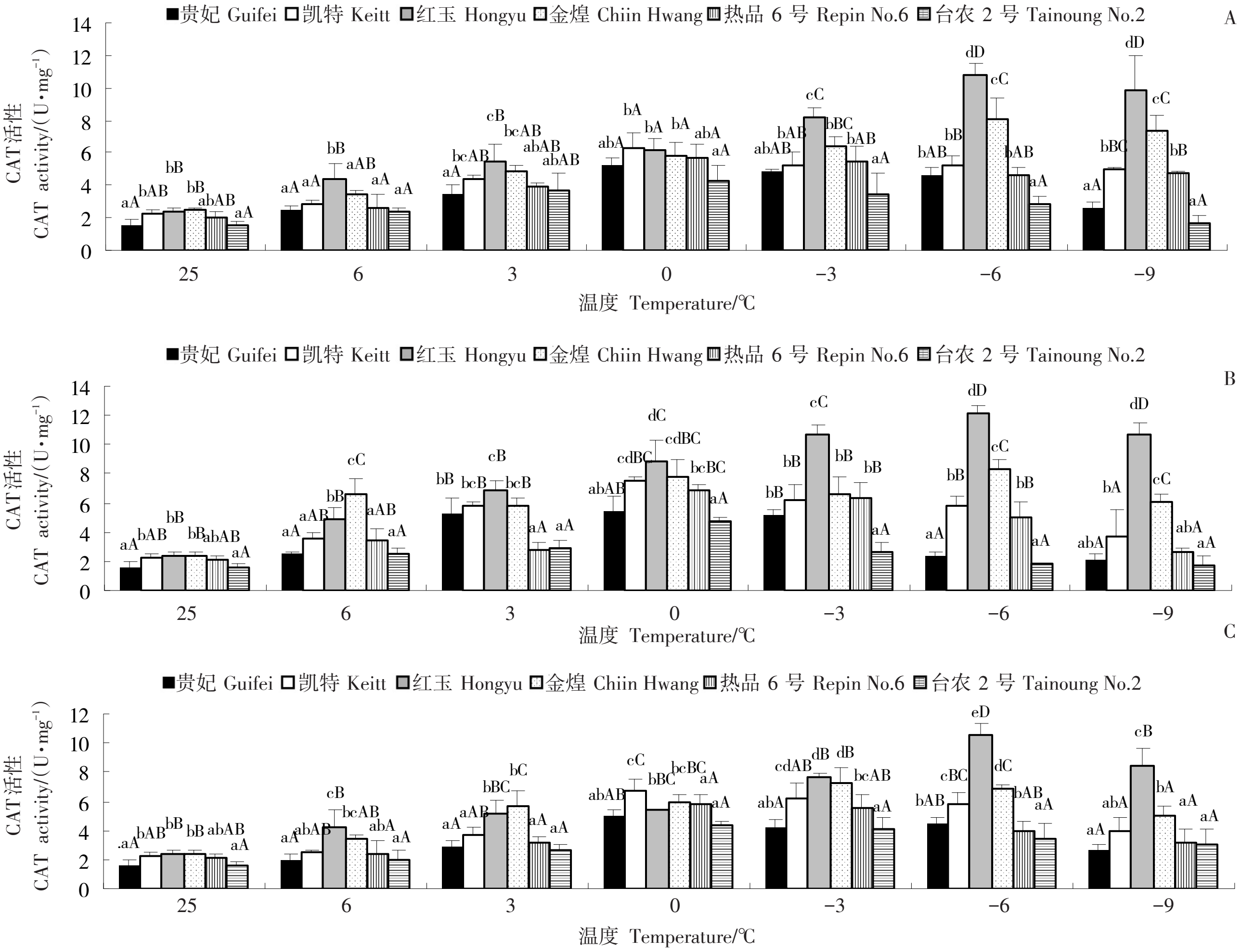


图 5　低温胁迫对 6 个杧果品种叶片 CAT 活性的影响

Fig. 5 Effect of low temperature on CAT activity in leaves of six mango cultivars

3 讨　论

电导率是间接鉴定植物耐寒性的一个重要指标，结合Logistic方程可获得植物的低温半致死温度。LT_{50}可用于判断品种间的耐寒性，近年来被用于草莓[19]、枣[20]、莲雾[21-22]、洋梨[23]、油桃[24]、核桃[25]等不同果树的耐寒性研究。本研究结果表明，杧果叶片电导率随温度下降而升高，但不同品种叶片电导率出现显著变化的处理温度和时间不同，这与品种的耐寒性相关。由得出的LT_{50}可知，6个杧果品种的耐寒性由强到弱依次为‘红玉’>‘金煌’>‘凯特’>‘热品6号’>‘贵妃’>‘台农2号’。其中‘红玉’比‘凯特’耐寒性强的结论与黄云等[4]的报道相符合。‘红玉’和‘金煌’的LT_{50}较低，与唐力生等[16]的结果相差较大，出现这种现象可能与品种、树龄、叶片成熟度有关。此外，笔者还在同等条件下测定了‘紫花杧果’的LT_{50}，为0.23 ℃，高于‘金煌’的LT_{50}，说明其耐寒性弱于‘金煌’，这与刘清国等[6]的观察结果一致。可见LT_{50}可作为一种衡量品种耐寒性的指标。

MDA含量高低可作为植物受逆境伤害程度的指标。在果树上的研究表明，随温度降低，植物叶片或枝条的MDA含量变化不同：草莓[19]、核桃[26]等呈现不断上升的趋势；南果梨[27]、燕山板栗[28]等上升后下降；龙眼[29]等下降后上升。本研究结果显示，所有杧果品种叶片MDA含量均随温度降低而升高，只是各品种MDA含量增加的幅度不同。相同处理时间下，与对照相比，‘贵妃’叶片MDA含量变化幅度最大，而‘台农2号’降温过程中其值总体（-9 ℃处理3 h时低于‘热品6号’）高于其他品种，这与本研究LT_{50}结果较一致，说明2者的耐寒性弱于其他品种。

SOD能特异性催化超氧阴离子（$O_2^{\cdot-}$），将其转化为H_2O_2，POD和CAT等可将H_2O_2转化为H_2O和O_2，从而抵御$O_2^{\cdot-}$和H_2O_2的毒害作用[30]，是植物体内自由基的有效清除剂，与植物抗逆性密切相关[31-33]。研

究表明在低温胁迫中,随温度降低,许多植物的SOD[26-27,33]、POD[24,34]和CAT[24,26]活性呈现升高后下降的趋势,活性上升是植物主动适应轻度胁迫的一种体现,而活性下降则与胁迫超出植物承受能力、酶自身遭受破坏有关[24]。本研究中不同杧果品种叶片3种保护酶总体上呈此变化,SOD活性到达峰值早,与赵蕾[35]和逯明辉等[36]的研究一致,说明SOD酶发挥作用主要是在受冷害的早期阶段。在低温胁迫期间POD和CAT活性升高的幅度高于SOD,但整体活性低于SOD,且耐寒性强的品种SOD均保持较高的活性,'红玉'极显著高于其他品种,'凯特'和'金煌'次之,这与LT_{50}结果一致,因而SOD酶活性的高低基本反映出了杧果品种间耐寒性的差异。

4 结 论

杧果叶片在低温胁迫过程中膜质过氧化加剧,REC、MDA含量升高,CAT活性先升后降,SOD和POD活性不同品种变化不同,品种间存在显著差异。6个杧果品种的耐寒性由强到弱依次为'红玉'>'金煌'>'凯特'>'热品6号'>'贵妃'>'台农2号'。

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