

陇东地区不同矮化中间砧对‘长富2号’苹果抗寒性的影响

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摘要:【目的】筛选在甘肃陇东地区适宜‘长富2号’苹果树体安全越冬的抗寒矮化中间砧, 并初步确立不同矮化中间砧‘长富2号’苹果抗寒性鉴定的综合评价方法。【方法】以9种不同矮化中间砧(SH₁、SC₁、SH₆、SH₃₈、M₂₆、M₇、M₉、T₃₃₇、JM₇)‘长富2号’(‘长富2号’/中间砧/新疆野苹果)的1 a(年)生枝条为试材, 采用人工模拟低温的方法, 测定不同温度(-15、-20、-25、-30、-35、-40 ℃)处理下枝条相对电导率(REC)、丙二醛(MDA)、可溶性糖、可溶性蛋白、花青苷含量以及超氧化物歧化酶(SOD)、过氧化物酶(POD)和过氧化氢酶(CAT)活性, 并分析各抗寒相关指标变化趋势。【结果】随着处理温度的降低, 9种中间砧‘长富2号’的REC、可溶性糖含量、MDA呈现上升趋势, 可溶性蛋白、花青苷含量以及SOD、POD和CAT活性呈现先上升后下降的趋势; 电导率结合Logistic方程计算出了各中间砧‘长富2号’半致死温度(LT₅₀), SH₆中间砧‘长富2号’LT₅₀最低, 为-35.293 ℃, JM₇中间砧‘长富2号’LT₅₀最高, 为-23.759 ℃; 主成分分析、隶属函数分析、聚类分析等多元方法成功应用于综合鉴定各中间砧‘长富2号’的抗寒性。【结论】不同中间砧‘长富2号’抗寒性可划分为3类: 第1类为SH₆、SH₁、SH₃₈、SC₁, 抗寒性最强; 第2类为M₇、M₂₆、M₉, 抗寒性较强; 第3类为JM₇、T₃₃₇, 抗寒性较弱。

关键词: ‘长富2号’苹果; 抗寒性; 矮化中间砧; LT₅₀; 主成分分析; 隶属函数法; 聚类分析

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Effects of different dwarfing interstocks on cold resistance of ‘Changfu 2’ apple in Longdong area

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Abstract: 【Objective】A study was conducted in attempting to screen the cold-resistant dwarf interstocks suitable for the ‘Changfu 2’ apple tree in the Longdong area of Gansu province, and to establish the method for evaluation of cold resistance of apples with different dwarfing interstocks. 【Methods】In this study, annual dormant branches from ‘Changfu 2’ (‘Changfu 2’/ interstocks / *Malus sieversii*) of nine dwarfing interstocks (SH₆, SH₃₈, SC₁, SH₁, M₂₆, M₉, M₇, T₃₃₇ and JM₇) were used as the experimental materials, which were even in size and free from diseases and pests. 8 physiological indexes, including relative electrical conductivity (REC), malondialdehyde (MDA) content, soluble sugar content, soluble protein content, anthocyanin content and superoxide dismutase (SOD) activity, peroxidase (POD) activity and catalase (CAT) activity of annual dormant branches were determined under different temperature treatments (-15, -20, -25, -30, -35, and -40 ℃), and the temperature at time of harvesting in the orchard was used as the control temperature. According to the change in each index in response to low temperature stresses, difference in cold resistance of ‘Changfu 2’ with nine dwarf interstocks was analyzed. 【Results】As the treatment temperature dropped, the relative electrical conductivity increased and

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showed a "slow-fast" trend. The relative conductivity of 'Changfu 2' / JM₇ / *Malus sieversii* was always the highest, while that of 'Changfu 2' / SH₆ / *Malus sieversii* was always the lowest. Based on REC, the semi-lethal temperatures (LT₅₀) of 'Changfu 2' with different interstocks were calculated by logistic equation, and the order of LT₅₀ was SH₆ > SH₃₈ > SC₁ > SH₁ > M₂₆ > M₉ > M₇ > T₃₃₇ > JM₇. With the decrease in temperature, the soluble sugar content of all interstock treatments showed an increasing trend, but there were differences among interstocks. 'Changfu 2' / SH₆ / *Malus sieversii* had the largest increase (196.99%); 'Changfu 2' / T₃₃₇ / *Malus sieversii* had the smallest increase (132.97%); the other seven had an increase between 142.46% and 174.97%. Soluble protein content in all treatments increased sharply and then decreased slowly, but with an overall rising trend with the decrease in temperature. The peak appeared at -30 °C, the highest peak was found in 'Changfu 2' / SH₆ / *Malus sieversii*, and the lowest peak in 'Changfu 2' / JM₇ / *Malus sieversii*. The variation pattern of anthocyanin content with temperature drop in the treatments was basically the same, increasing first and then decreasing. Except for 'Changfu 2' / T₃₃₇ / *Malus sieversii* which peaked at -20 °C, all other treatment peaked at -25 °C, and the peak values of each sample were in the rank of SC₁ > SH₆ > SH₃₈ > M₂₆ > SH₁ > M₉ > T₃₃₇ > M₇ > JM₇. Except for 'Changfu 2' / T₃₃₇ / *Malus sieversii*, 'Changfu 2' / M₂₆ / *Malus sieversii* and 'Changfu 2' / SH₁ / *Malus sieversii* whose MDA content decreased under treatment at -40 °C, all the treatments had an MDA content increasing with the decrease in temperature. The MDA contents 'Changfu 2' / T₃₃₇ / *Malus sieversii* and 'Changfu 2' / JM₇ / *Malus sieversii* were always higher than those of the other 7 treatments under various low temperatures; the contents of 'Changfu 2' / SH₃₈ / *Malus sieversii* and 'Changfu 2' / SH₆ / *Malus sieversii* increased steadily but remained at a low level. The activities of SOD, POD and CAT the nine treatments increased first but decreased later and the peak appeared at the critical temperature of -30 or -35 °C. Then, as the temperature continued to decrease, the degree of damage on the plant increased, and the enzyme activities decreased. All the 7 physiological indexes could reflect the cold resistance of 'Changfu 2' with different interstocks, but there were correlations among various physiological indicators, and the information reflected by the statistics overlapped to some extent. Therefore, in order to simplify the indicators and more accurately compare the differences in cold resistance between 'Changfu 2' of the nine interstocks, a principal evaluation system was established using principal component analysis. The principal component analysis of the original data showed that the two principal components were extracted from the eight physiological indicators with an eigenvalues of 5.832 and 1.128, respectively, and the contribution rates were 72.896% and 14.103%, respectively. The cumulative contribution rate was 86.998%, and the factor analysis results were ideal. A total of 86.998% of the total variance of the original variables was explained, covering most of the information of the original variables. The first factor reflected the soluble protein, antioxidant enzyme, and semi-lethal temperature, and the second factor soluble sugar and anthocyanin. Then, we used the membership function method to calculate the membership value of each comprehensive index, and the weights of the two comprehensive indicators were obtained according to the contribution rate of the comprehensive index, and were 0.838 and 0.162 respectively. Using the formula, the D value of each intermediate anvil Fuji was obtained. The D value was a comprehensive evaluation value of cold resistance, reflecting the difference in cold resistance of each intermediate anvil Fuji. The higher the D value, the stronger the cold resistance. Among the nine interstock, the D value of SH₆ was the highest, 0.947, and the cold resistance was the strongest; the D value of JM₇ was the lowest, 0.140, and the cold resistance was the weakest. The D values of the interstock was clustered by the inter-group connection method, and the clustering tree diagram was established. 【Conclusion】The results showed that the nine

interstocks were classified into three categories according to the cold resistance. The first type included SH₆, SH₁, SH₃₈ and SC₁, which had the strongest cold resistance; the second type consisted of M₇, M₂₆ and M₉, which had strong cold resistance; and the third type included JM₇ and T₃₃₇, which had weak cold resistance.

Key words: ‘Changfu 2’ apple; Cold resistance; Dwarfing rootstock; LT₅₀; Principal component analysis; Membership function method; Cluster analysis

矮化砧栽培是世界苹果栽培技术的主要方向,我国苹果主产区矮砧栽培多以矮化中间砧栽培为主^[1-2]。甘肃陇东地区是西北黄土高原苹果优势产业带中最具发展潜力的新兴苹果产区^[3]。该地区苹果栽培现仍以乔砧密植为主,矮化中间砧栽培技术体系尚不成熟^[4]。砧木抗性差、砧穗组合不合理、冬季干寒的气候已成为限制陇东地区苹果产业发展的关键因素。筛选出适合陇东地区的苹果抗寒矮化中间砧,对陇东苹果产业的健康持续发展具有重要的实践意义。

我国北方苹果产区冻害频发,当地苹果产业因此蒙受巨大经济损失,温度已成为限制苹果地理位置分布的主要环境因子之一^[5-6]。砧木对果树接穗品种抗逆性影响明显,选用适宜的砧木能提高树体的抗寒性^[7-8]。我国引进的苹果矮化中间砧多为国外培育的M系和MM系,从国外引入的这些矮化砧木,存在抗寒力较差、适应能力不强的缺点,在我国北部的苹果产区有不同程度的冻害发生^[9]。因此,进行砧木区域化试验进而筛选出适宜当地的抗寒矮化中间砧对于苹果产业提质增效很有必要。目前,关于冻害发生机制,大量研究均表明,细胞膜系统是植物冻害发生的主要部位^[10-11],低温引起细胞组织结冰导致了细胞膜结构的损伤,从而引起植物组织的损坏和死亡^[10, 12-13]。因此,维持细胞膜结构的稳定是植物抵御寒冷的关键,植物在响应低温逆境过程中,通过调节渗透物质的积累、抗氧化酶的产生、激素代谢的改变等途径防止膜损伤来提高其抗寒性^[14-16]。关于园艺作物抗寒性鉴定的方法已有不少报道:电导法作为植物抗寒性鉴定的常用方法,已广泛应用于苹果^[17-19]、杏^[20]、梨^[21-22]、葡萄^[23]等果树的抗寒性测定。高爱农等^[17]利用电导法结合 Logistic 方程,求出 51 个苹果品种的半致死温度并以此鉴定抗寒性,结论与恢复生长法、组织褐变法试验所得结论一致。王玮等^[24]对 7 个优良梨品种进行不同低温

处理,结合低温冻害调查结果验证求得的各品种半致死温度的正确性,结果表明半致死温度与其相近温度处理的枝条冻害指数呈显著负相关,从而确定半致死温度能准确反映梨品种的抗寒力。李翠红等^[9]测定不同低温处理下 8 个矮化中间砧一年生枝条砧木电导率,游离脯氨酸、可溶性糖、蛋白质含量,以及丙二醛、SOD、POD 酶活性等生理指标的变化,发现抗寒性与游离脯氨酸含量、可溶性蛋白质含量、可溶性糖含量、POD 和 SOD 活性存在极显著的正相关。梅立新等^[25]认为电导率、丙二醛含量和 SOD 酶活性可作为砧木早期抗寒性的实验室综合鉴定指标,能有效鉴定苹果矮化砧木的抗寒性。由于果树的抗寒性是多个数量和质量性状共同决定的,单一指标评价苹果抗寒性是片面的,多指标多种方法综合评价才能更准确地鉴定其抗寒性^[26]。WANG 等^[27]利用主成分分析法评估了 9 种苹果矮化中间砧的抗寒性,而关于陇东地区主栽品种富士还没有进行系统的砧木区域化抗寒性鉴定实验,针对以上问题,笔者以甘肃陇东地区 9 种不同矮化中间砧‘长富 2 号’一年生枝条为实验材料,通过测定 8 种生理指标,并利用主成分分析法、隶属函数法、聚类分析法综合评价 9 种不同矮化中间砧‘长富 2 号’的抗寒性差异,筛选出适合陇东地区‘长富 2 号’生长的抗寒矮化中间砧,为建立抗寒性鉴定评价方法提供依据。

1 材料和方法

1.1 试验地点

本试验于 2019 年 1 月在甘肃省庆城县苹果试验示范站(36°0′13″N, 107°54′56″E)进行。此地位于陇东黄土高原中部地带,属温带大陆性季风气候,海拔 1 285 m,年均降雨量 507 mm,年平均气温 9.4℃,冬天极端低温可达-25.1℃,年均无霜期 166 d,年均日照时数 2 420 h。黄绵土,土壤 pH 7.4~8.4,

果园土壤肥力均匀,行内清耕并用黑色地布覆盖,行间种植三叶草,施肥、灌水及病虫害防控与常规管理一致。

1.2 试验材料

供试试验品种为5 a(年)生晚熟富士品系‘长富2号’,基础为新疆野苹果,供试的9种矮化中间砧分别是SH₁、SH₆、SH₃₈、SC₁、M₂₆、M₉、T₃₃₇、M₇、JM₇。行株距为4 m×2 m,授粉品种为‘金世纪’,9种中间砧嫁接长度均为25 cm,起垄后露出地面长度均为10 cm,树形采用细长纺锤形。试验采用随机区组设计,选取生长发育正常一致的植株为试材,3株为1次重复,每处理3次重复。

1.3 试验采样

2019年1月4日苹果树体进入深度休眠期,采集每株试验树中部东南西北各方位的长势良好且成熟度、粗度一致的1 a生春梢枝条,每品种采集50枝,采集的枝条剪口处用石蜡封闭,并用湿报纸完全包裹,裹上保鲜膜后立即带回实验室,进行后续处理。

1.4 低温处理

将供试的枝条用蒸馏水冲洗干净,并用吸水纸擦干,然后将枝条分割成段,每段长约15 cm,石蜡封闭剪口。每种中间砧‘长富2号’枝条分7份,每份约12段,纱布包裹,置于超低温冰箱模拟低温处理。分别在-15、-20、-25、-30、-35、-40℃不同温度梯度下模拟低温处理,并以田间采集的未做处理枝条为对照(田间温度(-10±1)℃)。起始温度为0℃,低温处理以4℃·h⁻¹的速率降至目的温度后持续12 h,之后再以4℃·h⁻¹升温至0℃放置12 h进行解冻,随后进行相关指标的测定。

1.5 测定方法

相对电导率的测定参考潘晓云等^[28]的方法,可溶性糖含量的测定采用丁雪梅等^[29]的改进方法,花青苷的提取和含量测定参照谢兴斌^[30]的方法,可溶性蛋白的提取和含量测定参照邓丽莉等^[31]的优化方法,丙二醛(MDA)含量的测定采用硫代巴比妥酸(TBA)法^[32]、超氧化物歧化酶(SOD)活性的测定采用氮蓝四唑光化还原法^[32]、过氧化物酶(POD)活性的测定采用愈创木酚法^[32]、过氧化氢酶(CAT)活性的测定采用紫外吸收法^[32]。

1.6 半致死温度的计算

对不同低温处理下休眠枝的电解质渗出率进

行logistic回归分析,利用曲线的拐点所对应温度作为半致死温度(LT₅₀)。Logistic方程的表达式为: $y = k/(1 + ae^{-bt})$,其中y代表细胞伤害率,t代表处理温度,k为细胞伤害率的饱和容量,a、b为方程参数, $LT_{50} = -(\ln a/b)$ 。

1.7 数据统计分析

1.7.1 主成分分析 依据数据降维算法进行主成分分析,将原来多个相关的指标转换成新的少数几个互不相关的综合指标,并建立综合指标F_j的得分函数: $F_j = \sum_{i=1}^m a_{ij} \times x_i$ (i=1, 2, …, m, j=1, 2, …, n, n≤m) (1)。

1.7.2 隶属函数分析 利用隶属函数法综合评价不同中间砧富士抗寒性差异。计算公式如下:

$$D = \sum_{i=1}^m [u(x_i) \times (r_j / \sum_{j=1}^n |r_j|)], \quad (2)$$

$$\text{其中: } u(x_j) = (x_j - x_{\min}) / (x_{\max} - x_{\min})。 \quad (3)$$

x_j表示第j个指标值;x_{min}表示某中间砧富士第j个综合指标的最小值;x_{max}表示第j个综合指标的最大值;D为各中间砧富士用综合指标评价的抗寒性度量值;r_j为各中间砧富士第j个综合指标的贡献率;u(x_j)为第j个综合指标的隶属函数值;r_j/∑_{j=1}ⁿ |r_j|为各综合指标的权重,表示第j个综合指标在所有综合指标的重要程度。

1.7.3 聚类分析 对各中间砧富士的D值进行聚类,根据聚类结果将9种不同矮化中间砧富士的抗寒性进行分类。

采用Excel 2010、SPSS 23.0对数据进行分析处理。

2 结果与分析

2.1 低温胁迫对不同中间砧‘长富2号’电解质渗透率的影响及半致死温度计算

如图1所示,以田间采集[田间温度(-10±1)℃]未低温处理的‘长富2号’枝条为对照,在-15~-40℃下处理不同中间砧‘长富2号’1 a生枝条,随着处理温度下降,不同中间砧‘长富2号’枝条的相对电导率持续增加,并呈现出“慢-快”的趋势。在-15~-20℃低温处理后,所有不同中间砧‘长富2号’枝条电导率缓慢递增,在-25℃及以下低温处理后,中间砧JM₇、T₃₃₇、M₇、M₉、M₂₆‘长富2号’枝条电导率急剧升高,在-30℃及以下低温处理后,中间砧SH₆、SH₃₈、SC₁、SH₁‘长富2号’枝条电导率

开始急剧升高。中间砧 JM₇‘长富 2 号’枝条的相对电导率始终高于其他中间砧‘长富 2 号’枝条,而中间砧 SH₆‘长富 2 号’枝条的相对电导率始终低于同低温处理下的其他中间砧‘长富 2 号’。结合 Logistic 方程,对在不同低温处理下的各中间砧‘长富 2 号’枝条的相对电导率进行回归分析,计算出了各中间砧‘长富 2 号’的半致死温度,相关数据如表 1 所示。表中所有回归方程的相关系数在 0.927~0.983, *F* 值检验表明,各回归方程的拟合值均极显著,说明不同温度对各中间砧‘长富 2 号’相对电导率的影响符合 Logistic 方程变化规律,并且拟合结果可靠,精确度较高。从表中数据可得,各中间砧‘长富 2 号’半致死温度为-23.7593~-35.2927 °C,‘长富 2 号’LT₅₀在-30 °C 以上的中间砧有:SH₆、SH₁、SH₃₈、SC₁;在-25~-30 °C 的有:M₂₆、M₉、M₇、T₃₃₇; -25 °C 以下的是 JM₇。

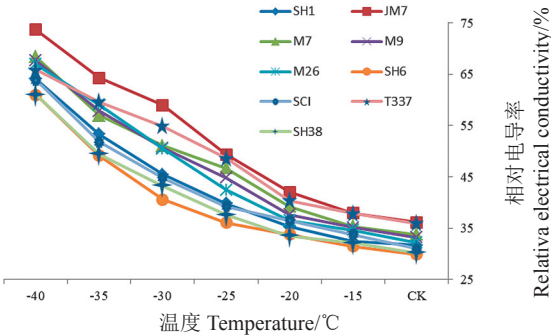


图1 低温胁迫对9种中间砧‘长富2号’REC的影响
Fig.1 Effect of low temperature stresses on REC of ‘Changfu 2’ apple with nine interstocks

表1 拟合方程和半致死温度(LT₅₀)
Table 1 Logistic equation and semi-lethal low temperature (LT₅₀)

中间砧 Interstocks	拟合方程 Logistic equation	半致死温度 Semi-lethal low temperature/°C	拟合度 R ²
SH ₁	$y=100/(1\pm4.0796e^{-0.045x})$	-31.244	0.958**
SH ₆	$y=100/(1\pm4.2503e^{-0.041x})$	-35.293	0.927**
SH ₃₈	$y=100/(1\pm4.1083e^{-0.041x})$	-34.463	0.950**
SC ₁	$y=100/(1\pm3.9393e^{-0.043x})$	-31.884	0.959**
M ₂₆	$y=100/(1\pm4.1041e^{-0.05x})$	-28.240	0.975**
M ₉	$y=100/(1\pm3.8152e^{-0.048x})$	-27.896	0.980**
M ₇	$y=100/(1\pm3.6147e^{-0.047x})$	-27.340	0.982**
JM ₇	$y=100/(1\pm3.6074e^{-0.054x})$	-23.759	0.983**
T ₃₃₇	$y=100/(1\pm3.0864e^{-0.043x})$	-26.209	0.980**

注:**表示在 *p* < 0.01 水平上差异显著。
Note:** indicate significant difference at *p* < 0.01.

2.2 低温胁迫对不同中间砧‘长富2号’可溶性糖含量的影响

随处理温度的降低,各中间砧‘长富 2 号’可溶性糖含量总体上呈现出增加的趋势,但不同中间砧‘长富 2 号’之间的变化存在差异(图 2)。随处理温度的降低,SH₆、SC₁、SH₃₈中间砧‘长富 2 号’可溶性糖含量持续快速增加;SH₁、M₂₆、JM₇、M₉中间砧‘长富 2 号’呈现先快速上升后缓慢下降的趋势,SH₁、M₂₆、JM₇中间砧‘长富 2 号’在-30 °C 达到峰值,M₉中间砧富士在-25 °C 达到峰值;T₃₃₇中间砧‘长富 2 号’平缓上升并在-30 °C 保持稳定;M₇中间砧‘长富 2 号’呈现“S”形上升的趋势。其中,SH₆中间砧‘长富 2 号’涨幅最大,达 196.99%;T₃₃₇中间砧‘长富 2 号’涨幅最小,为 132.97%;其他 7 种供试样品涨幅为 142.46%~174.97%。

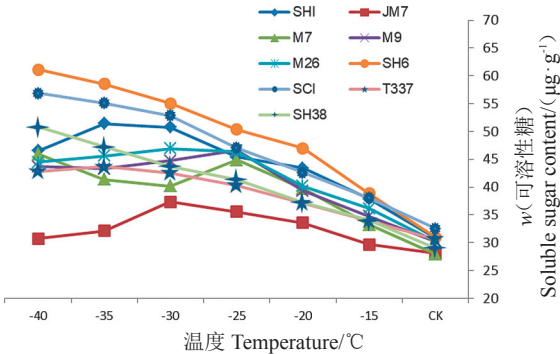


图2 低温胁迫对9种中间砧‘长富2号’可溶性糖含量的影响
Fig.2 Effect of low temperature stresses on soluble sugar content in ‘Changfu 2’ apple with nine interstocks

2.3 低温胁迫对不同中间砧‘长富2号’花青苷含量的影响

随着处理温度的降低,各中间砧‘长富 2 号’枝条花青苷含量变化趋势基本一致,都呈现出先增加后减少的趋势(图 3)。其中,在未进行低温处理前,各供试材料花青苷含量有所差异,如图中对照所示,M₂₆、SC₁、SH₆、SH₃₈中间砧‘长富 2 号’含量较高,均在 38 μg·g⁻¹ 以上;SH₁、T₃₃₇中间砧‘长富 2 号’次之,含量为 35.027 7 μg·g⁻¹ 和 36.034 1 μg·g⁻¹;JM₇、M₇、M₉中间砧‘长富 2 号’含量较低,均在 35 μg·g⁻¹ 以下。除 T₃₃₇中间砧‘长富 2 号’在-20 °C 达到峰值外,其他所有中间砧‘长富 2 号’均在-25 °C 达到峰值,各样品峰值从大到小依次排列为:SC₁>SH₆>SH₃₈>M₂₆>SH₁>M₉>T₃₃₇>M₇>JM₇。-25 °C 以下温度时,各中间砧富士花青苷含量不断下降。

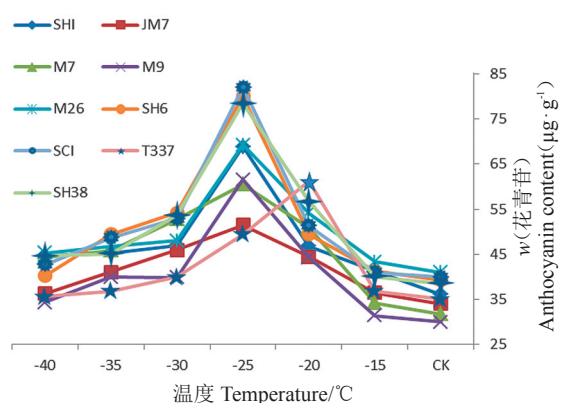


图3 低温胁迫对9种中间砧‘长富2号’花青苷含量的影响

Fig. 3 Effect of low temperature stresses on anthocyanin content in ‘Changfu 2’ apple with nine interstocks

2.4 低温胁迫对不同中间砧‘长富2号’可溶性蛋白含量的影响

随着处理温度的下降,9种中间砧‘长富2号’枝条含量都先大幅上升后缓慢下降,但整体仍呈上升趋势(图4)。供试的9种枝条在-15~-30℃的低温处理下,可溶性蛋白含量逐渐上升,在-30℃下达到了最高值,处理温度继续下降,可溶性蛋白含量也开始下降。供试的各砧木‘长富2号’可溶性蛋白含量变化趋势一致,但变化幅度存在差异。在-30℃低温处理下,SH₆、SC₁、SH₁中间砧‘长富2号’可溶性蛋白含量达到对照的1.85、1.82、1.75倍,其他中间砧‘长富2号’则是其对照的1.44~1.57倍。其中,增长幅度最大的是SH₆中间砧‘长富2号’,增长幅度最小的是JM₇中间砧‘长富2号’。

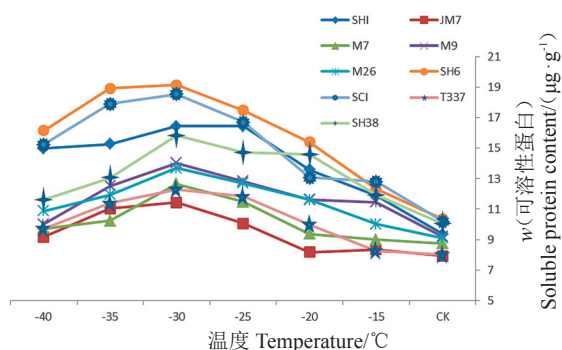


图4 低温胁迫对9种中间砧‘长富2号’可溶性蛋白含量的影响

Fig. 4 Effect of low temperature stresses on soluble protein content in ‘Changfu 2’ apple with nine interstocks

2.5 低温胁迫对不同中间砧‘长富2号’的丙二醛(MDA)含量的影响

除 T₃₃₇、M₂₆、SH₁中间砧‘长富2号’在-40℃处

理后,枝条MDA含量下降外,其他不同中间砧‘长富2号’枝条随着处理温度的降低,MDA含量呈增加趋势(图5)。T₃₃₇和JM₇中间砧‘长富2号’MDA含量在-20℃处理时开始骤增,并且在各个低温处理下MDA含量始终高于其他7种供试样品;M₂₆、M₇、M₉、SH₁、SC₁中间砧‘长富2号’枝条在-25℃处理时,MDA含量开始快速升高;SH₃₈中间砧‘长富2号’在-35℃时MDA含量升高速率开始变大;SH₆中间砧‘长富2号’始终保持平缓上升。

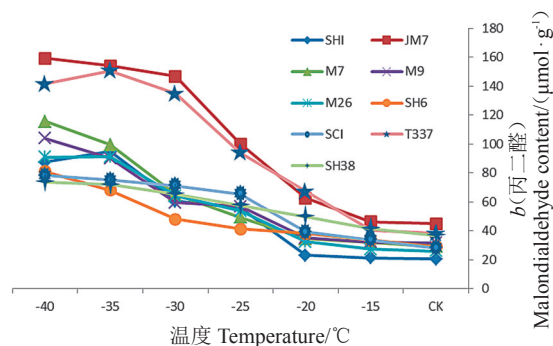


图5 低温胁迫对9种中间砧‘长富2号’丙二醛含量的影响

Fig. 5 Effect of low temperature stresses on malondialdehyde content in ‘Changfu 2’ apple with nine interstocks

2.6 低温胁迫对不同中间砧‘长富2号’超氧化物歧化酶(SOD)活性的影响

随着处理温度的降低,各中间砧‘长富2号’枝条SOD酶活性变化基本一致,都呈现先升高后降低的趋势(图6)。其中,M₉、M₂₆、M₇、T₃₃₇、JM₇中间砧‘长富2号’在-30℃处理下,SOD酶快速升高,达到峰值后开始降低,SH₆、SH₃₈、SC₁、SH₁中间砧‘长富2号’在-35℃处理下SOD酶活性快速升高,随后开始降低。中间砧JM₇‘长富2号’枝条的SOD酶活性峰值最低,而SH₆中间砧‘长富2号’峰值最高。

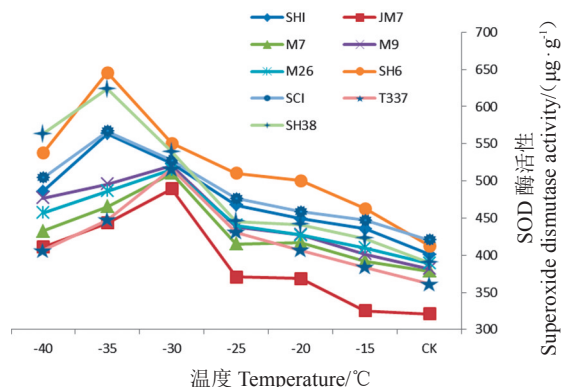


图6 低温胁迫对9种中间砧‘长富2号’SOD酶活性的影响

Fig. 6 Effect of low temperature stresses on superoxide activity in ‘Changfu 2’ apple with nine interstocks

2.7 低温胁迫对不同中间砧‘长富2号’的过氧化物酶(POD)活性的影响

随着处理温度的降低,9种中间砧‘长富2号’枝条 POD 酶活性都呈现先升高后降低的趋势(图7)。POD 酶活性的最高值出现在-35℃,各供试样品峰值从大到小排列为:SC₁>SH₆>SH₃₈>SH₁>M₂₆>M₇>M₉>JM₇>T₃₃₇。虽然各样品 POD 酶活性变化趋势基本一致,但是,变化幅度有所差异。SC₁、SH₆、SH₃₈中间砧‘长富2号’涨幅最大,分别为203.83%、193.67%、185.11%;SH₁、M₇、M₂₆中间砧‘长富2号’次之,涨幅分别为174.58%、168.81%、166.10%;T₃₃₇、JM₇中间砧‘长富2号’增长幅度最小,分别为153.03%、140.22%。

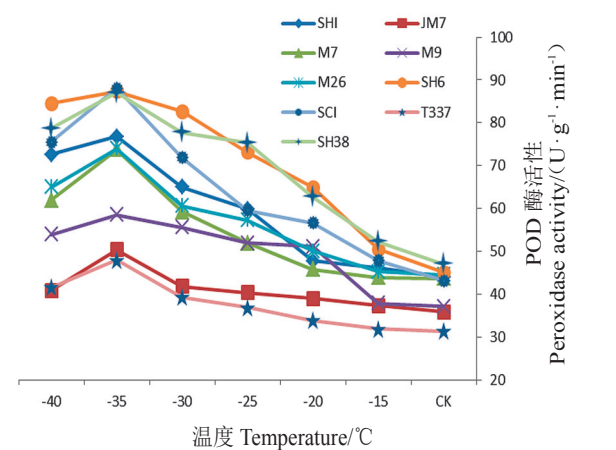


图7 低温胁迫对9种中间砧‘长富2号’POD含量的影响
Fig. 7 Effect of low temperature stresses on peroxidase activity in ‘Changfu 2’ apple with nine interstocks

幅度最小,峰值为对照的3.34,其他砧木的增长幅度在3.74~5.69倍。

2.8 低温胁迫对不同中间砧富士的过氧化氢酶(CAT)活性的影响

随着处理温度的降低,9种中间砧‘长富2号’枝条中 CAT 酶活性变化趋势基本一致,都呈先增大后降低的“单峰型”曲线,但各中间砧‘长富2号’的 CAT 活性达到峰值时的处理温度和变化幅度存在差异(图8)。SH₆、SH₃₈、SH₁、SC₁中间砧‘长富2号’在-35℃处理时 CAT 含量到达峰值,而 M₂₆、M₇、M₉、T₃₃₇、JM₇中间砧‘长富2号’在-30℃处理时, CAT 酶活性快速增加达到峰值,随后活性开始降低。经低温处理后,9种中间砧‘长富2号’枝条中 CAT 酶活性的增长量存在差异,SH₃₈中间砧‘长富2号’增长幅度最大,峰值为对照的6.71倍, JM₇增长

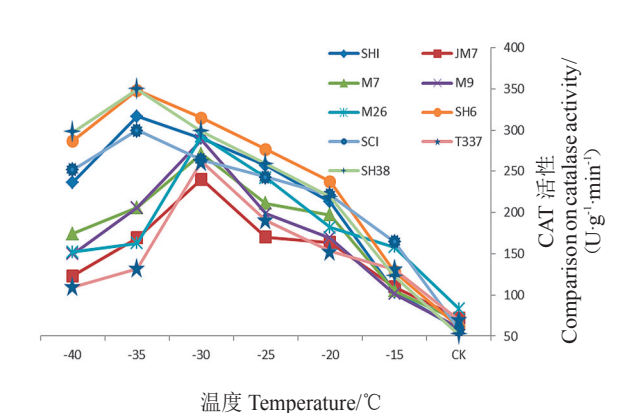


图8 低温胁迫对9种中间砧‘长富2号’CAT含量的影响
Fig. 8 Effect of low temperature stresses on Comparison on catalase activity in ‘Changfu 2’ apple with nine interstocks

对原始变量进行主成分分析,分析结果如表2

表2 解释的总方差
Table 2 Total variance explained

成分 Component	初始特征值 Initial eigenvalues			提取平方和载入 Extraction sums of squared loadings		
	特征值 Eigenvalue	方差 Variance/%	累积率 Cumulative/%	特征值 Eigenvalue	方差 Variance/%	累积率 Cumulative/%
1	5.832	72.896	72.896	5.832	72.896	72.896
2	1.128	14.103	86.998	1.128	14.103	86.998
3	0.546	6.827	93.825			
4	0.299	3.735	97.560			
5	0.160	2.000	99.560			
6	0.021	0.259	99.819			
7	0.008	0.097	99.917			
8	0.007	0.083	100.000			

所示,遵循特征值大于 1 的原则,从 8 项生理指标中共提取出 2 个主成分,特征值分别为 5.832、1.128,贡献率分别为 72.896%、14.103%,累积贡献率达 86.998%,因子分析结果理想,共解释了原有变量总方差的 86.998%,提取出的 2 个综合指标概括了 8 个生理指标的绝大部分信息。

成分得分系数矩阵如表 3 所示,第 1 主成分中主要解释了可溶性蛋白、抗氧化酶、半致死温度这些指标,其特征向量的绝对值较大,所占信息为总信息的 72.896%。第 2 主成分中,可溶性糖、花青苷这两个指标的特征向量绝对值最大,分别达 0.393、0.865,第 2 主成分主要解释了与渗透调节物质相关的变量,所占信息为总信息的 14.103%。

根据成分得分系数矩阵,可得出各综合指标得分函数:

$$F1=0.349X_1 + 0.379X_2 + 0.131X_3 + 0.388X_4 + 0.397X_5+0.357X_6-0.349X_7-0.400X_8$$

$$F2=-0.393X_1-0.212X_2+0.865X_3-0.088X_4+0.177X_5+0.103X_6-0.035X_7-0.047X_8$$

表 3 综合指标的系数及贡献率

Table 3 Comprehensive indicator score coefficient matrix									
	X_1	X_2	X_3	X_4	X_5	X_6	X_7	X_8	P
F_1	0.349	0.379	0.131	0.388	0.397	0.357	-0.349	-0.400	0.729
F_2	-0.393	-0.212	0.865	-0.088	0.177	0.103	-0.035	-0.047	0.141

注: X_1 : 可溶性糖; X_2 : 可溶性蛋白; X_3 : 花青苷; X_4 : 超氧化物歧化酶; X_5 : 过氧化物酶; X_6 : 过氧化氢酶; X_7 : 丙二醛; X_8 : 半致死温度; P : 贡献率。

Note: X_1 : Soluble suger; X_2 : Soluble protein; X_3 : Anthocyanin; X_4 : Superoxide dismutase; X_5 : Peroxidase; X_6 : Catalase; X_7 : Malondialdehyde; X_8 : Semi-lethal low temperature; P : Contribution.

2.10 隶属函数分析

利用主成分分析法,提取出能反映所有原始变量绝大部分信息的 2 个综合指标,根据公式(3)计算出各综合指标的隶属函数值(表 4),再根据综合指标的贡献率,求出了 2 个综合指标的权重,分别为 0.838,0.162(表 4)。利用公式(2)得到了各中间砧‘长富 2 号’的 D 值,D 值是抗寒性的综合评价价值,反映了各中间砧‘长富 2 号’的抗寒性差异,D 值越高,抗寒性越强。9 种中间砧‘长富 2 号’中,SH₆ D 值最高,为 0.947,抗寒性最强;JM₇ D 值最低,为 0.140,抗寒性最弱。

2.11 聚类分析

采用组间连接法对各中间砧‘长富 2 号’的 D

表 4 9 种中间砧‘长富 2 号’的综合指标值、权重、隶属函数值、D 值及综合评价

Table 4 The comprehensive index value, weight, membership function value, D value and comprehensive evaluation of ‘Changfu 2’ apple with nine interstocks

中间砧 Interstocks	x_1	x_2	$U(x_1)$	$U(x_2)$	D 值 D value	抗寒性 Cold resistance
SH ₁	1.130	-0.429	0.649	0.359	0.602	较强 Strong
JM ₇	-4.046	0.945	0.000	0.866	0.140	较弱 Weak
M ₇	-1.082	1.309	0.372	1.000	0.473	适中 Moderate
M ₉	-0.404	-1.078	0.457	0.121	0.402	适中 Moderate
M ₂₆	-0.064	0.161	0.499	0.577	0.512	适中 Moderate
SH ₆	3.930	0.423	1.000	0.674	0.947	最强 Strongest
SC ₁	1.283	-1.406	0.668	0.000	0.559	较强 Strong
T ₃₃₇	-2.642	-1.165	0.176	0.089	0.162	较弱 Weak
SH ₃₈	1.895	-1.241	0.745	0.061	0.634	较强 Strong
权重 Weight	-	-	0.838	0.162	-	-

值进行聚类分析,建立了聚类树状图(图 9)。聚类分析结果显示 9 种中间砧‘长富 2 号’材料划分为 4 类:第 1 类为 SH₆;第 2 类为 SH₁、SH₃₈、SC₁;第 3 类为 M₇、M₂₆、M₉;第 4 类为 JM₇、T₃₃₇。

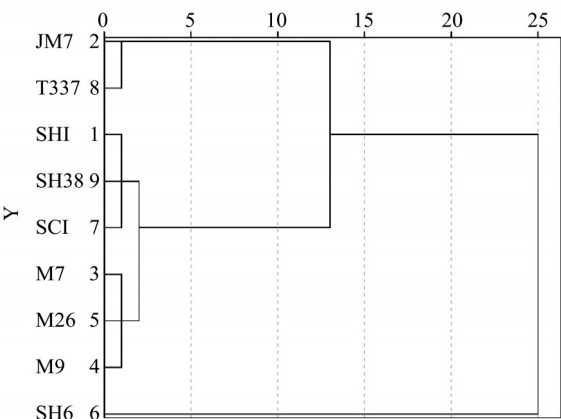


图 9 9 种中间砧‘长富 2 号’抗寒性的聚类分析

Fig. 9 Cluster diagram of ‘Changfu 2’ apple with nine interstocks

3 讨 论

大量研究表明,细胞膜系统是植物冻害发生的主要部位^[10]。植物受到低温胁迫时,一方面膜脂物相发生改变,由液晶相变为凝胶相,膜结构破坏、通透性增强;另一方面细胞内外结冰,造成了质膜的机械损伤,膜系统被破坏,同时与膜结合的功能蛋白(酶)活性丧失。导致细胞内一系列生理、代谢功能的紊乱,造成植株的伤害和死亡^[33-34]。通过电导法测定电解质渗出率的变化可反映细胞及膜系统受损伤的程度^[35]。本试验结果表明随着处理温度的

降低,各中间砧‘长富2号’枝条电解质渗透率持续升高,受伤程度加深,这与刘贝贝等^[36]和曹建东等^[37]研究结果一致。Whitlow等^[38]、孙程旭等^[39]和卜庆雁等^[40]等认为低温胁迫下电解质渗透率可作为判断植物抗寒性的可靠办法。本试验结果表明,‘长富2号’/SH₆/新疆野苹果电解质渗透率增长最缓,抗寒性最强;‘长富2号’/JM₇/新疆野苹果电解质渗透率增长最快,抗寒性最差。

半致死温度(LT₅₀)是目前广泛应用于评价植物抗寒性的重要指标^[41-43]。在本研究中,不同中间砧‘长富2号’的半致死温度的排列顺序为:‘长富2号’/SH₆/新疆野苹果>‘长富2号’/SH₃₈/新疆野苹果>‘长富2号’/SC₁/新疆野苹果>‘长富2号’/SH₁/新疆野苹果>‘长富2号’/M₂₆/新疆野苹果>‘长富2号’/M₉/新疆野苹果>‘长富2号’/M₇/新疆野苹果>‘长富2号’/T₃₃₇/新疆野苹果>‘长富2号’/JM₇/新疆野苹果。这与Wang等^[27]对不同砧木半致死温度的大小排列的估算结果极为接近,其LT₅₀排列次序为:SH₃₈>SH₆>SH₁>SC₁>M₇>M₂₆>JM₇>T₃₃₇>M₉。可以发现,抗寒性较强的砧木,其嫁接的接穗也表现出较强的抗寒性。SH系砧木及其嫁接‘长富2号’的LT₅₀都普遍高于M系或其他砧木及其嫁接‘长富2号’。这也反映出砧木对接穗抗寒性的影响极为重要,但可能由于砧穗间的互作、亲和性或立地条件等差异,砧木并不能绝对决定接穗品种的抗寒性。

低温胁迫下,植物体内通过生物合成或吸收来积累渗透调节物质,提高胞内溶质的浓度,降低胞内渗透势,从而减少脱水结冰来增强植物体抵御低温的能力^[35,44]。可溶性糖作为植物细胞的重要渗透调节物质,通过2个方面来提高植物的抗寒性,一方面胞内可溶性糖的积累,细胞液浓度升高,胞内渗透势降低,提高保水能力,冰点下降;另一方面可溶性糖还是冰的保护剂,对细胞的生命物质和细胞膜起保护作用^[45-46]。在本研究中,随着处理温度的降低,可溶性糖含量总体都呈上升趋势,不同中间砧‘长富2号’在低温胁迫下的可溶性糖含量及增幅与抗寒性呈正相关关系,该研究中SH系中间砧‘长富2号’可溶性糖含量和增幅都呈现较高水平,可溶性糖含量高,抗寒性强,可溶性糖增长幅度大,抗寒性强。这与Wang等^[27]在苹果砧木上的研究一致,同时,这一点也被曹建东等^[37]在葡萄上的研

究证实。花青苷在低温等逆境下发挥着重要作用^[47-48]。Leng等^[49]、卜庆雁等^[40]认为,植物1a生枝皮层内花青苷含量可作为植物抗寒性鉴定的有效办法。在本研究中,-25℃低温胁迫下,各中间砧‘长富2号’花青苷含量都达到峰值,峰值越高,抗寒性越强。可溶性蛋白的亲水性较强,植物细胞内含量增大时,不仅可以降低组织和细胞的冰点温度,还能增强细胞的保水能力,避免低温胁迫下原生质的严重脱水而结冰致死^[37]。在本试验中,低温胁迫下,9种不同中间砧‘长富2号’可溶性蛋白含量都先大幅上升而后缓慢降低,但整体仍呈上升趋势。其中,抗寒性强的中间砧‘长富2号’可溶性蛋白含量较高且增加幅度大,抗寒性弱的砧穗组合可溶性蛋白含量始终处于较低水平,增加幅度也不明显,不同中间砧‘长富2号’可溶性蛋白含量与抗寒性之间呈显著正相关关系。这与高京草等^[50]在枣树上的研究一致,曹建东等^[37]、王淑杰等^[46]在葡萄上的研究也得到了一致的结果。

正常情况下,植物体内的活性氧保持稳定的状态,而当植物遭受低温等逆境时,活性氧大量累积,细胞膜和膜蛋白遭到破坏,造成脂膜过氧化。丙二醛(MDA)是脂膜过氧化的最终产物之一,所以植物体内MDA含量可表示细胞膜系统的受伤害程度^[51]。随着处理温度的降低,中间砧T337、JM7‘长富2号’的MDA含量变化最快,积累最高,表明细胞受伤害程度不断加深,且LT₅₀最低,表明其可抵御温度最低,抗寒性最弱;‘长富2号’/SH系/新疆野苹果的MDA含量一直稳定在较低水平,LT₅₀高,表明可抵御温高,抗寒性强。不同中间砧‘长富2号’的MDA含量与抗寒性呈负相关,这与梅立新等^[25]、王延秀等^[27]在苹果砧木抗寒性上的研究一致。抗氧化酶可通过清除活性氧、防止脂膜过氧化来保护植物免受损害,因此,超氧化物歧化酶(SOD)、过氧化物酶(POD)、过氧化氢酶(CAT)在低温胁迫下的含量变化可作为植物抗寒性强弱判定的有效指标。在本试验中,随着处理温度的降低,9种中间砧‘长富2号’的SOD、POD、CAT酶活性都呈现出不断增高而后降低的趋势,峰值出现在-30℃或-35℃,抗寒性越强的‘长富2号’酶活性峰值越高。这可能说明,抗氧化酶系统在低温胁迫下,共同协作抵御低温逆境,酶活性不断攀升,在临界温度时达到峰值,随后,处理温度继续降低,植

物体受伤害程度不断加深,酶活下降。这与梅立新等^[25]在苹果矮化砧木上的研究一致。

果树的抗寒性是多个数量和质量性状共同决定的^[26],以上各单项指标虽与抗寒性存在相关关系,但是用来单一评价苹果抗寒性是片面的,所以本研究使用主成分分析法将指标简化,再采用隶属函数法对提取的综合指标进行综合分析,最后对综合评价进行聚类分析,以期更准确地鉴定各砧穗组合‘长富2号’的抗寒性。根据主成分分析结果,从8项生理指标中共提取出2个主成分,累积贡献率达86.998%,概括了原始变量的绝大部分信息,结果理想。从综合分析结果来看,‘长富2号’/SH系(SH₆、SH₃₈、SC₁、SH₁)/新疆野苹果抗寒性较强,SH系砧木可为甘肃陇东地区‘长富2号’适宜中间砧的选择提供参考,‘长富2号’/M系(M₂₆、M₉、M₇、T₃₃₇)/新疆野苹果抗寒性次之,‘长富2号’/JM₇/新疆野苹果砧穗组合的抗寒性最差,M系、JM₇砧木在甘肃陇东地区的推广需综合研究考虑。

4 结 论

本研究确定了9种不同中间砧‘长富2号’的半致死温度,并对抗寒相关生理指标进行了比较分析,利用主成分分析法、隶属函数法、聚类分析等方法综合鉴定了9种不同矮化中间砧‘长富2号’的抗寒性。综合半致死温度和数据分析结果,依据抗寒性强弱,将供试的砧穗组合划分为3类:第1类为SH₆、SH₁、SH₃₈、SC₁,抗寒性最强;第2类为M₇、M₂₆、M₉,抗寒性中等;第3类为JM₇、T₃₃₇,抗寒性较弱。综合评价结果可为中国不同气候区域富士系矮化中间砧的选择提供依据。

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