

3种梨野生砧木资源叶片抗氧化酶活性分析

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摘要:【目的】探究杜梨、木梨、秋子梨3种重要梨野生砧木资源抗氧化酶(SOD、POD、CAT)活性差异及盐碱胁迫处理后的变化。【方法】采集大树和实生苗期杜梨、木梨、秋子梨资源叶片, 进行盐碱胁迫处理, 测定处理前后SOD、POD、CAT活性并比较其差异。【结果】SOD活性, 杜梨>秋子梨>木梨, 胁迫处理后均明显上升; POD活性, 秋子梨>木梨>杜梨, 胁迫处理后均显著上升; 而CAT活性, 木梨>杜梨>秋子梨, 处理后仅杜梨活性升高。杜梨叶片抗氧化酶活性强于木梨、秋子梨。山西杜梨1和山西杜梨3耐盐碱性强。【结论】盐碱胁迫处理后, SOD平均活性增加最多, 其次为POD平均活性, CAT平均活性降低。杜梨抗氧化酶活性及耐盐碱性强于木梨、秋子梨。

关键词: 梨; 砧木; 抗氧化酶

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Analysis of antioxidant enzyme activity in the leaves of 3 species of *Pyrus* as rootstock

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Abstract:【Objective】*P. betulaefolia*, *P. ussuriensis* and *P. xerophila* are the main rootstocks in the North, Northeast and Northwest of China. Antioxidant enzymes have close connection with plant growth and development. The aim of the study was to investigate the antioxidant enzyme (SOD, POD and CAT) activities among 3 species of pyrus and the connection between the antioxidant enzyme (SOD, POD and CAT) activities and the salt resistance. 【Methods】Six accessions of each species were collected from “National Repository of Apple and Pear” in Research Institute of Pomology, Chinese Academy of Agricultural Sciences in August 2019. The seeds of 8 accessions were collected and stratified and then sown in the soil for germination. 60-day old seedlings were treated with saline-alkali solution. The activities of SOD, POD and CAT of both the leaves from the adult trees of 18 accessions and from the leaves seedlings of 8 accessions were measured at about 25 °C through ultraviolet spectrophotometer. The mean value and standard error of antioxidant enzyme activities and relevance were calculated with SPSS 26.0 software, one-way ANOVA was used to analyze the significant differences, and LSD (minimum significance method) was used to test the significant differences among the accessions at $\alpha=0.05$ level. Origin 2019b APP was used for principal component analysis. 【Results】Among the 18 wild pear accessions, the activity range of SOD was $37.55\text{--}206.64 \text{ U} \cdot \text{mL}^{-1}$, with the average level $121.57 \text{ U} \cdot \text{mL}^{-1}$, and the highest SOD activity was detected in the leaves of *P. betulaefolia*-Wujiazhuang 1. The activity range of POD was $32.71\text{--}455.60 \text{ U} \cdot \text{mL}^{-1}$, with the average level $177.55 \text{ U} \cdot \text{mL}^{-1}$, and the highest POD activity was found in *P. xerophila*-Huangtupo. The CAT activity range was $58.72\text{--}237.71 \text{ U} \cdot \text{mL}^{-1}$.

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$\text{U} \cdot \text{mL}^{-1}$, with the average level $101.41 \text{ U} \cdot \text{mL}^{-1}$, and the highest CAT activity was found in *P. xerophila*-Lingkong 1. Among the activities of POD, SOD and CAT of the 18 wild pear accessions, POD was the highest, followed by SOD and CAT in turn. There were significant differences in SOD and POD activities among 18 accessions, but the differences in CAT activity among some accessions were not obvious. The average activity of SOD, POD, and CAT for *P. betulaefolia* were 155.99 , 98.78 and $89.26 \text{ U} \cdot \text{mL}^{-1}$, respectively, the level were 94.39 , 189.73 and $138.43 \text{ U} \cdot \text{mL}^{-1}$ respectively, as for *P. ussuriensis*, the level were 114.31 , 257.27 and $79.68 \text{ U} \cdot \text{mL}^{-1}$ for *P. xerophila*, respectively. The overall levels of SOD activity in the leaves of three wild pear species were *P. betulaefolia* > *P. ussuriensis* > *P. xerophila*. There were significant differences between *P. betulaefolia* and *P. xerophila*. The overall levels of POD activity were, *P. ussuriensis* > *P. xerophila* > *P. betulaefolia*, and the significant differences existed among *P. ussuriensis*, *P. xerophila* and *P. betulaefolia*. The overall levels of CAT activity were *P. xerophila* > *P. betulaefolia* > *P. ussuriensis*. According to principal component analysis, the first discriminant function explained 44.7% of the activities of the three antioxidant enzymes in wild rootstock accessions in the dataset, whereas the second function explained 38.8%. Therefore, the two principal components could represent the information content of 83.5%, which could well represent the data information. Examination of the coefficients of the discriminant functions indicated that CAT and SOD were the highest contributors to the first function. But in terms of second function the highest contributors were SOD and POD. Then we discovered that *P. betulaefolia* and *P. xerophila* could be distinguished by PC1 (CAT and SOD). The accessions of *P. betulaefolia* mainly clustered in the first quadrant, while *P. xerophila* mainly clustered in the quadrant 3 and 4. PC2 (SOD and POD) could distinguish the accessions of *P. betulaefolia* and *P. ussuriensis*. The accessions of *P. ussuriensis* clustered in the quadrant 2 and 3, and the accessions of *P. ussuriensis* and *P. xerophila* clustered in the same quadrant, mainly due to the POD activity. Based on the principal component analysis and we calculated the score of each accession using the comprehensive evaluation function. At first, we calculated the average scores of the accessions of *P. ussuriensis*, *P. xerophila* and *P. betulaefolia*, and found that the scores of the accessions of *P. betulaefolia* were significantly higher than those of *P. ussuriensis* and *P. xerophila*, and the scores of accessions of *P. xerophila* were slightly higher than those of *P. ussuriensis*, and *P. betulaefolia*-Wujiazhuang 1 was the highest, while the score of *P. ussuriensis*-Sunwu 10 was the lowest. After saline-alkali treatment, *P. ussuriensis* and *P. xerophila* appeared wilting first, and *P. betulaefolia* was the most resistant. The SOD activities of *P. betulaefolia*, *P. ussuriensis* and *P. xerophila* showed upward trends, *P. betulaefolia* rose by $525.69 \text{ U} \cdot \text{mL}^{-1}$, *P. xerophila* increased by $447.52 \text{ U} \cdot \text{mL}^{-1}$, *P. ussuriensis* rose by $569.32 \text{ U} \cdot \text{mL}^{-1}$. At the same time, their POD activities also presented rising trends, *P. betulaefolia* rose by $102.17 \text{ U} \cdot \text{mL}^{-1}$, *P. xerophila* increased by $472.19 \text{ U} \cdot \text{mL}^{-1}$, *P. ussuriensis* rose by $408.05 \text{ U} \cdot \text{mL}^{-1}$. However, the CAT activity of *P. betulaefolia* only increased by $52.95 \text{ U} \cdot \text{mL}^{-1}$, the CAT activity of *P. ussuriensis* decreased slightly by $18.60 \text{ U} \cdot \text{mL}^{-1}$, and the CAT activity of *P. xerophila* decreased the most, by $104.51 \text{ U} \cdot \text{mL}^{-1}$. The average activity of SOD increased significantly, with an average increase of $507.28 \text{ U} \cdot \text{mL}^{-1}$, and the average activity of POD also showed an upward trend, with an average increase of $317.34 \text{ U} \cdot \text{mL}^{-1}$, which was significantly lower than the SOD activity. But the average activity of CAT showed a downward trend, with an average decrease of $23.98 \text{ U} \cdot \text{mL}^{-1}$. **【Conclusion】** There were differences in antioxidant enzyme activities among various wild pear species and accessions. The highest SOD activity was found in *P. betulaefolia*-Wujiazhuang 1, the highest POD activity in *P. xerophila*-Huangtupo, and the highest CAT activity in *P. xerophila*-Lingkong 1. The antioxidant enzyme activities in leaves of *P. betulaefolia* were higher than those of *P. ussuriensis* and *P. xerophila* as a whole, and the antioxidant enzyme activities of *P. ussuriensis* and *P. xerophila* were higher than those of *P. betulaefolia*.

riensis was slightly higher than that of *P. xerophila*. The antioxidant enzyme activities of *P. betulaefolia*-Wujiazhuang 1 was the highest, while *P. ussuriensis*-Sunwu 10 the lowest. The activity of SOD and POD showed a trend of increase after the high-concentration salt-alkali treatment. The CAT activity of *P. betulaefolia* showed an increase trend, and the salt-alkali resistance of *P. betulaefolia* was stronger than that of the other two species.

Key words: *Pyrus*; Rootstocks; Antioxidant enzyme

梨为蔷薇科(Rosaceae)梨亚科(Pomaceae)梨属(*Pyrus* L.)植物,是我国主栽果树之一,栽培面积和产量仅次于苹果。梨树繁殖方法主要为嫁接,需要采用合适的砧木。由于梨野生资源的抗逆性较强,常被用作砧木材料。杜梨(*Pyrus betulaefolia* Bge.)分布于新疆、河北、山西、甘肃等省,分布广泛,适应胁迫能力强,成为北方广泛应用的砧木类型^[1]。秋子梨(*Pyrus ussuriensis* Maxim.)主要分布在黑龙江、吉林、辽宁和甘肃等地,在朝鲜、日本、俄罗斯等欧亚国家也有分布^[2],可在寒冷、干燥地区生长,因而被用作抗寒砧木,是优异的抗寒种质资源^[3]。木梨(*Pyrus xerophila* Yü)产于我国西北各省区,是西北各省区梨的主要砧木,与秋子梨、新疆梨、西洋梨等栽培品种的嫁接亲和力强。木梨适应性强,喜冷凉气候,较耐寒,抗旱,抗病虫害^[4]。

抗氧化酶与植物生长息息相关,前人研究主要集中在几种关键抗氧化酶活性方面。严善春等^[5]用紫外分光光度法对日本落叶松、兴安落叶松和长白落叶松的杂交后代,共2代7个家系杂种落叶松针叶内的过氧化氢酶(CAT)、过氧化物酶(POD)和超氧化物歧化酶(SOD)的活性及其差异进行比较分析,发现7个家系/子代中,保护酶活性较高的,其潜在的抗虫性较强。冯佳等^[6]通过对6个不同品系地木耳抗氧化酶活性的比较研究,发现不同品系地木耳的CAT活性不同,这可能是由于其生活环境的差异所致,采自山西宁武荷叶坪品系的CAT活性最大,其次是秋千沟品系,说明这两个品系比其他品系的抗逆性强。吴岳等^[7]对山药综合抗氧化酶活性比较分析发现,山药品种SHQ1具有比其他品种更高的抗氧化活性,并表现出更强的抗逆性。在非胁迫环境中,周鹏等^[8]发现玉簪品种火与冰和梦想的SOD、POD活性显著高于其他品种;CAT活性品种间差异不显著。陈展宇等^[9]发现盐碱生境能提高幼苗时期甜高粱叶片中SOD、CAT、POD的活性,不同品种抗氧化酶活性增幅不同。在对梨的研究中,刘艳等^[10]

测定了18个梨品种的抗氧化酶活性,发现不同品种抗氧化酶总活性之间存在差异,推测总活性最高的品种抗逆性可能大于总活性最低的。路斌等^[11]研究表明,随着盐胁迫程度的加重,盐害指数逐渐增高,SOD与POD活性增强,随着胁迫时间延长,SOD活性呈现先升高后降低的趋势,POD活性呈降低的趋势。

有关梨野生资源抗氧化酶活性差异的研究还未见报道,笔者测定了杜梨、木梨、秋子梨盐碱胁迫处理前后的抗氧化酶活性,利用主成分分析法对不同品种梨进行抗氧化酶活性评价,为进一步研究梨野生砧木资源抗逆性及探究其抗逆响应机制奠定基础,为抗逆砧木的选育提供参考。

1 材料和方法

1.1 材料

2019年8月在中国农业科学院果树研究所国家果树种质兴城梨、苹果圃同一立地条件下采集杜梨、木梨、秋子梨资源各6份(表1),取其叶片,编号装入塑封袋中,迅速放入装有冰袋的泡沫塑料箱,带回实验室。叶片在液氮条件下研磨,-80℃保存。2019年9月,根据果实成熟情况及代表性,选取其中8份材料(表2),取成熟期果实,层积处理,穴盘育苗,实生苗生长60 d后,对其进行盐碱胁迫处理,采集叶片。

1.2 方法

1.2.1 酶活性测定 SOD活性参照王学奎等^[12]的NBT(氮蓝四唑)光化还原法测定。POD活性采用李合生^[13]的愈创木酚比色法测定。CAT活性参照李小方等^[14]的比色法测定。

1.2.2 种子育苗及盐碱胁迫处理 种子进行层积处理,梨树不同砧木种子所需层积时间不同,杜梨为60~80 d、秋子梨、木梨为50~60 d。

育苗及处理:采用基质块育苗,各品种选取适当株数的苗沙培,进行盐碱胁迫处理(根据盐碱地主要盐分组成特点,选定中性盐NaCl和Na₂SO₄及两种碱性盐NaHCO₃和Na₂CO₃,质量比为NaCl:Na₂SO₄:

表1 试验材料

Table 1 Experimental materials

材料 Accessions	种 Species	材料 Accessions	种 Species
早熟山梨 <i>P. ussuriensis</i> -Zaoshu	秋子梨 <i>P. ussuriensis</i>	车鸣峪山梨7号 <i>P. xerophila</i> -Chemingyu7	木梨 <i>P. xerophila</i>
胜山铁梨 <i>P. ussuriensis</i> -Shengshan	秋子梨 <i>P. ussuriensis</i>	灵空山梨1号 <i>P. xerophila</i> -Lingkong1	木梨 <i>P. xerophila</i>
五常山梨1号 <i>P. ussuriensis</i> -Wuchang1	秋子梨 <i>P. ussuriensis</i>	黄土坡山梨3号 <i>P. xerophila</i> -Huangtupo3	木梨 <i>P. xerophila</i>
安图山梨3号 <i>P. ussuriensis</i> -Antu3	秋子梨 <i>P. ussuriensis</i>	平安酸梨1号 <i>P. xerophila</i> -Pingan1	木梨 <i>P. xerophila</i>
红面山梨 <i>P. ussuriensis</i> -Hongmian	秋子梨 <i>P. ussuriensis</i>	吴家庄山梨 <i>P. xerophila</i> -Wujiazhuang	木梨 <i>P. xerophila</i>
孙吴山梨10号 <i>P. ussuriensis</i> -Sunwu10	秋子梨 <i>P. ussuriensis</i>	赵俺村糖梨 <i>P. xerophila</i> -Zhaoancun	木梨 <i>P. xerophila</i>
山西杜梨3号 <i>P. betulaefolia</i> -Shanxi3	杜梨 <i>P. betulaefolia</i>	武家庄杜梨1号 <i>P. betulaefolia</i> -Wujiazhuang1	杜梨 <i>P. betulaefolia</i>
山西杜梨1号 <i>P. betulaefolia</i> -Shanxi1	杜梨 <i>P. betulaefolia</i>	一平垣乡杜梨2号 <i>P. betulaefolia</i> -Yipingshanxiang2	杜梨 <i>P. betulaefolia</i>
一平垣乡杜梨1号 <i>P. betulaefolia</i> -Yipingshanxiang1	杜梨 <i>P. betulaefolia</i>	太林乡杜梨1号 <i>P. betulaefolia</i> -Tailinxiang1	杜梨 <i>P. betulaefolia</i>

表2 盐胁迫材料

Table 2 Experimental materials for salt-alkali treatment

材料 Accessions	种 Species	材料 Accessions	种 Species
山西杜梨3号 <i>P. betulaefolia</i> -Shanxi3	杜梨 <i>P. betulaefolia</i>	灵空山梨 <i>P. xerophila</i> -Lingkong1	木梨 <i>P. xerophila</i>
山西杜梨1号 <i>P. betulaefolia</i> -Shanxi3	杜梨 <i>P. betulaefolia</i>	平安酸梨 <i>P. xerophila</i> -Pingan1	木梨 <i>P. xerophila</i>
太林乡杜梨1号 <i>P. betulaefolia</i> -Tailinxiang1	杜梨 <i>P. betulaefolia</i>	早熟山梨 <i>P. ussuriensis</i> -Zaoshu	秋子梨 <i>P. ussuriensis</i>
赵俺村糖梨 <i>P. xerophila</i> -Zhaoancun	木梨 <i>P. xerophila</i>	红面山梨 <i>P. ussuriensis</i> -Hongmian	秋子梨 <i>P. ussuriensis</i>

$\text{NaHCO}_3:\text{Na}_2\text{CO}_3=1:9:9:1$,根据杜梨、木梨、秋子梨耐盐碱能力,确定浓度为 $300 \text{ mmol} \cdot \text{L}^{-1}$,预计处理14 d,根据品种盐害情况结束处理,取胁迫处理后实生苗的叶为试验材料。

1.2.3 数据分析 使用SPSS 26.0计算抗氧化酶活性的平均值、标准误和相关性,采用One-way ANOVA差异显著性分析,用LSD(最小显著性差异法)在 $\alpha=0.05$ 水平下检验品种之间的差异显著性;使用Origin 2019b中的PCA进行主成分分析。

2 结果与分析

2.1 18份梨野生资源抗氧化酶活性

如表3所示,18份梨野生资源的SOD活性为

$37.55\sim206.64 \text{ U} \cdot \text{mL}^{-1}$,平均SOD活性为 $121.57 \text{ U} \cdot \text{mL}^{-1}$;POD活性为 $32.71\sim455.60 \text{ U} \cdot \text{mL}^{-1}$,平均POD活性为 $177.55 \text{ U} \cdot \text{mL}^{-1}$;而CAT活性为 $58.72\sim237.71 \text{ U} \cdot \text{mL}^{-1}$,平均CAT活性为 $101.41 \text{ U} \cdot \text{mL}^{-1}$ 。

18份梨野生资源的POD平均活性最大,SOD平均活性次之,CAT平均活性最小。其中,SOD活性最高的是武家庄杜梨1号,为 $206.64 \text{ U} \cdot \text{mL}^{-1}$,孙吴山梨10号SOD活性最低;POD活性最高的是黄土坡山梨,为 $455.60 \text{ U} \cdot \text{mL}^{-1}$,车鸣峪山梨7号的POD活性最低;CAT活性最高的是灵空山梨1号,为 $237.71 \text{ U} \cdot \text{mL}^{-1}$,活性最低的是孙吴山梨10号;在 $p < 0.05$ 水平下进行方差分析,发现各种质间SOD活性、POD活性大都存在显著差异,而CAT活性在部分种质间不存在显著差异。

2.2 梨不同种野生砧木资源抗氧化酶活性比较

如图1所示,杜梨的平均SOD活性为 $155.99 \text{ U} \cdot \text{mL}^{-1}$,平均POD活性为 $98.78 \text{ U} \cdot \text{mL}^{-1}$,平均CAT活性为 $89.26 \text{ U} \cdot \text{mL}^{-1}$;木梨平均SOD活性为 $94.39 \text{ U} \cdot \text{mL}^{-1}$,平均POD活性为 $189.73 \text{ U} \cdot \text{mL}^{-1}$,平均CAT活性为 $138.43 \text{ U} \cdot \text{mL}^{-1}$;秋子梨平均SOD活性为 $114.31 \text{ U} \cdot \text{mL}^{-1}$,平均POD活性为 $257.27 \text{ U} \cdot \text{mL}^{-1}$,平均CAT活性为 $79.68 \text{ U} \cdot \text{mL}^{-1}$ 。

以一个酶的酶活性为变量研究不同种梨野生资源,发现杜梨、木梨、秋子梨的CAT活性低于另两种酶活性,木梨CAT活性最高,杜梨次之,秋子梨CAT

表3 不同梨野生砧木资源 SOD、POD、CAT 活性比较

Table 3 Comparison of SOD, POD and CAT activities of different pears wild rootstock accessions

材料 Accession	SOD 活性 SOD activities	POD 活性 POD activities	CAT 活性 CAT activities
早熟山梨 <i>P. ussuriensis</i> -Zaoshu	131.09±10.21 d	111.11±6.94 g	66.98±1.48 d
胜山铁梨 <i>P. ussuriensis</i> -Shengshan	199.65±1.67 a	408.93±10.13 a	61.70±1.69 f
五常山梨1号 <i>P. ussuriensis</i> -Wuchang1	104.39±12.95 g	147.94±12.86 d	79.16±2.45 bc
安图山梨3号 <i>P. ussuriensis</i> -Antu3	112.91±6.77 e	175.56±20.37 b	99.81±2.38 a
红面山梨 <i>P. ussuriensis</i> -Hongmian	100.29±9.68 i	376.93±23.92 a	111.43±3.20 a
孙吴山梨10号 <i>P. ussuriensis</i> -Sunwu10	37.55±5.13 m	323.16±32.76 b	58.72±3.99 g
车鸣峪山梨7号 <i>P. xerophila</i> -Chemingyu7	152.71±20.41 d	32.71±3.69 m	135.27±10.88 a
灵空山梨1号 <i>P. xerophila</i> -Lingkong1	79.30±8.79 l	56.41±9.23 j	237.71±7.50 a
黄土坡山梨3号 <i>P. xerophila</i> -Huangtupo3	84.28±10.11 k	455.60±2.51 a	101.70±1.96 a
平安酸梨1号 <i>P. xerophila</i> -Pingan1	97.49±8.80 j	339.02±11.90 a	90.13±2.92 b
吴家庄山梨 <i>P. xerophila</i> -Wujiazhuang	103.38±14.85 h	93.81±29.43 h	145.65±10.88 a
赵庵村糖梨 <i>P. xerophila</i> -Zhaoancun	49.19±1.99 l	160.86±6.04 c	122.74±2.95 a
山西杜梨3号 <i>P. betulaefolia</i> -Shanxi3	174.31±5.81 a	140.17±3.62 e	99.72±2.54 b
山西杜梨1号 <i>P. betulaefolia</i> -Shanxi1	107.73±4.74 f	93.81±29.43 i	70.33±5.99 c
一平垣乡杜梨1号 <i>P. betulaefolia</i> -Yipingyuanxiang1	170.36±7.01 b	132.39±13.24 f	62.94±3.24 e
武家庄杜梨1号 <i>P. betulaefolia</i> -Wujiazhuang1	206.64±28.98 a	46.51±8.06 k	102.08±2.21 a
一平垣乡杜梨2号 <i>P. betulaefolia</i> -Yipingyuanxiang2	155.33±5.96 c	68.09±8.42 i	76.65±3.74 a
太林乡杜梨1号 <i>P. betulaefolia</i> -Tailinxiang1	121.56±9.68 e	32.93±8.15 l	102.61±3.31 c
平均值 Average value	121.57	177.55	101.41

注:不同小写字母表示在 $p < 0.05$ 水平上差异显著。

Note: Different small letters indicate significant difference at $p < 0.05$.

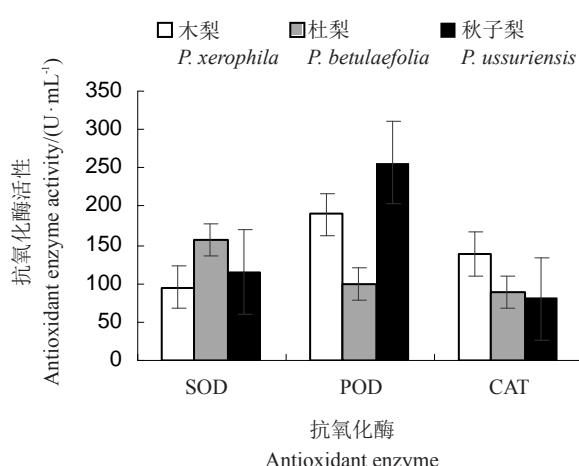


图1 梨野生砧木资源抗氧化酶活性

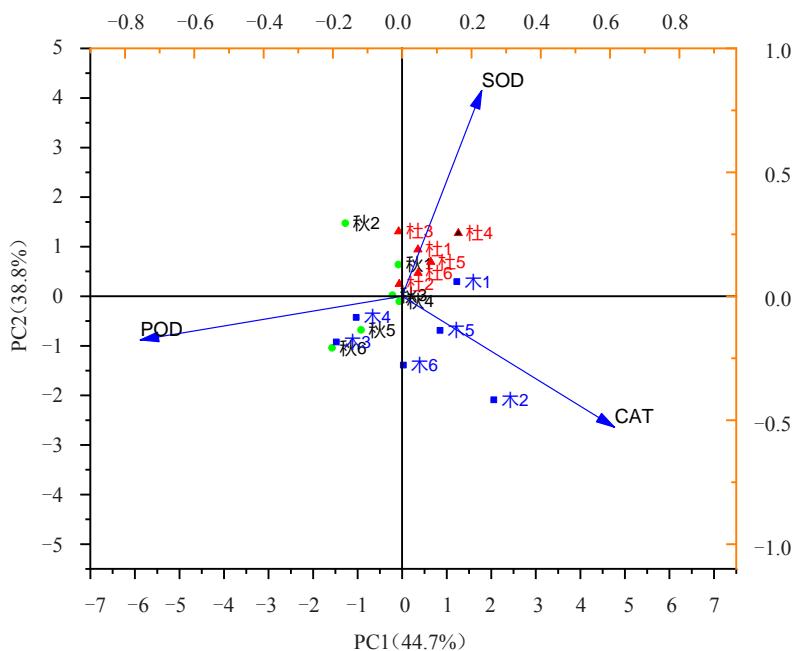
Fig. 1 Antioxidant enzyme activities of wild pears rootstock accessions

活性最低,同时,木梨、杜梨与秋子梨存在显著差异;对于 POD 活性而言,秋子梨>木梨>杜梨,并且杜梨、木梨、秋子梨之间存在显著差异;杜梨 SOD 活性最高,木梨最低,且杜梨与木梨之间存在显著差异。

2.3 18份梨野生资源叶片内抗氧化酶活性的主成分分析

经方差分析可知,梨野生资源种内及种间叶片内抗氧化酶活性差异显著,同时 SOD、POD、CAT 3 种酶的酶活性也存在较大差异,不易于评价其抗氧化酶活性,因此利用抗氧化酶活性进行主成分分析,挖掘抗氧化酶活性最高的品种。从主成分分析结果(图2)可看出,第1主成分的方差占所有主成分方差的44.7%,第2主成分占38.8%,主成分1和主成分2的累计方差贡献率达到83.5%,即2个主成分可代表其83.5%的信息含量,能够很好地代表所有数据信息。第1主成分中CAT活性的特征值最大,其次是SOD活性,说明第1主成分主要反映CAT、SOD活性,并且将杜梨、木梨分开,杜梨主要集中于第1象限,木梨主要集中于3、4象限;第2主成分中SOD活性具有最大载荷,其次是POD,说明第2主成分主要反映了SOD、POD酶活性,同时将杜梨与秋子梨分开,秋子梨集中于2、3象限。部分木梨、秋子梨聚集在第3象限,与其POD活性相关。

运用综合评价函数计算不同种梨野生种质抗



杜 1. 山西杜梨 3 号 *P. betulaefolia-Shanxi3*; 杜 2. 山西杜梨 1 号 *P. betulaefolia-Shanxi1*; 杜 3. 一平坦乡杜梨 1 号 *P. betulaefolia-Yipingyuanxiang1*; 杜 4. 武家庄杜梨 1 *P. betulaefolia-Wujiazhuang1*; 杜 5. 一平坦乡杜梨 2 号 *P. betulaefolia-Yipingyuanxiang2*; 杜 6. 太林乡杜梨 *P. betulaefolia-Tailinxiang*; 木 1. 车鸣峪山梨 7 号 *P. xerophila-Chemingyu7*; 木 2. 灵空山梨 1 号 *P. xerophila-Lingkong1*; 木 3. 黄土坡山梨 3 号 *P. xerophila-Huangtupo3*; 木 4. 平安酸梨 *P. xerophila-Pingan*; 木 5. 吴家庄山梨 *P. xerophila-Wujiazhuang*; 木 6. 赵俺村糖梨 *P. xerophila-Zhaoancun*; 秋 1. 早熟山梨 *P. ussuriensis-Zaoshu*; 秋 2. 胜山铁梨 *P. ussuriensis-Shengshan*; 秋 3. 五常山梨 1 号 *P. ussuriensis-Wuchang1*; 秋 4. 安图山梨 3 号 *P. ussuriensis-Antu3*; 秋 5. 红面山梨 *P. ussuriensis-Hongmian*; 秋 6. 孙吴山梨 10 号 *P. ussuriensis-Sunwu10*。

图 2 不同梨野生砧木资源抗氧化酶活性主成分分析

Fig. 2 Principal component analysis of antioxidant enzyme activity in different wild rootstocks of pear

氧化酶活性的综合得分,得分越高,说明该品种的抗氧化酶活性越高。由表 4 可知,武家庄山梨 1 号得分最高,推测其抗氧化酶活性最高,部分品种得分为负数,其潜在抗氧化能力可能较弱,其中孙吴

山梨 10 号得分最低,其抗氧化能力最弱。杜梨整体得分优于秋子梨、木梨,木梨略微优于秋子梨,因此杜梨抗氧化酶活性高于木梨,木梨高于秋子梨。

表 4 不同梨野生砧木资源主成分分析得分

Table 4 Principal component analysis of different pear wild rootstock accessions

材料 Accession	PC1	PC2	得分 Score	材料 Accession	PC1	PC2	得分 Score
武家庄杜梨 1 <i>P. betulaefolia-Wujiazhuang1</i>	1.26	1.27	3.17	山西杜梨 1 号 <i>P. betulaefolia-Shanxi1</i>	-0.06	0.25	0.20
车鸣峪山梨 7 号 <i>P. xerophila-Chemingyu7</i>	1.23	0.29	1.99	胜山铁梨 <i>P. ussuriensis-Shengshan</i>	-1.28	1.47	0.00
一平坦乡杜梨 2 号 <i>P. betulaefolia-Yipingyuanxiang2</i>	0.64	0.69	1.67	安图山梨 3 号 <i>P. ussuriensis-Antu3</i>	-0.06	-0.10	-0.21
山西杜梨 3 号 <i>P. betulaefolia-Shanxi3</i>	0.36	0.94	1.57	五常山梨 1 号 <i>P. ussuriensis-Wuchang1</i>	-0.22	0.02	-0.27
一平坦乡杜梨 1 号 <i>P. betulaefolia-Yipingyuanxiang1</i>	-0.08	1.31	1.41	赵俺村糖梨 <i>P. xerophila-Zhaoancun</i>	0.03	-1.39	-1.58
太林乡杜梨 <i>P. betulaefolia-Tailinxiang</i>	0.36	0.47	1.03	平安酸梨 <i>P. xerophila-Pingan</i>	-1.03	-0.43	-1.88
早熟山梨 <i>P. ussuriensis-Zaoshu</i>	-0.08	0.64	0.63	红面山梨 <i>P. ussuriensis-Hongmian</i>	-0.92	-0.68	-2.03
吴家庄山梨 <i>P. xerophila-Wujiazhuang</i>	0.85	-0.69	0.34	黄土坡山梨 3 号 <i>P. xerophila-Huangtupo3</i>	-1.48	-0.92	-3.06
灵空山梨 1 号 <i>P. xerophila-Lingkong1</i>	2.06	-2.09	0.34	孙吴山梨 10 号 <i>P. ussuriensis-Sunwu10</i>	-1.57	-1.04	-3.32

2.4 盐碱胁迫处理的梨野生砧木资源叶片抗氧化酶活性变化情况分析

如图3所示,盐碱胁迫后,秋子梨SOD活性上升幅度最大,为 $569.32 \text{ U} \cdot \text{mL}^{-1}$,木梨上升幅度最小,仅为 $447.52 \text{ U} \cdot \text{mL}^{-1}$,同时杜梨上升了 $525.69 \text{ U} \cdot \text{mL}^{-1}$,

杜梨、秋子梨显著高于木梨;同时杜梨、木梨、秋子梨的POD活性也都呈现出上升趋势,分别上升了 102.17 、 472.19 、 $408.05 \text{ U} \cdot \text{mL}^{-1}$,秋子梨、木梨显著高于杜梨;但是对于CAT活性而言,仅杜梨的CAT活性上升,上升了 $52.95 \text{ U} \cdot \text{mL}^{-1}$,秋子梨CAT活性略微

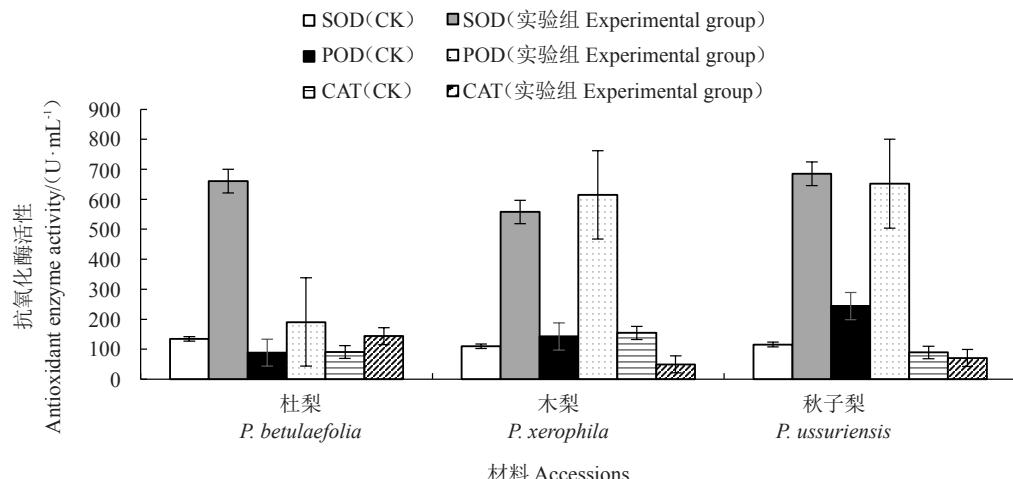


图3 杜梨、木梨、秋子梨野生砧木资源抗氧化酶活性变化情况

Fig. 3 Changes of antioxidant enzyme activity in wild rootstock of *P. betulaefolia*, *P. xerophila* and *P. ussuriensis*

下降,下降了 $18.60 \text{ U} \cdot \text{mL}^{-1}$,而木梨CAT活性下降幅度最大,下降了 $104.51 \text{ U} \cdot \text{mL}^{-1}$ 。

如图4所示,盐碱胁迫处理后SOD活性显著升高,平均上升了 $507.28 \text{ U} \cdot \text{mL}^{-1}$,POD活性也呈现上升趋势,平均上升了 $317.34 \text{ U} \cdot \text{mL}^{-1}$,明显低于SOD活性。CAT活性却呈现下降趋势,平均下降了 $23.98 \text{ U} \cdot \text{mL}^{-1}$ 。胁迫处理后,SOD活性最高,POD活性次之,CAT活性最低。

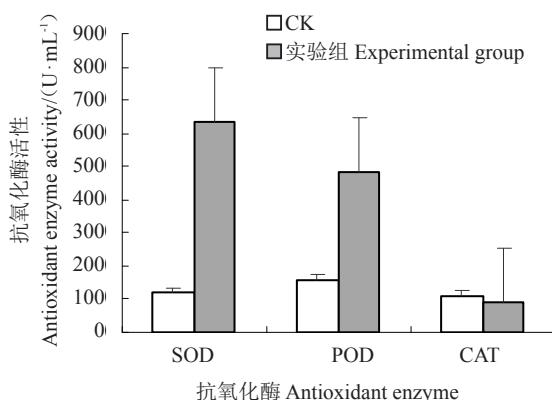


图4 不同抗氧化酶活性盐碱胁迫前后变化

Fig. 4 Changes of antioxidant enzyme activity before and after salt-alkali treatment

如表5所示,山西杜梨1抗氧化酶活性得分为2.56,山西杜梨3得分2.18,山西杜梨1与山西杜梨3

表5 盐碱胁迫后不同梨野生砧木资源主成分分析得分

Table 5 Principal component analysis score statistics table of different pear wild rootstock accessions after salt-alkali treatment

材料 Accessions	PC1	PC2	得分 Scores
山西杜梨1 <i>P. betulaefolia</i> -Shanxi1	1.70	-0.37	2.56
山西杜梨3 <i>P. betulaefolia</i> -Shanxi3	1.37	-0.16	2.18
早熟山梨 <i>P. ussuriensis</i> -Zaoshu	-0.10	2.37	1.99
太林乡山梨1 <i>P. betulaefolia</i> -Tailinxiang	-0.04	-0.17	-0.23
平安酸梨1 <i>P. xerophila</i> -Pingan	-0.84	0.04	-1.39
红面山梨 <i>P. ussuriensis</i> -Hongmian	-0.52	-0.60	-1.44
赵庵村糖梨 <i>P. xerophila</i> -Zhaoancun	-0.88	-0.20	-1.68
灵空山梨 <i>P. xerophila</i> -Lingkong1	-0.69	-0.90	-2.00

的抗氧化酶活性得分明显优于其他资源;同时,盐碱胁迫处理后,秋子梨最先发生盐害症状,为2~3 d,其次木梨为3~5 d,杜梨出现盐害症状至少需6 d,其中山西杜梨1与山西杜梨3相较于其他资源出现盐害症状最晚,为其中耐盐碱性最强的资源。

3 讨 论

超氧化物歧化酶(SOD)是清除活性氧过程中第一个起作用的抗氧化酶,能将超氧化物阴离子自由基 O_2^- 迅速歧化为过氧化氢和分子氧。尽管过氧化氢仍对机体有害,但体内的过氧化氢酶(CAT)和过氧化物酶(POD)会立即将其分解为完全无害的水。

这样3种酶就组成了一个防氧化链条^[15]。

从试验的主成分分析可看出,第1主成分中CAT活性系数最大,SOD活性次之,即第一主成分主要反映SOD、CAT活性,而第2主成分中SOD载荷最大,即第2主成分主要反映SOD活性,因此在两个主成分中SOD活性都具有地位,而SOD在活性氧清除反应中处于核心地位,能将超氧化物阴离子自由基快速氧化为过氧化氢和分子氧。在逆境条件下,抗氧化酶能被活性氧诱导产生,从而减轻对细胞膜的伤害。Wang等^[16]在烟草中转入SOD基因后,发现其光合效率在盐胁迫下比对照显著提高,同时抗逆水平也有所提高。对苗期紫花苜蓿的研究发现,不同浓度混合盐碱胁迫下,SOD、POD和CAT活性均有不同程度的增加。在逆境胁迫下,抗逆性强的品种对自由基的清除能力增强,抗逆性弱的品种的清除能力下降。抗逆性强的品种抗氧化酶活性相对增加较多,从而适应逆境,而抗逆性弱的品种可能缺乏这种应激机制,活性反而下降,较难适应逆境^[17]。本试验中,3个品种的SOD活性在逆境条件下均上升明显,与前人研究一致。

POD的主要作用是催化过氧化氢的分解,从而减少过氧化氢对细胞膜的破坏,提高植物抗性。当植物受到轻微的环境胁迫时,POD活性会增加,从而增强植物对活性氧的去除能力^[18]。当受到严重胁迫时,POD活性会大大降低,导致活性氧的积累和细胞损伤。POD在很多方面都发挥着作用,譬如促进木质化、控制细胞生长、消除ROS等^[19-20]。有研究发现,POD活性越高的高粱品种,其盐胁迫耐受性越强^[21]。由继红等^[22]研究表明,苜蓿中SOD、POD活性高及POD同工酶酶带数量多的品种抗寒性强。笔者发现秋子梨、木梨的平均POD活性较高,秋子梨属于优异抗寒资源,推测其POD活性与抗寒性存在关系,而木梨主要生长于西北地区,西北地区盐碱状况严重,因此推测木梨POD活性可能与其在原生境环境条件下的驯化情况存在关系。对白刺^[23]、青山杨^[24]、四翅滨藜^[25]、合欢^[26]等的研究均发现,POD活性均随着盐浓度和pH的增加表现出先上升后下降的趋势。此结论与本试验情况相似,高浓度盐碱处理时,存在POD酶活性下降的现象。

CAT由第2主成分反映,是一类广泛存在于生物体防御系统的关键酶之一,植物细胞中的CAT在清除活性氧、维持植物体内活性氧代谢平衡方面起

着重要作用^[27]。不适宜的温度会引起CAT活性的变化。研究表明,高温条件下,CAT活性与杜鹃种间耐热性呈正相关^[28]。干旱胁迫下ROS大量累积,引发氧化胁迫,植物抗氧化酶系统启动自我防御功能,提高抗氧化酶活性,平衡活性氧代谢,保护膜系统,增强抗旱能力。王宏等^[29]研究表明,活性氧信号参与了翠冠、苏翠1号和华酥对黑斑病的响应,而CAT作为解毒酶,参与了翠冠和苏翠1号对黑斑病的抗性响应。许多研究发现,CAT与盐胁迫耐受相关。本试验中仅杜梨面对高浓度盐碱胁迫时,CAT活性明显上升,推测其耐盐碱性强与CAT活性高存在很大关系。

4 结 论

梨野生资源种内及种间叶片内抗氧化酶活性差异显著。盐碱胁迫处理后的SOD、POD平均活性上升,SOD活性上升幅度最大,POD活性次之;CAT平均活性却呈现出下降趋势,然而,杜梨CAT活性呈现出上升趋势。杜梨抗盐碱性以及抗氧化酶活性依次强于木梨、秋子梨。综上所述,杜梨在处理前后抗氧化酶活性均强于木梨和秋子梨,更适宜作为盐碱性土地的砧木。

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