

新疆野核桃种质资源对核桃腐烂病的抗性评价

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摘要:【目的】了解新疆野核桃对核桃腐烂病的抗性, 挖掘抗性种质。【方法】以新疆巩留野核桃自然保护区中7个类型的28份新疆野核桃种质1年生枝条为材料, 通过室内人工接种的方法, 记录野核桃在接种核桃腐烂病菌后第12天的发病情况, 测定韧皮部POD、SOD、CAT酶活性以及MDA含量等生理指标, 最后采用主成分分析法和聚类分析法综合评价野核桃种质资源对核桃腐烂病的抗病能力。【结果】新疆野核桃种质间的抗病能力显示出较大差异($p<0.05$), 其中J-1、J-2、XT-3、XT-4等12个野核桃单株在病斑表征上显示为高抗病种质; 发病枝条(平均水平)的POD酶活性、未发现SOD酶活性、CAT酶活性、MDA含量相比未发病枝条(平均水平)均有不同程度上升, 分别为未发病枝条的260.6%、118.9%、283.6%和192.6%, 其中POD酶活性、CAT酶活性、MDA含量与枝条发病程度呈极显著正相关($p<0.01$), SOD与枝条发病程度呈显著线性关系。主成分分析结果显示抗病能力为0.93~1.32的野核桃种质抗病性较强, 为XT-4、J-1、J-2、XY-4、XY-1、XY-2、T-2; 聚类分析结果显示, 28份野核桃种质的抗病性可分为3类, 其中B类核桃抗病性最强, C类抗病性最弱, A类属于中间类型。【结论】从28份新疆野核桃材料中初步筛选了7个抗病种质, 研究结果可为新疆野核桃抗病育种研究提供基础性参考。

关键词:新疆野核桃; 种质筛选; 核桃腐烂病; 生理响应

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Evaluation of resistance of Xinjiang wild walnuts to walnut canker

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Abstract:【Objective】In recent years, walnut canker disease (*Cytospora chrysosperma*) has become one of the important diseases affecting the development of walnut trees in Xinjiang. The investigation on the natural incidence of wild walnut trees in Xinjiang wild walnut populations suggested that Xinjiang has a rich wild walnut germplasm resource, and there are obvious differences in disease resistance among different varieties, but there is still a lack of systematic research. Through the disease resistance evaluation with different methods, the disease-resistant germplasm resources can be screened and can lay a solid foundation for resistance breeding of cultivated walnut, and also provide a scientific basis for the protection and utilization of wild walnut in Xinjiang.【Methods】The annual branches of 28 Xinjiang wild walnut accessions of 7 types were used as experimental materials. Three wounds up to the xylem were made on each branch with a punch, about 5 mm in diameter. Then, agar plates with pathogen purified for 6 days were inoculated through the wound, and the lesion length was recorded on the 12th day. The phloem tissue around the branch lesion was taken and stored in the refrigerator at -40 °C after quick freezing with liquid nitrogen. The activities of POD, SOD, CAT, and MDA in the phloem were measured. The resistance of Xinjiang wild walnuts to walnut canker was evaluated by principal component analysis and cluster analysis.【Results】After inoculation with walnut canker pathogen for 12 days,

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the disease degree of the 28 isolated branch materials showed a significant difference. Among them, the lowest length of disease spot in the branch was 5 mm and the highest was 100 mm. The disease spots on the surface of branches were obvious, and the color of the diseased part of branches turned black and lose water. According to the multiple comparison results, the difference between L-1 with the largest disease spot on branches and the nine single plants without disease was the most significant. The tested materials were divided into 8 groups according to the differences. Among the 28 wild walnut materials, the highest SOD enzyme activities were found in X-4, XT-2, XY-5, and T-1, and all showed susceptible individual plants. The SOD activity in L-1, XT-1, and X-1 with the largest lesion was the lowest, which might have reached the physiological threshold with the infection of pathogenic bacteria. The highest POD enzyme activity was in T-1, XT-1, and X-3, and the three accessions were susceptible materials. The lowest POD activity was found in X-5, XT-4, and J-1, and none of the three materials displayed disease symptom. The highest CAT enzyme activities were in XT-2, L-2, and X-1, of which L-2 and X-1 were severely diseased. The lowest CAT enzyme activity was in J-3, J-1, and XT-5, among them, J-1 and XT-5 were not infected, and the disease of J-3 was mild. The highest MDA content was detected in XY-3, XT-1, L-1, and X-1, which were all susceptible materials, and the lesion length of XT-1, L-1, and X-1 were longest. The lowest content of MDA was in XT-4, T-2, XY-1, XY-4, and XY-2, which were all non-diseased materials. Compared with the non-diseased branches, the average POD enzyme activity, SOD enzyme activity, CAT enzyme activity, and MDA content in diseased branches increased by 160.6%, 18.9%, 183.6%, and 92.6%, respectively. The POD enzyme activity, CAT enzyme activity, and MDA content were significantly positively correlated with the size of the disease spots ($p<0.01$). Although the SOD activity of diseased plants was higher than that of non-diseased plants, there was no significant linear relationship between SOD and branch lesion length. The SOD activity of diseased plants of wild walnut was higher than that of the non-diseased plants, but there was no significant linear relationship between SOD and lesion length. The results of the principal component analysis showed that two principal component matrices with eigenvalues greater than 1 were extracted, and the variance contribution rate of the two principal components reached 73.42%. According to the matrix formula, the disease resistance of Xinjiang wild walnut was -1.45-1.32, among which XT-4 had the highest disease resistance and XT-1 had the lowest disease resistance. Xinjiang wild walnut germplasm with a score of 0.93-1.32 had good disease resistance, including seven individual plants XT-4, J-1, J-2, XY-4, XY-1, XY-2, and T-2. The results of cluster analysis showed that the disease resistance of 28 wild walnut accessions could be divided into three categories at 4 Euclidean distances, among which class B walnut had the best disease resistance, class C walnut had the worst disease resistance, and class A belonged to the intermediate type. Most of the 7 high resistance germplasms belonged to class B. **【Conclusion】** The annual branches of the 28 Xinjiang wild walnuts showed different disease resistance after inoculation with walnut canker. The activities of POD, CAT, and content in MDA can be used as physiological indexes for the preliminary identification of walnut canker resistance. Finally, 7 high disease resistant materials were selected from the 28 wild walnut materials in Xinjiang. The results provide reference for disease resistance breeding of walnut.

Key words: Xinjiang wild walnut; Germplasm screening; Walnut canker disease; Physiological response

核桃腐烂病又称烂皮病、黑水病等,主要为金黄壳囊孢菌 *Cytospora chrysosperma*(有性型 *Valsa sor-dida*)引发的真菌病害^[1]。该病在新疆栽培核桃园中发生较为普遍,主要危害枝干的皮层,当发现皮下出现黑色黏稠的汁液时,皮下已扩展数厘米以上的病斑,轻者主枝枯死,重者整株枯萎死亡^[2-4]。对核桃腐烂病的发病情况进行调查发现,部分重病园发病率高达 90%^[5],已成为影响新疆核桃树健康生长发育的重要病害之一。筛选出抗病种质进而选育栽培抗病新品种,是控制植物病害发生的有效途径^[6]。在以往的研究中,主要通过离体评价初步筛选抗病种质,但离体材料的抗性可能弱于完整植株,鉴定结果可能会有所偏差^[7]。研究发现植物在受到病原物侵染后,膜系统发生伤害,细胞膜透性增加,丙二醛(MDA)累积,它和过氧化物酶(POD)、超氧化物歧化酶(SOD)、过氧化氢酶(CAT)等抗氧化酶均参与了植物的防御,可能与植物的抗病性有关,可以作为植物抗病性鉴定的生理指标^[8]。在此基础上利用主成分分析、聚类分析相结合的方法,筛选出所需的高抗种质,有效避免了传统方法的不足^[9-10]。

新疆野核桃(*Juglans regia* L.)是胡桃科(Juglandaceae)、胡桃属(*Juglans*)植物,多年生落叶乔木,王磊等^[11]按种子特征将其划分有 14 个类型。在我国仅成片分布于巩留野核桃林自然保护区,被认为是中亚栽培核桃(*Juglans regia* L.)的直系祖先^[12],亦是研究栽培核桃之起源、进化以及遗传变异的重要基因库。目前,对新疆野核桃的地理分布^[13]、抗性生理^[14-15]、遗传多样性^[16-17]、营养品质^[18]、年龄结构及生长特性^[19]、核心种质的构建^[16, 20]以及遗传图谱^[21]等方面进行了研究。王肇延^[16]调查发现,该地区野核桃不同种质间的抗核桃腐烂病能力存在明显差别,根据患病差异可划分为 4 个等级。野核桃树种质资源丰富,但与其他林果种质资源相比,野核桃树种质资源抗病资源研究较少,尚需进一步挖掘。本研究以新疆野核桃 7 个类型的 28 份种质为材料,通过人工接种,记录不同发病情况,测定过氧化物酶(POD)、超氧化物歧化酶(SOD)、过氧化氢酶(CAT)活性和丙二醛含量(MDA)等生理生化指标,采用主成分分析、系统聚类分析对新疆野核桃抗病综合评价,筛选抗性较强的种质,以期为后续野核桃种质资源的合理开发利用提供科学依据。

1 材料和方法

1.1 试验材料

1.1.1 采样 以已构建的新疆野核桃种质资源基础数据库为采样依据^[16],2021 年 5 月下旬在新疆伊犁巩留县野核桃林(82°17'56"E, 43°27'21"N)选取 28 份树势良好的种质,采摘中部外围无病害且粗细长短、含水量一致的 1 年生枝条,将采集的枝条剪成 20 cm 小段,每份资源取 5 根枝段,切面用乳胶封口,记好编号放入编织袋中,带回实验室,用无菌水擦拭并用 75% 乙醇消毒备用。

1.1.2 培养基 PDA 培养基:马铃薯(切块去皮)200 g,琼脂 13 g,葡萄糖 15 g,无菌水 1000 mL,用于菌株纯化^[4]。致病菌株由新疆农业大学林木病理实验室提供。

菌饼:本试验所用核桃腐烂病菌株为新疆农业大学马荣研究组分离鉴定的金黄壳囊孢(*Cytospora chrysosperma*)(编号 XJAU-936)^[1],该菌株置于斜面 PDA 培养基 4 °C 冰箱保存,保存于新疆农业大学林木病理实验室。试验时挑取菌丝接种于 PDA 培养基上,在恒温培养箱中 25 °C 黑暗培养 6 d,在菌落边缘打取直径 5 mm 的菌饼作为接种体。

1.2 试验处理

将 28 份新疆野核桃枝条去掉梢端和末端,每份样本 3 个重复,采用枝条打孔法^[4],每根枝条用灭菌的打孔器制造 3 个深达木质部的伤口,约 5 mm 大小,随后接种纯化 6 d 的 PDA 菌饼于伤口处,纱布保湿并用保鲜膜二次固定,将枝条插入浸有无菌水的花泥中,置于恒温 25 °C 保湿培养。隔天取下接种体,每天早晚喷雾保湿各 1 次,第 12 天通过十字交叉法使用软皮尺记录病斑长度和宽度^[22],并计算发病程度(公式 1)^[22-23]。取枝条病斑外围的韧皮部,液氮速冻后 -40 °C 冰箱保存,用于测量生理指标。

$$\text{发病程度} = \Sigma \text{每孔测量病斑直径} / \text{总接种伤口数} \quad (1)$$

1.3 实验方法

测定方法参考刘家尧等^[24]编著的《植物生理学实验教程》。POD 活性采用愈创木酚显色法测定,SOD 活性采用氮蓝四唑法测定,MDA 含量采用硫代巴比妥酸法测定。CAT 活性采用北京 Solarbio (CAT)ELISA 试剂盒说明测定。

1.4 分析方法

主成分分析法: 使用 SPSS 统计分析软件, 通过描述统计的方法将数据进行标准化, 将标准化的数据通过降维的方式进行主成分分析^[25]。在主成分提取表中, 根据特征根大于 1 确定主成分的数量, 特征根用 λ 表示。主成分系数等于各变量因子载荷向量除以各自主成分的特征根的算术平方根, 见公式(2):

$$t_i = a_i / \sqrt{\lambda_i} \quad (i=1,2) \quad (2)$$

因子载荷值是指确定各个指标对各成分的影响, 在成分矩阵中表示。计算各品种的综合得分, 见公式(3):

$$Q = (F_1 \times W_1 + F_2 \times W_2 + \dots + F_i \times W_i) / (W_1 + W_2 + \dots + W_i) \quad (3)$$

Q 为综合得分, W_i 为主成分提取表中的方差贡献率, F_i 表示主成分的特征根对应的特征向量的和。利用 Between-groups linkage(组间距离)法对所得结果进行系统聚类评价^[26], 比较新疆野核桃种质抗病能力强弱, 对生理指标与发病程度进行 Pearson 相关性分析^[27]。

1.5 数据处理

采用 Excel 2016 对数据进行统计, 采用 SPSS 22.0 数据分析软件进行多重比较分析、相关性分析、主成分分析及聚类分析。

2 结果与分析

2.1 新疆野核桃种质资源离体枝条接种后发病情况

表 1 核桃腐烂病菌侵染后野核桃枝条发病程度

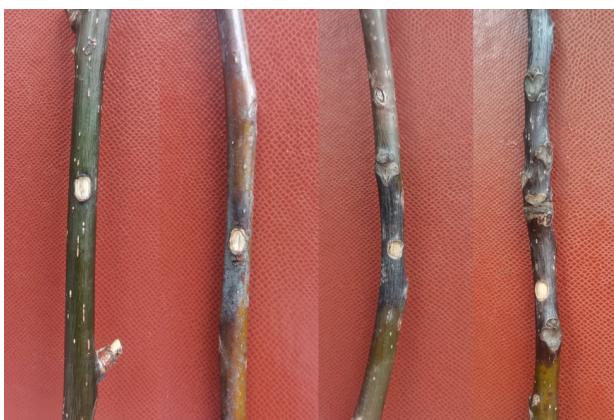
Table 1 The severity of disease of walnut branches infected by walnut canker

样本编号 Sample	类型 Type	发病程度 Disease degree/mm	样本编号 Sample	类型 Type	发病程度 Disease degree/mm
J-1	尖果 Pointed fruit	5.00±0.00 a	XT-2	小椭圆 Small oval	18.60±2.57 abcd
J-2	尖果 Pointed fruit	5.00±0.00 a	XT-3	小椭圆 Small oval	5.00±0.00 a
J-3	尖果 Pointed fruit	11.00±0.65 ab	XT-4	小椭圆 Small oval	5.00±0.00 a
J-4	尖果 Pointed fruit	29.00±2.45 d	XT-5	小椭圆 Small oval	5.00±0.00 a
L-1	卵圆 Eggs round	100.00±14.15 h	XY-1	小圆 Small round	5.00±0.00 a
L-2	卵圆 Eggs round	69.70±3.19 f	XY-2	小圆 Small round	5.00±0.00 a
L-3	卵圆 Eggs round	18.20±1.96 abcd	XY-3	小圆 Small round	25.40±3.41 d
P-1	平底圆 Flat round	14.20±1.80 abc	XY-4	小圆 Small round	5.00±0.00 a
P-2	平底圆 Flat round	19.00±1.80 bcd	XY-5	小圆 Small round	46.10±2.41 e
P-3	平底圆 Flat round	69.30±3.59 f	X-1	心形 Heart	91.30±7.01 g
T-1	椭圆 Oval	47.90±1.80 e	X-2	心形 Heart	45.50±4.32 e
T-2	椭圆 Oval	9.30±0.88 ab	X-3	心形 Heart	44.30±4.24 e
T-3	椭圆 Oval	25.00±2.43 cd	X-4	心形 Heart	42.10±2.81 e
XT-1	小椭圆 Small oval	84.00±7.76 g	X-5	心形 Heart	5.00±0.00 a

注: 同列不同小写字母表示不同菌株间差异显著($p < 0.05$), 表内数据为(平均值±标准误差)。下同。

Note: Different small letters in the same column indicate significant difference between different strains at $p < 0.05$, the data in the Table are the mean ± standard error. The same below.

测定结果表明, 将培养 6 d 的核桃腐烂病菌 PDA 菌饼接种于枝条韧皮部, 25 °C 保温保湿培养 12 d, 能够有效地侵染 1 年生枝条, 且显现出不同程度的差异(图 1)。



图中为部分抗病性差异明显的野核桃资源枝条感染腐烂病的图片, 从左到右依次为高抗、中抗、中感、高感。

The picture shows some wild walnut resource branches infected with canker disease with obvious difference in disease resistance, from left to right: highly resistant, mediumly resistant, mediumly sensitive, and highly sensitive.

图 1 新疆野核桃种质资源感病情况

Fig. 1 The level of infection and canker disease of Xinjiang wild walnuts

由表 1 可见, 28 份材料离体枝条接种核桃腐烂病原菌后发病程度呈明显差异, 其中枝条病斑最小值为 5 mm, 最大值为 100 mm, 枝条表面病斑明显, 病部枝条颜色变黑失水。J-1、J-2、XT-3、XT-4、XT-5、XY-

1、XY-2、XY-4、X-5、T-2、J-3、P-1 的病斑长度均小于 15 mm, 其中除 T-2、J-3、P-1 外, 其余 9 份材料均未形成病斑; L-3、XT-2、P-2、T-3、XY-3、J-4 形成的病斑长度在 15~35 mm 之间; X-4、X-3、X-2、XY-5、T-1 形成的病斑长度在 35~55 mm 之间; P-3、L-2、XT-1、X-1、L-1 形成的病斑长度均超过 55 mm。多重比较结果显示, 枝条病斑最长的 L-1 与未发病的 9 份材料之间差异显著 ($p < 0.05$), 其中侵染病斑长度小于 15 mm 的单株占供试单株的比例较大, 占总供试单株的 42.9%, 而侵染病斑长度超过 55 mm 抗病能力弱的单株, 仅有 5 株 (17.9%)。由核桃腐烂病病原菌形成的病斑长度差异, 反映出野核桃种质间的抗病能力存在差异。

2.2 核桃腐烂病菌对新疆野核桃种质生理指标的影响

接种核桃腐烂病菌 12 d 后, 不同新疆野核桃种质的抗病生理响应表现出差异。表 2 显示, 28 份野

核桃材料中 SOD 酶活性最高为 X-4、XT-2、XY-5、T-1, 且都表现为感病材料, 病斑的最长的 L-1、XT-1、X-1 的 SOD 酶活性最低, 可能随着病原菌侵染, 已达到生理阈值; POD 酶活性最高的为 T-1、XT-1、X-3, 3 个种质均为感病材料, POD 酶活性最低的为 X-5、XT-4、J-1, 且 3 个材料均未发病; CAT 酶活性最高的为 XT-2、L-2、X-1, 其中 L-2、X-1 为重度患病材料; CAT 酶活性最低的为 J-3、J-1、XT-5, 其中 J-1、XT-5 均未发病, J-3 患病较轻; MDA 含量最高的为 XY-3、XT-1、L-1、X-1, 均为感病材料, 且 XT-1、L-1、X-1 3 个材料的病斑长度都表现为较高, MDA 含量最低的为 XT-4、T-2、XY-1、XY-4、XY-2, 均为未发病材料。发病材料枝条的平均 POD 酶活性、SOD 酶活性、CAT 酶活性、MDA 含量相比未发病枝条均有不同程度上升, 分别为未发病枝条 (平均) 的 260.6%、118.9%、283.6% 和 192.6%, 其中 POD 酶活性、CAT

表 2 生理指标测定结果

Table 2 The results of physiological indicators

样本编号 Sample	类型 Type	超氧化物歧化酶活性 SOD activity/(U·g ⁻¹ ·min ⁻¹)	过氧化物酶活性 POD activity/(U·g ⁻¹ ·min ⁻¹)	过氧化氢酶活性 CAT activity/(U·g ⁻¹ ·min ⁻¹)	b(丙二醛) MDA content/(μmol·mg ⁻¹)
J-1	尖果 Pointed fruit	31.67±2.98	127.60±11.35	13.60±1.05	13.18±0.39
J-2	尖果 Pointed fruit	33.57±1.11	205.73±50.16	22.06±3.95	11.70±0.52
J-3	尖果 Pointed fruit	42.92±0.54	234.38±37.19	8.24±1.44	25.74±1.87
J-4	尖果 Pointed fruit	40.34±0.23	1 106.77±238.38	107.98±5.24	18.10±0.69
L-1	卵圆 Eggs round	27.68±0.64	429.69±11.35	115.26±4.94	29.33±3.98
L-2	卵圆 Eggs round	55.14±0.10	1 018.23±497.90	146.19±10.82	27.74±0.68
L-3	卵圆 Eggs round	31.67±1.76	205.73±15.41	55.61±2.52	23.23±1.88
P-1	平底圆 Flat round	40.89±0.95	609.38±168.69	43.03±1.83	13.70±1.27
P-2	平底圆 Flat round	41.32±0.39	161.46±23.87	34.89±1.37	16.44±0.61
P-3	平底圆 Flat round	45.08±0.84	2 190.10±370.59	94.21±3.53	22.61±2.02
T-1	椭圆 Oval	51.45±0.45	3 492.19±368.46	58.32±5.38	21.30±2.53
T-2	椭圆 Oval	33.46±2.02	151.04±15.63	60.08±1.13	7.91±1.09
T-3	椭圆 Oval	48.85±1.02	1 039.06±345.43	59.02±2.45	17.51±1.41
XT-1	小椭圆 Small oval	8.15±1.65	3 385.42±647.64	114.60±23.21	30.17±1.18
XT-2	小椭圆 Small oval	58.74±0.74	328.13±67.71	201.91±5.92	14.97±0.50
XT-3	小椭圆 Small oval	30.61±2.21	945.31±373.07	65.37±3.45	15.84±1.40
XT-4	小椭圆 Small oval	28.76±0.91	106.77±13.53	24.26±2.11	6.02±0.58
XT-5	小椭圆 Small oval	40.8±1.55	1 039.06±410.46	13.34±1.73	13.85±0.62
XY-1	小圆 Small round	39.12±2.21	507.81±67.66	28.87±0.42	8.41±0.40
XY-2	小圆 Small round	30.73±1.61	638.02±131.63	45.67±1.94	8.87±0.32
XY-3	小圆 Small round	37.03±1.32	2 309.90±68.06	81.68±3.62	30.63±0.86
XY-4	小圆 Small round	28.36±1.03	1 158.85±107.09	23.49±2.51	8.64±0.97
XY-5	小圆 Small round	56.33±4.03	1 028.65±40.59	25.94±7.14	22.92±0.40
X-1	心形 Heart	12.21±1.79	2 138.02±485.32	133.87±7.88	28.96±1.64
X-2	心形 Heart	45.17±0.51	1 731.77±258.78	75.34±1.54	14.52±0.42
X-3	心形 Heart	43.86±0.93	2 888.02±746.21	58.19±12.59	25.73±0.75
X-4	心形 Heart	60.57±0.64	2 028.65±256.68	58.39±6.17	19.14±0.57
X-5	心形 Heart	47.63±0.53	83.33±14.73	19.29±5.07	14.46±1.13

注: 表内数据为平均值±标准误差。

Note: Data in the Table are means ± standard error.

酶活性、MDA含量与枝条病斑长度的相关系数达0.765、0.567、0.614(表3),呈极显著正相关($p<0.01$),枝条发病越严重,韧皮部POD活性、CAT活性、MDA含量越高,抗性越差;野核桃发病材料SOD活性相比未发病虽有不同程度提高,但未发现与枝条病斑长度呈显著线性关系。

表3 新疆野核桃生理指标的相关性分析

Table 3 Correlation analysis of physiological indexes of Xinjiang wild walnuts

指标 Index	发病 程度 Disease degree	超氧化 物歧化 酶活性 SOD activity	过氧化 物酶活性 POD activity	丙二醛 含量 MDA content	过氧化 氢酶 活性 CAT activity
发病程度 Disease degree	1.000				
超氧化物歧化酶活性 SOD activity	-0.152	1.000			
过氧化物酶活性 POD activity	0.576**	-0.057	1.000		
丙二醛含量 MDA content	0.764**	-0.061	0.541**	1.000	
过氧化氢酶活性 CAT activity	0.614**	0.015	0.291	0.463*	1.000

注: 相关系数为 Pearson 相关性分析所得,* $p < 0.05$; ** $p < 0.01$ 。下同。

Note: The correlation coefficient is obtained by Pearson correlation analysis, * $p < 0.05$; ** $p < 0.01$. The same below.

2.3 主成分分析法综合评价

2.3.1 新疆野核桃生理指标的主成分分析 对新疆野核桃抗病生理指标进行主成分分析,得到5个主成分集合(表4),由表4所示,前2个主成分集合特征值都大于1,且方差贡献率累计达73.42%,对原始数据解释率较高,可作为评价新疆野核桃抗病性的综合指标。选出前两个主要成分给出的负载矩阵(表5),根据1.4计算公式,利用2个主成分特征值与表5中的相关性负荷量构建评价模型如下:

$$F_1 = -0.5674 \times Z_1 - 0.5601 \times Z_2 - 0.4479 \times Z_3 - 0.4351 \times$$

表4 新疆野核桃抗病生理指标的特征值和贡献率

Table 4 Eigenvalues and contribution rates of disease resistance related physiological indexes of Xinjiang wild walnut

主成分 Principal component	特征值 Eigenvalue	贡献率 Contribution rate/%	累计贡献率 Cumulative contribution rate/%
1	2.663	53.257	53.257
2	1.008	20.164	73.421
3	0.710	14.203	87.624

表5 新疆野核桃抗病生理指标的负荷量

Table 5 Loads of physiological indexes for disease resistance of Xinjiang wild walnut

主成分 Principal component	发病程度 Morbidity level	超氧化物 歧化酶 活性 SOD activity	过氧化物 酶活性 POD activity	丙二醛 含量 MDA content	过氧化 氢酶 活性 CAT activity
主成分1 PC1	-0.926	0.135	-0.731	-0.865	-0.710
主成分2 PC2	0.042	-0.980	0.010	-0.034	-0.210

$$Z_4 + 0.0827 \times Z_5;$$

$$F_2 = 0.0418 \times Z_1 - 0.0339 \times Z_2 + 0.01 \times Z_3 - 0.2092 \times Z_4 - 0.9761 \times Z_5;$$

$Z_1 \sim Z_5$ 为原始数据标准化处理,以主成分对应贡献率作为权重,算出综合得分:

$$F = F_1 \times 0.53257 + F_2 \times 0.20164.$$

其中 $Z_1 \sim Z_5$ 依次代表发病程度、MDA含量、POD酶活性、CAT酶活性、SOD酶活性, F 、 F_1 、 F_2 表示主成分的特征根对应的特征向量的和,通过计算得到不同种质新疆野核桃得分,综合得分值越大,抗病能力越好。表6显示,XT-1的抗病能力最低,达-1.45;为低抗病性。XT-4的抗病能力最高,达1.32,为高抗病单株,其中得分为0.93~1.32的新疆野核桃种质抗病能力较好,为XT-4、J-1、J-2、XY-4、XY-1、XY-2、T-2。

表6 28份新疆野核桃材料抗病能力比较

Table 6 Comparison of disease resistance among 28 Xinjiang wild walnut materials

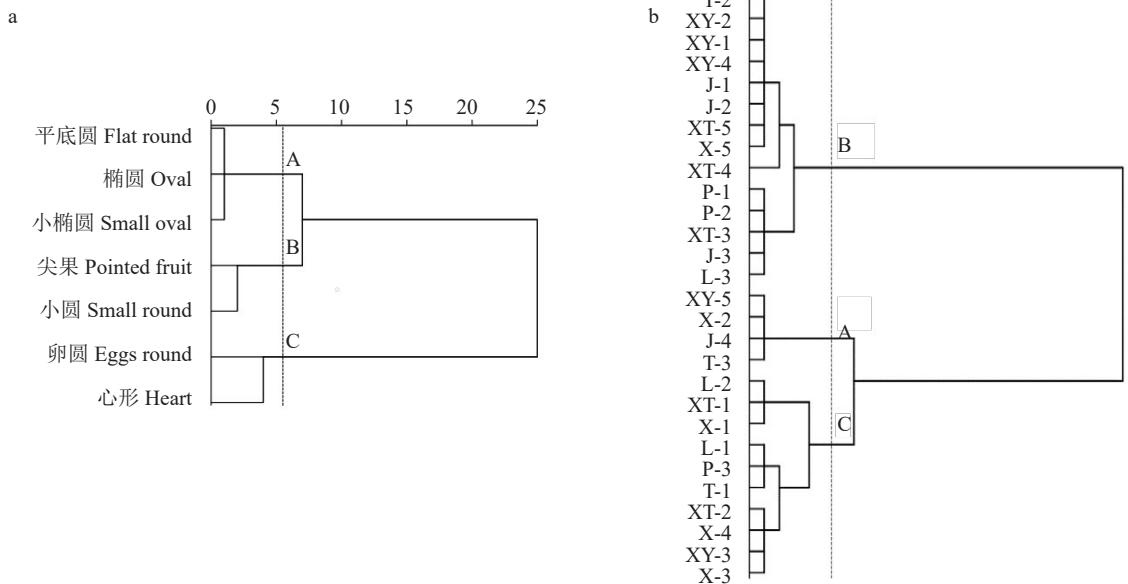
编号 No.	抗病能力 Disease resistance	编号 No.	抗病能力 Disease resistance
XT-4	1.32	T-3	0.01
J-1	1.07	J-4	-0.25
J-2	1.04	X-2	-0.29
XY-4	0.99	XY-5	-0.31
XY-1	0.98	X-4	-0.59
XY-2	0.94	XT-2	-0.62
T-2	0.93	XY-3	-0.78
X-5	0.80	X-3	-0.86
XT-5	0.72	T-1	-0.96
P-1	0.56	P-3	-1.06
P-2	0.55	L-1	-1.13
XT-3	0.49	X-1	1.35
J-3	0.39	L-2	-1.42
L-3	0.28	XT-1	-1.45
J-3	0.39	L-2	-1.42
L-3	0.28	XT-1	-1.45

2.3.2 对新疆野核桃聚类评价 根据表6野核桃抗病能力得分,算出不同类型核桃的综合得分,表7显示,7种类型新疆野核桃抗病能力依次为尖果形>小圆形>小椭圆形>平底圆形>椭圆形>心形>卵圆形。图2显示,28个样本的综合抗病能力在欧式距离4处可分为3类,A类包括XY-5、X-2等4个种质,B类包括XT-4、J-1、J-2等14个种质,C类包括L-2、XT-1等10个种质。结合表1结果可知,其中B类中的野核桃抗病能力最强,相比抗病能力最差的C类中的野核桃抗病能力最强,相比抗病能力最差的C

表7 新疆野核桃不同类型综合抗病能力分析

Table 7 Analysis on comprehensive disease resistance of different types of Xinjiang wild walnut

类型 Type	抗病能力 Disease resistance	类型 Type	抗病能力 Disease resistance
尖果 Pointed fruit	0.56	椭圆 Oval	-0.01
小圆 Small round	0.36	心形 Heart	-0.46
小椭圆 Small oval	0.09	卵圆 Eggs round	-0.76
平底圆 Flat round	0.02		



a. 新疆野核桃不同类型抗病能力聚类图;b. 28份新疆野核桃材料抗病能力聚类图。

a. Cluster map of different types of disease resistance of Xinjiang wild walnut; b. Cluster map of disease resistance of 28 Xinjiang wild walnut materials.

图2 新疆野核桃腐烂病抗性聚类分析

Fig. 2 Cluster analysis of Xinjiang wild disease resistance among

类,各单株受病理侵染胁迫程度较小,且多数未发病,属高抗病类型,A类核桃则属于中间类型。此外由主成分分析综合筛选的7个高抗种质XT-4、J-1、J-2、XY-4、XY-1、XY-2、T-2以及低发病水平的尖果型种质、小圆型种质也多属于B类,显示抗病性较强。

3 讨 论

核桃腐烂病的发生已成为影响新疆核桃树健康生长发育的重要病害之一^[28]。笔者在本研究中测定了新疆野核桃28个种质1年生离体枝条对核桃腐烂病病原菌的抗性,为抗病品种的筛选提供基础依据。结果显示核桃腐烂病菌株对各材料的致病力存在明显差异,表明野核桃单株拥有丰富的抗性变

异。此外,J-1、J-2、XT-3、XT-4等12个野核桃单株在病斑表征上显示为高抗病种质,在7个类型的新疆野核桃中,尖果型表现出比其他类型更高的抗性。

在植物与病原物长期协同进化过程中,植物形成了一系列复杂的防御机制抵御病原物的入侵。植物对病原物侵入的生理反应是以酶的催化活动实现的,体内的POD、CAT、SOD等防御酶都与抗病性有关^[29]。本研究显示,POD、CAT与病斑长度呈极显著正相关,发病程度越严重,枝条韧皮部POD酶、CAT酶活性水平越高,其抗性越差。说明野核桃受到腐烂病病原菌侵染产生了大量的活性氧,高抗品种能够抵御病原菌不被侵染,因此侵入的病原物较少,活性氧产生较低,而感病品种侵入的病原菌较多,导致

活性氧大量爆发。但是活性氧的爆发不仅可以抑制病原菌,还可以损伤植物本身。因此植物识别到活性氧大量爆发,反馈调节,提高了 POD 和 CAT 等酶的数量和活性,以抵御活性氧对树体造成的伤害^[30],这与冯雷等^[31]以及伏荣桃等^[32]基于植物抗病响应所表现出较高酶活性水平的研究结果基本一致。其中感染严重的 L-1 的 POD 酶活性低于平均水平,说明寄主-病原菌互作体系已经稳定,且酶活性一般在组织坏死前增加,至后期感染严重时下降,这与刘琳等^[33]研究结果一致。从植物自身的抗性而言,SOD 的活性水平已被证实与机体自身的抗性密切相关,是植物细胞内抵御活性氧(ROS)伤害的主要保护性酶类,对清除和阻止 ROS 的产生起到重要作用^[7],但也有研究认为二者没有显著线性关系。薛程^[34]研究发现梨树腐烂病病害程度与枝条韧皮部 SOD 活性无显著相关,蒋时姣^[35]通过人工接种核桃黑斑病发现 SOD 活性虽有不同程度提高,但与核桃发病程度无显著相关,这与本研究结果相似。造成此结果可能是当核桃腐烂病菌侵害植株时,活性氧参与了核桃对核桃腐烂病的防御反应,抑制了植株 SOD 酶相关基因上调表达,造成 SOD 酶系统紊乱^[36],且不同的酶系在寄主-病原物互作中的作用存在差异,不同病原物与寄主的组合、同一种酶活性变化也不一致^[37]。因此,一种酶在抗病中的作用不能一概而论,应根据具体情况具体分析。丙二醛是机体内脂质过氧化的最终产物之一,其含量高低可一定程度地反映植物受氧化伤害的程度^[38],笔者在本研究中发现新疆野核桃腐烂病 MDA 含量越高,核桃受侵染程度越严重,这与李波等^[39]研究结果相同。说明野核桃离体茎段在受到病原菌侵染后,MDA 的含量增加,因而加大膜质过氧化作用对细胞的伤害,降低了植物对病害的抗逆能力^[40]。

主成分分析法可以将有限信息的利用发挥到最大化,避免重复信息的干扰,聚类分析的结果满足相同组中数据对象的差距小,不同组中的数据差距大,两种方法结合可以尽可能避免传统人为方法的不足^[41]。早期菌丝在植物细胞壁间蔓延过程中,肉眼观察难以判断,如 XT-4 和 XT-3 的病斑大小一样,但主成分分析的结果中,两个数据却有相差,这可能是由于菌丝已经在 XT-3 韧皮部蔓延,但表征并未显现,以及综合评价中发病单株 T-2 却比未发病单株 X-5 得分高,这可能是由于核桃体内酚醌类物质含

量较高,接种后在伤口处上形成生理性的黑斑,与核桃腐烂病病斑混淆而影响抗性评价^[42]。通过主成分分析利用多个指标综合评价,所得结果可靠性较高,可有效避免人为主观判断的误差。通过核桃腐烂病病原菌侵染不同核桃种质指标的变化,初步确定部分抗病种质,与主成分分析、聚类分析的结果较为一致,其中 XT-1 的抗病能力最低,XT-4 的抗病能力最高。综合分析显示,得分为 0.93~1.32 的新疆野核桃种质抗病能力较好,分别为 XT-4、J-1、J-2、XY-4、XY-1、XY-2、T-2。28 份野核桃材料抗病能力可分为三类,其中 B 类核桃抗病能力最强,C 类抗病能力最差,A 类则属于中间类型。初步筛选出的 7 个高抗种质可作为未来高抗单株选育的优良亲本的备选材料。

4 结 论

28 份新疆野核桃材料 1 年生枝条接种核桃腐烂病后显示出不同的抗病力,通过自然发病情况调查、离体枝条接种、生理指标 POD 和 CAT 酶活性以及 MDA 含量测定对新疆野核桃对核桃腐烂病抗性进行了初步鉴定,筛选出的 7 个抗病种质可为新疆野核桃抗病种质的进一步筛选及抗病基因鉴定打下一定的基础。

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