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Grey Correlative Degree Analysis on the Cold-Resistant Traits of Parthenocarpic Eggplant

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Abstract

In order to breed new eggplant variety with parthenocarpic ability and strong cold-resistance, the six cold-resistant traits of fifteen eggplant resources with parthenocarpic ability were evaluated with grey correlative degree analysis. One non-parthenocarpic resource ‘Shenggao No. 2’ was used as control. The results indicated that ‘29’, ‘32’, ‘30’, ‘XBL’, ‘31-2-1’, ‘TXQ’ and ‘31-2-2’ had strong cold-resistance; ‘HLMQ’, ‘HQ’, ‘ZHQ’, control and ‘HXZ’ had weak cold-resistance; the other four resources had moderate cold-resistance. When suffered cold stress, the resources with strong cold-resistance had a larger increase of SOD, POD, CAT activities and a least increase of MDA content and EL. Among the strong cold-resistant resources, ‘29’, ‘30’, ‘31-2-1’, ‘TXQ’ and ‘31-2-2’ had purplish red fruits and significantly stronger cold-resistance than control, which were suitable for cultivation in south China. ‘32’ and ‘XBL’ with white fruits could be used as breeding materials.

Keywords: eggplant, cold-resistance, parthenocarpy, grey correlative degree analysis, antioxidase

1. Introduction

As an agronomically important solanaceous crop, eggplant (*Solanum melongena* L.) is a kind of thermophile vegetable and is widely grown in both north and south China. It often encounters chilling injury in winter and early spring cultivation, which results in delaying the growth, declining of yield and quality. The optimum growth temperature for eggplant is 22-30 ^{o}C. When temperature is lower than 17 ^{o}C, the eggplant growth will be slowed down; when lower than 10 ^{o}C, the plant will suffer metabolic disturbance (Song, 2011).

Under cold stress, eggplant resources with parthenocarpic ability can bear large number of fruits without pollination while the non-parthenocarpic resources can’t fruit. Parthenocarpy can overcome loosing flowers and fruits, improve yield and reduce cost. Meanwhile, parthenocarpic fruits have no seed, which improves quality (Gao et al., 2012). To mitigate these defects in fruit set under low temperature, the breeding of parthenocarpic variety may be a cost-effective alternative.

Parthenocarpic round eggplant with strong cold-resistance was screened out from local varieties (Liu et al., 2005). Pan et al. (2005) found parthenocarpic material from inbred lines of round eggplant. Some parthenocarpic lines suitable for protected cultivation were selected from abroad eggplants (Zhang et al., 2008; Yang et al., 2009). However, the parthenocarpic lines selected already are suitable for cultivation in north China, the purplish red and long eggplant with parthenocarpic ability and suitable for cultivation in south China has not been selected yet.

The antioxidant enzymes such as peroxidase (POD), superoxide dismutase (SOD) and catalase (CAT) have been extensively studied for their roles in plant defense (Aniszewski et al., 2008; Kong et al., 2015; Shi et al., 2015; Wei et al., 2016). Malondialdehyde (MDA) content, electrolyte leakage (EL) and chilling injury (CI) index are closely related to cold-resistance (Shi et al., 2015; Wei et al., 2016). Grey correlative degree analysis is one of the multi-parameter diagnostic methods, which was conducted to make a comprehensively quantitative evaluation on sixteen eggplant resources according to their six main cold-resistant traits. It would help to know the relationship between parthenocarpy and cold-resistance, which would also lay foundation for breeding new variety with strong cold-resistance and suitable for cultivation in south China.

2. Materials and Methods

2.1 Plant Materials

Sixteen eggplant resources were used as materials, the characters of which were showed in Table 1. 'Shenggao No. 2' without parthenocarpic ability was used as control. The seedlings with four leaves of sixteen eggplant resources were cultivated in incubators.

Table 1. The characters of sixteen eggplant resources

Eggplant resource	With or without parthenocarpic ability	Fruit shape	Fruit color	Eggplant resource	With or without parthenocarpic ability	Fruit shape	Fruit color
HXZ	Yes	Long cylindric	Light purple	31-2-2	Yes	Long cylindric	Purplish red
HL	Yes	Short cylindric	Blackish purple	32	Yes	Long cylindric	White
HQ	Yes	Long stick	Purplish red	ZHQ	Yes	Long cylindric	Purplish red
XBL	Yes	Long cylindric	White	49	Yes	Long cylindric	Green
Control	No	Long cylindric	Purplish red	TXQ	Yes	Long cylindric	Purplish red
29	Yes	Long cylindric	Purplish red	HLMQ	Yes	Long cylindric	Blackish purple
30	Yes	Long cylindric	Purplish red	GQ	Yes	Long cylindric	Blackish purple
31-2-1	Yes	Long cylindric	Purplish red	59-1	Yes	Long cylindric	Blackish purple

2.2 Experimental Treatment and Sample Preparation

Firstly, the seedlings were precultured at 20 ^{o}C/10 ^{o}C (light 12 h/dark 12 h) for 2 days ; then treated at 5 ^{o}C/5 ^{o}C (light 12 h/ dark 12 h) for 4 days; finally, recovered at 30 ^{o}C/20 ^{o}C for 2 days. At the four stages: after preculture (2 d), treated at 5 ^{o}C for 2 days (4 d) and 4 days (6 d), recovered for 2 days (8 d), the leaves were taken for analyzing the activities of antioxidase and MDA content. The weight of each sample was 0.5 g. After weighing, leaves were immediately frozen in liquid nitrogen, then stored at -80 ^{o}C until use. At the above four stages, fresh leaves were taken respectively and immediately used for EL analysis. The experiment was repeated three times (three independent samples).

2.3 CI Index

On the sixth and eighth day, external CI symptoms were visually analyzed. CI was determined according to the following scale: 0 = no damage, 1 = the edge of the leaf wilting, 2 = one leaf wilting, 3 = two leaves wilting, 4 = over two leaves wilting. Observations were made on 20 plants for each resource. The CI index was calculated according to the following equation:

$$\text{CI Index} = \frac{\sum(\text{Injury level} \times \text{Number of plants at that level})}{\text{The highest level} \times \text{total number of plants}} \tag{1}$$

2.4 The Activity of SOD, POD and CAT

For sample preparation, 0.5 g leaves were ground in a mortar with 5 ml 0.2 mol/L phosphate buffer solution (pH = 7.0). The suspension was transferred to a tube, then mixed and centrifuged at 12,000 rpm for 15 min at 4 ^{o}C. The supernatant was collected. Three extracts were done for each resource.

The SOD activity was measured using Total Superoxide Dismutase assay kit (Hydroxylamine method) (Nanjing jiancheng bioengineering institute) following the manufacturer's instructions. SOD activity was calculated as the Equation (2):

$$\text{SOD activity } (u \cdot g^{-1} \text{ fresh weight}) = \frac{2 \times (Ack - Ae) \times Vt \times V2}{Ack \times V1 \times W} \tag{2}$$

Where,

Ack: the absorbance of control; Ae: the absorbance of sample; Vt: the volume of the reaction liquid (ml); W: the fresh weight (FW) of leaves (g); V1: the volume of the enzyme solution in reaction liquid (ml); V2: the total volume of the enzyme extraction (ml).

The POD activity was measured by guaiacol method (Wu et al., 2006), which was calculated as the following equation:

$$\text{POD activity } (\Delta A_{470}\cdot g^{-1}\text{ FW}\cdot \text{min}^{-1}) = \frac{\Delta A_{470} \times V2}{W \times V1 \times t} \tag{3}$$

Where,

ΔA_{470}: the variation of the absorbance during the reaction time; V2: the total volume of the enzyme extraction (ml); W: the FW of leaves (g); V1: the volume of the enzyme solution in reaction liquid (ml); t: the reaction time (min).

The CAT activity was measured by ultraviolet absorption method (Li, 2005), which was calculated as the Equation (4):

$$\text{CAT activity } (u\cdot g^{-1}\text{ FW}\cdot \text{min}^{-1}) = \frac{\Delta A_{240} \times V2}{0.1 \times W \times V1 \times t} \tag{4}$$

Where,

ΔA_{240}: the variation of the absorbance during the reaction time; V2: the total volume of the enzyme extraction (ml); W: the FW of leaves (g); V1: the volume of the enzyme solution in reaction liquid (ml); t: the reaction time (min).

2.5 MDA Content

MDA content was measured using thiobarbituric acid (TBA) method (Ma, 2009). 0.5 g leaves were ground in a mortar with 5 ml 5% trichloroacetic acid (TCA). The suspension was transferred to a tube and centrifuged at 3,000 rpm for 10 min at room temperature. 3 ml supernatant and 3 ml TBA were mixed and boiling for 30 min. The absorbance of supernatant at 450 nm, 532 nm and 600 nm was measured. Blank control: 3 ml TCA + 3 ml TBA. Three extracts were done for each resource. MDA content was calculated as Equation (5):

$$\text{MDA content } (\mu\text{mol}\cdot g^{-1}\text{ FW}) = \frac{[6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}] \times Vt \times V2}{1000 \times V1 \times W} \tag{5}$$

Where,

Vt: the volume of the reaction liquid (ml); V2: the total volume of the enzyme extraction (ml); W: the FW of leaves (g); V1: the volume of the enzyme solution in reaction liquid (ml).

2.6 Electrolyte Leakage

EL was analyzed as described in the experimental guide (Chen & Wang, 2006). Ten discs (1 cm × 1 cm) from the leaves were obtained with a hole-punch and rinsed with deionized water for three times. When dried out with filter paper, the leaves were soaked in 10 ml deionized water for 2.5 h. The conductivity of the bathing solution was measured with a conductimeter (R1). Afterwards, the bathing solution was boiled for 15 min, when cooled to room temperature, the conductivity of the bathing solution was measured (R2). EL was calculated as: EL = R1/R2 × 100%. Measurements were done in triplicate.

2.7 Data Analysis

Microsoft Excel 2010 and DPS v7.05 were used for data collecting and analyzing.

Grey correlative degree analysis: Sixteen eggplant resources could be considered as a grey system, in which each resource was a contributing factor. Now suppose there were i eggplant resources and j main cold-resistant traits which would be analyzed. 'Reference resource' had the best cold-resistant traits, which consisted of the reference data array X_0, and each cold-resistant trait of test resources consisted of Xi accordingly. Kj represented each cold-resistant trait. The averages of cold-resistant traits on the sixth day were showed in Table 2.

Table 2. Main cold-resistant traits of reference resource and test resources

Serial number	Eggplant resource	K_1	K_2	K_3	K_4	K_5	K_6
		SOD activity	POD activity	CAT activity	MDA content	EL	CI index
X_0	Reference resource	1078.000	20.800	76.000	0.000	0.150	0.110
X_1	HXZ	872.600	12.100	43.650	0.018	0.263	0.730
X_2	HL	896.600	19.000	28.280	0.018	0.204	0.460
X_3	HQ	875.600	20.400	31.540	0.019	0.351	1.000
X_4	XBL	951.700	19.570	41.120	0.018	0.156	0.500
X_5	Control	953.700	16.130	39.880	0.025	0.328	0.910
X_6	29	1075.500	20.770	64.140	0.014	0.225	0.217
X_7	30	885.000	14.050	75.880	0.012	0.371	0.140
X_8	31-2-1	963.700	13.040	71.360	0.012	0.285	0.516
X_9	31-2-2	961.400	17.840	64.140	0.009	0.282	0.786
X_{10}	32	1018.900	15.270	72.270	0.015	0.237	0.117
X_{11}	ZHQ	817.000	16.900	52.390	0.008	0.311	0.956
X_{12}	49	908.100	15.220	54.200	0.016	0.206	0.930
X_{13}	TXQ	943.900	11.980	62.270	0.013	0.269	0.317
X_{14}	HLMQ	1026.500	14.150	27.100	0.014	0.206	0.950
X_{15}	GQ	930.800	17.610	42.260	0.015	0.228	0.875
X_{16}	59-1	992.000	16.020	22.580	0.010	0.177	0.875

MDA content, EL and CI index were negative traits, and the data would be converted according to '1-actual data'. For contrast, all the data of cold-resistant traits would be initialized. Equal-weighted correlative degree would be calculated according to the equation below:

$$\varepsilon_i(k) = \frac{\min_{i \dots}\min_{k}|x_0(k) - x_i(k)| + \rho\max_{i \dots}\max_{k}|x_0(k) - x_i(k)|}{|x_0(k) - x_i(k)| + \rho\max_{i \dots}\max_{k}|x_0(k) - x_i(k)|} \tag{6}$$

$$r_i = \frac{1}{n}\sum_{k=1}^{n} \varepsilon_i(k) \tag{7}$$

In the formula, the letter 'n' stands for the number of cold-resistant traits,'$\varepsilon_i(k)$' stands for the correlative coefficient of X_i to X_0, 'ρ' represents identification coefficient (artificial setting 0.5), and 'r_i' represents the equal-weighted correlative degree of test resources (Table 2).

3. Results

3.1 Chilling Injury Index

On the sixth day, the CI indexes of '30', '32', '29', 'TXQ', 'HL', 'XBL', '31-2-1', 'HXZ' and '31-2-2' were significantly lower than that of control, which indicated they had stronger cold-resistance than control (Figure 1). However, the CI indexes of 'HQ', 'ZHQ' and 'HLMQ' were significantly higher than that of control, and visible symptoms of CI were detected in 'HQ'. When recovered for 2 days, only the CI index of 'HL' kept increasing to 0.74, which suggested the recovery capability of 'HL' was relatively weak; the CI index of other resources decreased rapidly.

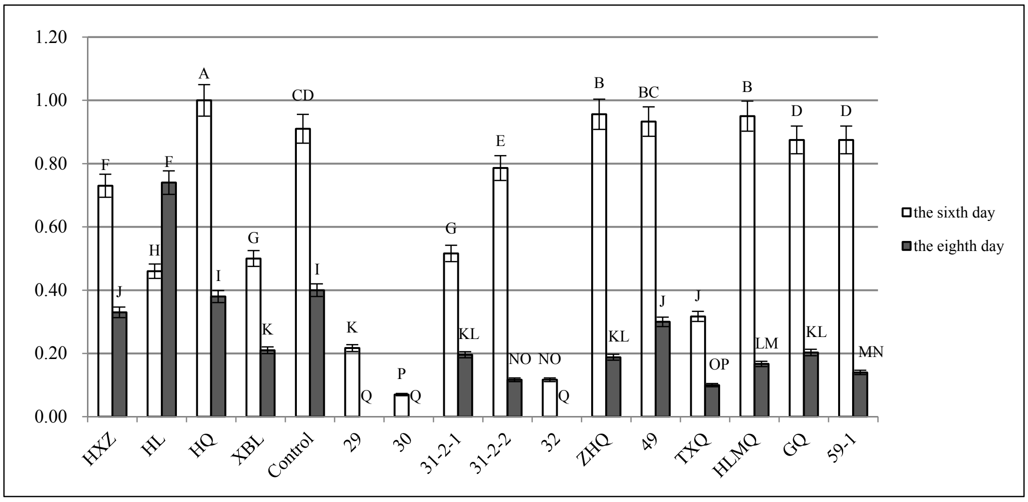

Figure 1. Chilling injury index of sixteen resources on the sixth and eighth day

Note. The capital letters show significantly different ($P < 0.01$). Percent error line (5%).

3.2 The SOD, POD and CAT Activities

Under cold stress, SOD, POD and CAT activities of all resources increased (Appendix A-C). However, the SOD, POD and CAT activities of some resources reached the peak on the fourth day, some on the sixth day. The resources with stronger cold-resistance had a larger increase of SOD, POD and CAT activities than those with weaker cold-resistance.

3.3 MDA Content and EL

At the first three stages, the MDA content and EL of all resources increased (Appendix D-E), when recovered for 2 days, all MDA content and EL decreased. The resources with stronger cold-resistance had a least increase of MDA content and EL, which suggested they had the least damage.

3.4 Grey Correlative Degree Analysis

According to the equal-weighted correlative degree, the test resources were ranked (Table 3). The results showed '29' had the largest correlative degree (r = 0.899) with the reference resource, which revealed '29' had the strongest cold-resistance, followed by '32' and '30', with the value of 0.877 and 0.820 respectively. 'HXZ' had the smallest correlative degree of 0.665, which was smaller than that of control.

Table 3. The equal-weighted correlative degree of test resources

Serial number	Eggplant resource	Equal-weighted correlative degree	Rank	Serial number	Eggplant resource	Equal-weighted correlative degree	Rank
X6	29	0.899	1	X15	GQ	0.712	9
X10	32	0.877	2	X12	49	0.710	10
X7	30	0.820	3	X16	59-1	0.710	10
X4	XBL	0.787	4	X14	HLMQ	0.695	12
X8	31-2-1	0.759	5	X3	HQ	0.689	13
X13	TXQ	0.754	6	X11	ZHQ	0.680	14
X9	31-2-2	0.752	7	X5	Control	0.673	15
X2	HL	0.744	8	X1	HXZ	0.665	16

3.5 Cold-Resistance Grade Classification

According to the correlative degree (Table 3), test resources could be divided into three grades. When the correlative degree was over 0.750, the test resources had strong cold-resistance, so '29', '32', '30', 'XBL', '31-2-1', 'TXQ' and '31-2-2' had strong cold-resistance. When the correlative degree was more than 0.700 and less than 0.750, the test resources had moderate cold-resistance, 'HL', 'GQ', '49' and '59-1' belonged to this kind. When less than 0.700, the test resources had weak cold-resistance.

4. Discussion

Visible symptom of chilling injury is wilting of leaves. CI index had significant negative correlation with cold-resistance, which was also an important index to evaluate the cold-resistance in seedling stage (Wang et al., 2015b). The result of CI index was highly consistent with that of grey correlative degree. One main difference was 'HL' had strong cold-resistance according to CI index and moderate cold-resistance according to the latter. So combined with other cold-resistant traits, CI index could evaluate more objectively.

In order to clear away the harmful materials caused by cold stress, the activity of antioxidase such as SOD, POD and CAT will increase rapidly (Fu et al., 2010; Hao et al., 2014; Yan et al., 2011). The variety with strong cold-resistance had a larger increase of SOD and POD activities (Zhang & Yan, 2013).Wang et al. (2013) reported that rice with extremely strong chilling-tolerance had a bigger jump of SOD and POD activities than chilling-sensitive rice. Under cold water stress, CAT activity of chilling-sensitive rice and chilling-tolerant rice increased significantly (Wang, 2015). Jia et al. (2011) reported that CAT activity of wild Lily species with strong cold-resistance increased and maintained a higher level when treated at 11 ^{o}C/5 ^{o}C (day/night). The results in this work were in accordance with these reports.

Low temperature is harmful to the cell membrane system which causes the selectivity of cell membrane lost and MDA produced. Electrolyte and small molecule organic compounds will permeate out, which lead to the increase of relative conductivity. Under cold stress, materials with strong cold-resistance had a smaller change in MDA content and EL (Zhang et al., 2012, 2013; Wang et al., 2013).

Grey correlative degree analysis is one of the multi-parameter diagnostic methods, which was applied in many crops (Yang et al., 2012; Wang, 2013; Long, 2013; Xing, 2015; Lu et al., 2016; Wang et al., 2015a; Chen et al., 2016). Through grey correlative degree analysis, Chen et al. (2015) selected two varieties which were better than control in agronomic traits and disease-resistance. In this work, cold-resistance of sixteen resources was analyzed. Among the fifteen resources with parthenocarpic ability, only one resource had weaker cold-resistance than control, so most of the resources with parthenocarpic ability had strong cold-resistance.

According to the equal-weighted correlative degree, the resources were divided into three grades. Seven resources had strong cold-resistance and five resources including control had weak cold-resistance. '29', '30', '31-2-1', 'TXQ' and '31-2-2' had purplish red fruits and significantly stronger cold-resistance than control, which were suitable for cultivation in south China. '32' and 'XBL' with white fruits could be used as breeding materials.

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Appendix

Appendix A. The mean values of CAT activity

CAT activity	2 d	4 d	6 d	8 d
HXZ	28.80	34.40	43.65	17.70
HL	24.88	42.60	28.28	29.28
HQ	30.68	33.80	31.54	34.46
XBL	18.36	26.52	41.12	25.48
Control	25.24	34.8	39.88	29.82
29	25.29	72.27	64.14	19.88
30	26.65	57.81	75.88	18.07
31-2-1	29.81	69.56	71.36	44.26
31-2-2	36.13	69.56	64.14	13.55
32	32.07	60.52	72.27	19.88
ZHQ	14.36	50.59	52.39	24.39
49	37.94	45.17	54.20	28.91
TXQ	44.26	72.27	62.27	23.49
HLMQ	18.07	30.71	27.10	14.45
GQ	26.20	29.81	42.26	13.55
59-1	16.26	20.78	22.58	26.20

Appendix B. The mean values of POD activity

POD activity	2 d	4 d	6 d	8 d
HXZ	5.05	10.60	12.10	8.20
HL	6.64	11.30	19.00	15.60
HQ	13.27	16.54	25.50	19.50
XBL	6.70	11.10	20.30	18.50
Control	13.70	15.60	16.13	26.70
29	8.16	19.40	20.77	21.64
30	5.61	12.29	14.05	12.11
31-2-1	6.02	7.54	13.04	19.53
31-2-2	4.54	6.67	17.84	15.16
32	6.32	14.36	15.27	15.75
ZHQ	9.96	14.12	16.90	18.26
49	8.03	13.39	15.22	22.06
TXQ	5.85	10.96	11.98	12.29
HLMQ	11.04	19.45	14.15	25.95
GQ	8.70	20.83	17.61	17.47
59-1	4.13	9.41	16.02	18.53

Appendix C. The mean values of SOD activity

SOD activity	2 d	4 d	6 d	8 d
HXZ	719.7	977.1	916.2	930.9
HL	623.1	717.6	941.4	832.2
HQ	789.1	895.2	919.4	834.3
XBL	716.6	954.0	999.3	842.7
Control	885.8	953.0	1001.4	904.7
29	684.0	983.9	1075.5	844.6
30	460.0	861.0	885.0	731.0
31-2-1	714.0	906.0	963.7	838.5
31-2-2	834.9	1007.0	961.4	966.0
32	616.7	754.0	1018.9	846.5
ZHQ	702.0	718.7	817.0	900.0
49	717.0	757.7	908.1	858.0
TXQ	670.3	742.6	943.9	822.0
HLMQ	950.0	999.0	1026.5	1040.9
GQ	768.6	957.5	930.8	706.3
59-1	726.1	835.0	992.0	974.8

Appendix D. The mean values of MDA content

MDA content	2 d	4 d	6 d	8 d
HXZ	0.0100	0.0114	0.0178	0.0110
HL	0.0122	0.0134	0.0180	0.0151
HQ	0.0080	0.0163	0.0193	0.0080
XBL	0.0060	0.0080	0.0176	0.0081
Control	0.0110	0.0141	0.0250	0.0206
29	0.0070	0.0118	0.0135	0.0120
30	0.0062	0.0103	0.0117	0.0114
31-2-1	0.0059	0.0125	0.0115	0.0072
31-2-2	0.0122	0.0156	0.0086	0.0081
32	0.0092	0.0117	0.0153	0.0081
ZHQ	0.0033	0.0115	0.0076	0.0050
49	0.0094	0.0114	0.0164	0.0093
TXQ	0.0111	0.0137	0.0129	0.0071
HLMQ	0.0103	0.0111	0.0141	0.0122
GQ	0.0059	0.0061	0.0152	0.0023
59-1	0.0099	0.0165	0.0100	0.0054

Appendix E. The mean values of EL

EL	2 d	4 d	6 d	8 d
HXZ	0.113	0.33	0.263	0.209
HL	0.112	0.337	0.204	0.156
HQ	0.176	0.416	0.351	0.184
XBL	0.094	0.250	0.156	0.103
Control	0.108	0.386	0.328	0.166
29	0.192	0.179	0.225	0.183
30	0.156	0.171	0.371	0.235
31-2-1	0.051	0.117	0.285	0.134
31-2-2	0.081	0.224	0.282	0.199
32	0.203	0.183	0.237	0.161
ZHQ	0.189	0.243	0.311	0.206
49	0.158	0.203	0.206	0.185
TXQ	0.182	0.266	0.269	0.175
HLMQ	0.294	0.203	0.206	0.195
GQ	0.071	0.159	0.228	0.127
59-1	0.194	0.201	0.177	0.166