Chilling resistance of corn and cold stress responses of salicylic acid-treated corn

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ABSTRACT: Breeding chilling-resistant corn coupled with the use of exogenous hormones to mitigate the damage caused by stress is important in successful corn farming. Here, we evaluated the germination rate of 45 corn varieties and profiled their chilling resistance level. Besides, to determine the effect of salicylic acid (SA) treatment on plants under cold stress, we treated the corn with SA and then selected chilling-resistant and chilling-sensitive varieties. Our data demonstrated that the germination energy (GE), germination percentage (GP), and germination index (GI) for most of the cold treated corn varieties were lower than those of the control group. The membership function (MF) and D-value results showed that *HengYu709* was a chilling resistant corn variety while *DongDan213* was sensitive to the chilling stimuli. Furthermore, treatment with SA enhanced the chilling resistance in both *HengYu709* and *DongDan213*. In addition, the data showed that 0.5 or 1.0 mmol/l SA treatment significantly increased photosynthesis and oxidase activity in corn under cold stress. However, a high concentration of SA could reduce the photosynthesis indexes. Taken together, our data demonstrate that SA treatment could alleviate the effect of cold stress in corn growth and development.

KEYWORDS: chilling stress, germination, salicylic acid, chilling resistance, maize

INTRODUCTION

Corn (Maize), a major food crop in many parts of the world [1], is the third most important food crop in China after wheat and rice [2]. Since corn is a warm-climate plant, chilling is one of the abiotic stimuli that influences its growth and development [3]. Therefore, effective measures to mitigate the damage caused by cold stress are crucial in corn production. The sensitivity of corn to temperature depends on the developmental stage. It has been reported that abiotic stress leads to accumulation of reactive oxygen species (ROS), which may cause oxidative damage in plant cells [4,5]. Under natural and robust artificial selection, plants have gradually developed defense mechanisms against oxidative stress [6]. With the development of various planting technologies, the use of exogenous hormones has proven effective in the alleviation of the damage caused by abiotic stress [7].

Salicylic acid (SA) is an endogenous regulator of plant metabolism and plays various roles in physiological and metabolic processes in plant cells [8]. Exogenous application of SA regulates the functions of antioxidative enzymes and escalates plant tolerance to abiotic stresses [9]. In addition, SA participates in the signaling processes associated with abiotic stress responses such as high or low temperatures, drought, or salinity [10]. Besides, previous studies have shown that SA pre-treatment could considerably counter the harmful impact of heat and high light stress on PSII in wheat leaves as well as stimulation of photosynthetic functions [11]. In addition, SA treatment can alleviate the damage caused by low temperatures. The effect of SA on low temperature stress was first demonstrated by addition of 0.5 mM SA to the hydroponic growth solution of maize seedlings under normal growth conditions. The data showed that SA substantially reduced damage of maize in subsequent chilling stress [12]. Besides, SA treatment increased the activity of glutathione reductase and guaiacol peroxidase, which could partially explain the increased cold tolerance [13]. To cope up with excessive production of ROS in plant cells, the activities of the superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) were shown to be activated in plant leaves under chilling stress [14]. Xu et al [15] noted that different varieties have different enzyme-specific oxidation activities in response to the chilling stress. Because higher activities of antioxidant enzymes and higher content of non-enzymatic antioxidants under stress are associated with genotype-dependent tolerance to chilling, it is important to evaluate the response of different crop cultivars to abiotic stress [16]. However, data on the use of SA to resist low temperature stress in crop production, especially corn, remain scant. Besides, there is limited knowledge on the response of different corn varieties to SA. Here, we tested the germination rate of 45 corn varieties and profiled their chilling resistance levels. We then subjected the chilling-resistant and chilling-sensitive varieties to SA treatment in order to evaluate the effect of SA treatment on plants under cold stress. We aimed to unearth an effective method that could be used in ranking cold resistance and reveal the genotype differences between cold-resistant and

cold-sensitive corn varieties under chilling stress.

MATERIALS AND METHODS

Assessment of low-temperature resistance

This study was conducted at the Crop Research Center of Jilin Agricultural University, Changchun, China. To screen cold-tolerant corn varieties, a total of 45 corn varieties in Northeast China were collected (Table S1). The corn seeds were surface sterilized in 1% sodium hypochlorite for 10 min and then washed with distilled water. Corn seed germination was evaluated by randomly placing 20 seeds in a petri dish (9 cm in diameter) containing 3 filter papers moistened with distilled water. A total of 9 germination dishes were randomly set up and placed in 3 artificial climate chambers. The experiment was conducted in triplicate with 20 repetitions in each artificial climate chamber. The light/dark (12 h/12 h) cycles, relative humidity (RH, 100%), or light intensity (100 lx) in the 3 artificial climate chambers were kept constant. On the other hand, the temperature of the 3 artificial climate boxes was set to 10°C (group 10°C), 15°C (group 15°C), or 25°C (group control) as shown in Fig. S1. The germination energy (GE, the percentage of normal germinated seeds in all tested seeds at the 3rd day), germination percentage (GP, the percentage of normal germinated seeds in all tested seeds at the end of the test) [17], and GI ($[G_1/1] + [G_2/2] + ... + [G_x/x]$); where G is the germination day 1, 2, ..., and xrepresents the corresponding day of germination [18] were calculated. Based on the germination test data, we determined the low-temperature resistance grade.

Corn treatment with salicylic acid

Following the corn germination tests, cold-tolerant (HengYu709) or cold-sensitive (DongDan213) corn was treated with SA to evaluate its ability to shield the plants from cold stress. A total of 160 HengYu709 and 160 DongDan213 corn plants were used in this study. Seeds were sown in plastic soil-filled pots with each pot having single seedlings. The soil substrate had pH 6.8, organic matter (OM) 12.1 g/kg, total nitrogen (TN) 1.093 g/kg, total phosphorus (TP) 381.83 mg/kg, alkaline hydrolyzable nitrogen (AhN) 65.27 mg/kg, available phosphorus (AP) 10.68 mg/kg, and available potassium (AK) 103.84 mg/kg. Conventional fertilization was implemented during planting such as the application of 2.5 g of urea, 1.2 g of diammonium phosphate, and 1 g of potassium chloride. At the booting stage, 150 plant samples from each variety were randomly divided into 5 groups (10 biological repeats in each) and then sprayed with 5 different concentrations of SA: 0.0 (control group, marked as CK), 0.5, 1.0, 1.5, or 2.0 mmol/l; 3 times a day (20 ml per day). After 2 days of SA treatment, the plants were randomly divided into 2 groups (5 plants in each group). One group was placed in an artificial climate chamber at 10 °C while the other was placed in an artificial climate chamber at 15 °C. After 5 days of stress, the flag leaves of the plants were harvested for subsequent experiments (Fig. S2).

Leaf gas exchange measurement

To test for the presence of measurement biases in the introduced T_{leaf} - T_{air} , net photosynthetic rate (Pn), transpiration rate (Tr), and stomatal conductance (Gs) were measured by LI-6400 XT portable photosynthesis system (LI-COR, Lincoln, NE, USA) with expanded temperature control kits.

Measurement of chlorophyll fluorescence parameters

The leaves were used for gas exchange measurement, and chlorophyll fluorescence parameters were evaluated using a pulse modulation fluorometer (FMS-2, Hansatech, UK) following the previously described protocol by Yin et al [19]. Before the chlorophyll fluorescence assays, the plants were dark-adapted for 25 min. Minimum fluorescence (F₀) was measured with a beam of light having an intensity of less than $0.05 \ \mu mol/m^2 s$. On the other hand, maximum fluorescence (F_m) was obtained by measuring chlorophyll fluorescence during a 2.5 s pulse of saturating light $(18\,000 \,\mu mol/m^2 s)$. Maximal PSII photochemical efficiency (F_v/F_m) and variable fluorescence (F_v) were also recorded [20]. The quantum efficiency of PSII (Φ PSII) was calculated using $(F'_m \pm F_s)/F'_m$ [21] while photochemical quenching (qP) was calculated using $(F'_m \pm F_s)/(F'_m \pm F_0)$ [22].

Determination of SOD, POD, and CAT

Enzyme activity was determined in 0.1 g of the leaves. Superoxide dismutase (SOD) activity was detected based on the inhibition of the photochemical reduction of nitro blue tetrazolium (NBT) [23]. Catalase (CAT) activity was obtained spectrophotometrically by measuring the decomposition of 45 μ M H₂O₂ in 0.05 M phosphate buffer (pH 7.0) at 240 nm [24]. On the other hand, peroxidase (POD) activity was estimated spectrophotometrically by measuring the increase in absorbance of the product of the reaction in 30% H₂O₂, 99% 2-methoxyphenol, and 0.05 M phosphate buffer (pH 6.0) at 470 nm [24]. In addition, we adopted the glucosinolates barbituric acid colorimetric method to determine Malondialdehyde (MDA) content [25]. Antioxidant enzyme activities and MDA content were determined following the instruction in the assay kit.

Comprehensive evaluation

Membership function (MF) and D-values were used to assess the seed germination chilling resistance index, following the previously described protocols [26, 27]. Besides, the relative trait values (RTV), % RTV = $X_L/X_N \times 100\%$, were also calculated. X_L represents

the index value measured at low temperature while $X_{\rm N}$ represents the index value measured at normal temperature.

RESULTS

Relative trait values (RTV) for GE, GP, and GI

To test the chilling stress response, we subjected the corn seedlings to low temperature stimuli. Our analysis showed that at 15 °C, the GE, GP, or GI values of most of the plants decreased compared with the control (RTV < 100%). However, several indicators in some plant materials were improved compared to the control (RTV > 100%) (Table S1). DongDan213had the highest GE value while ChangDan297 had the lowest GE value at 15 °C. On the other hand, JunDan6 and HengYu709 had the highest GI values while LvYu4117 and DongDan213 had the lowest GI values of all the varieties. Germination tests at 10 °C showed that HengYu709 and LiangYu99 had the highest and lowest GE, GP, and GI values, respectively. Besides, HengYu709 had a relatively high GI value at 15 °C and 10 °C. Thus, it was feasible to speculate that HengYu709 has a strong tolerance to cold stress during germination. Interestingly, while DongDan213 had the highest RTV for GE value at 15 °C, the values decreased sharply at 10 °C. In addition, the DongDan213 GI value was relatively low. Taken together, we show that DongDan213 was sensitive to cold stress during germination.

Membership function (MF) for GE, GP, and GI

To increase the confidence in the evaluation data, the membership function was established based on the GE, GP, and GI values. The larger the MF value, the stronger the resistance of the variety to cold stimuli. At 15°C, Jundan6, ChuangShi998, LiMin33, HW217, JiuDan100, LiNong24, DayuM737, JiDan33, HengYu709, and TieYan36 had higher GI values, thus these varieties were more resistant to cold stress. On the other hand, JiDan137, PingAn185, WeiKe3757, XianYu335, MeiHeDa529, W1521, JiDan50, LiangYu88, FuYou10, and LuYu4117 showed sensitivity to the low-temperature stress at 15 °C (Table S2). Further reduction of the temperature (10°C) led to higher GI values in Hengyu709, DaYuM737, JiNongDa885, LiMin33, JiDan47, ZhengDan958, JiDan550, HW217, HeYu3, and JiDan87, demonstrating more resistance to the cold stress. Conversely, XianYu335, ChangDan297, JiDan33, XianYu508, LuYu4117, LuAo150, LiangYu88, FuYou10, LiangYu66, and LiangYu99 were susceptible to the cold stress at 10 °C (Table S2).

Comprehensive evaluation of the cold tolerance using D-value

Comprehensive evaluation value or recovery index (D) for cold tolerance was computed at germination. Based on the D-value, the cold-tolerance capacity for every variety can be ranked; the larger the value of D, the stronger the cold tolerance. Among the examined varieties, *HengYu709* had the greatest mean D-value (6.96) while *LvYu4117* had the lowest mean D-value (0.8). Clustering of the varieties based on the D-values showed that *HengYu709*, *DaYuM737*, and *JiDan47* belonged to level I; *YanFeng508* and *ZhengDan958* belonged to level II; *DongDan213* and *DeDan1002* belonged to level III while *LiangYu66* and *LiangYu99* were clustered in level IV (Fig. 1). Taken together, data from the 3 evaluation indexes robustly demonstrated that *HengYu709* was more resistant to a low-temperature environment while *DongDan213* was more sensitive to the low temperatures.

Effects of salicylic acid treatment on leaf gas exchange

Here, we used HengYu709 and DongDan213 to assay the effect of SA treatment on the plants' response to cold. Cold stress significantly decreased the Pn value of flag leaves of both corn varieties; the lower the temperature, the lower the Pn value. An appropriate concentration of SA treatment was shown to significantly alleviate the decrease of Pn caused by the low temperature stress. At 15 °C, the plants' Pn value peaked at 0.5 mmol/l SA treatment while at 10°C, HengYu709 and DanDong213 attained Pn value peaks at 1.0 and 1.5 mmol/l SA treatment, respectively. For the cold-sensitive variety, the Pn value of DanDong213 at 15°Cwas significantly increased with 0.5 mmol/l SA treatment compared with 0 mmol/l, which was even higher than that at normal temperature (Fig. 2a). However, too high concentrations of SA lead to decreased Pn values. The Tr values showed similar trends as the Pn values. Cold stress reduced the Tr values for both corn varieties. Whereas the Tr value increased under low-temperature stress, it decreased after exceeding 1.0 mmol/ m^2 s (Fig. 2b). In addition, the Gs value for the plants at 15 °C peaked at 0.5 mmol/l SA treatment. Importantly, the Gs values for HengYu709 and DanDong213 peaked at 1.0 and 1.5 mmol/l SA treatment, respectively, at 10 °C (Fig. 2c).

Effects of salicylic acid treatment on chlorophyll fluorescence parameters

An increased concentration of SA at 15 °C led to the initial rise of F_v/F_m , Φ PSII, and qP values, which declined afterwards. Treatment with 0.5 mmol/l SA had the largest value of chlorophyll fluorescence parameters. There was, however, a negative effect when the SA concentration was increased to 2.0 mmol/l. No significant difference was found in F_v/F_m , Φ PSII, or qP between the 2 corn varieties at a given SA concentration (Fig. 3). At 10 °C, the F_v/F_m and Φ PSII values increased significantly compared with the unsprayed control. Furthermore, at the 10 °C environment coupled with the increased SA concentration,



Fig. 1 D-value evaluation of corn germination index. Different colors indicate different chilling resistance grades.



Fig. 2 Effects of salicylic acid on gas exchange parameters of corn leaves under chilling stress.

Fig. 3 Effects of salicylic acid on chlorophyll fluorescence parameters of corn leaves under chilling stress.

the F_v/F_m , Φ PSII, or qP value showed initial increase *Dan213*, respect before a decline. At 10 °C, the qP value peaked at 1.0 ment groups, the

or 1.5 mmol/l SA treatment in *Hengyu709* or *Dong-Dan213*, respectively. In the 0–1.0 mmol/l SA treatment groups, the qP value of the *HengYu709* leaves was



Fig. 4 Effects of salicylic acid on protective enzyme activities of maize leaves under chilling stress.

significantly higher than that of the *DongDan213* at a given concentration (Fig. 3).

Effects of salicylic acid treatment on SOD, POD, and CAT

There was no significant difference in the SOD activity between the 2 corn varieties under normal conditions. The POD and CAT activities in *HengYu709* were significantly higher than those in the *DongDan213*. Besides, treatment with SA at 15 °C had little effects on leaf SOD activity. However, 0.5 mM of SA treatment could significantly increase leaf POD and CAT activities. Moreover, at 10 °C coupled with the increased SA concentration, there was initial rise of SOD and POD activities, followed by a decline (Fig. 4).

DISCUSSION

Bud formation is one of the most sensitive stages to temperature in the growth and development of corn. Previous studies reported that identification of cold tolerance at bud formation might reflect field experiment [28]. On the other hand, seed germination is a dynamic and ordered process involving several biochemical pathways [29], which can be affected by abiotic stresses. Here, we evaluated the germination efficiency of different varieties of corn and their response to abiotic stimuli. The germination tests showed that the GE, GP, and GI values of most coldexposed corn varieties were lower than those in the control group, indicating that cold stress has a negative impact on seed germination. Interestingly, at 15 °C, the GE, GP, and GI values of some varieties was even higher than those at 25 °C but declined sharply at 10 °C. Thus, the GE, GP, and GI values are not sufficient in the assessment of cold resistance. To better dissect the response, we used the MF and D-value methods, which had been used in previous studies [30, 31]. The MF and D-value data was consistent with the cold resistance profile of the plants. Using the D-value data, we classified all the corn varieties into 4 grades, which were close to the production practices.

We then used SA to gain more insights into the role of hormones in plant growth. SA, an omnipresent plant growth hormone with additive effects on plant growth and development, can increase photosynthetic rate [32], regulate the antioxidant defense system through alleviation of oxidative stress [10], and improve PRO production [33]. To interrogate the effects of cold stress as well as the SA regulation on photosynthesis, we investigated gas exchange together with chlorophyll fluorescence parameters. Our data revealed that the corn plants, especially lowtemperature sensitive corn (DongDan213), subjected to cold stress experience sharp decline in photosynthesis. Besides, under a low temperature environment, most of the varieties showed a significant decrease in Pn value [34]. It has been reported that SA treatment could enhance low temperature resistance in many plant species such as rice, maize, wheat, and potato [35]. Sayyari et al [36] showed that exogenous application of SA can increase growth parameters and chlorophyll content in watermelon exposed to low temperatures, which was consistent with our findings. However, too high concentrations of SA reduced the photosynthesis indexes of the corn varieties under study. Previous studies reported that the Arabidopsis SA hyperaccumulation mutants showed susceptibility to cold stress, which might be due to the cell elongation and reduced cell proliferation [37]. In their study, Yang et al [38] reported that high concentrations of exogenous SA could reduce the activity of antioxidant enzymes, which weakened the ability of watermelon to confer resistance to cold. Similarly, our data showed that the treatment with 2.0 mmol/l of SA could reduce the activity of SOD, PRO, or CAT [38]. In addition, we found that 0.5 or 1.0 mmol/l SA treatment significantly increased photosynthesis and oxidase activity in corn under cold stress. Upon subjection to cold stress, there was accumulation of reactive oxygen species (ROS) in plant cells which could further induce membrane lipid peroxidation, thus leading to cell death or stagnation of plant growth and development [39]. The use of SA on cucumber seedlings under cold stress was shown to inhibit the accumulation of H_2O_2 [40]. Exogenous SA activates the antioxidant enzymes which remove excess H_2O_2 , thus improving the cold resistance by the cucumber seedlings. Under cold stress, maize varieties with high cold resistance activate their own resistance to adversity. The cold resistant corn consumes excess light energy through processes such as the lutein cycle or photorespiration to form self-protection. However, under strong cold stress, parts of the PSII response centers in corn leaves were damaged, which hindered their growth and development. In agreement with previous observations [41], we showed that SA treatment could alleviate the effects of cold stress and improve photosynthesis in corn.

CONCLUSION

Taken together, our data demonstrates that evaluation of seed germination rate at low temperature can reflect the chilling resistance of corn varieties. Besides, the use of comprehensive evaluation D-value method closely illuminates the production practice experience. In addition, SA treatment can significantly improve the cold tolerance of different corn varieties.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at http://dx.doi.org/10.2306/scienceasia1513-1874. 2022.018.

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Appendix A. Supplementary data

There were 45 varieties in total, and 3 groups of germination tests were carried out for each variety

Fig. S1 Experimental design for the germination tests. The circle represents the germination Petri dishes.



Fig. S2 Experimental design for the salicylic acid (SA) treatment.

	15 °C			10 °C		
Variety	RTV for GE	RTV for GP	RTV for GI	RTV for GE	RTV for GP	RTV for GI
	V _{15°C} , V _{25°C} ,	V _{15°C} , V _{25°C} ,	V _{15°C} , V _{25°C} ,	V _{10°C} , V _{25°C} ,	V _{10°C} , V _{25°C} ,	V _{10°C} , V _{25°C} ,
DeDan1002	105.56	96.49	121.82	31.58	61.40	54.41
JiSheng385	100.00	101.75	136.92	51.16	70.18	53.43
W1521	300.00	53.13	53.19	38.89	87.50	74.80
HW217	39.53	108.16	176.35	52.94	89.80	73.95
HengYu709	51.67	105.26	207.41	183.87	103.51	91.87
WeiKe3757	136.84	68.75	79.68	19.23	39.58	31.75
YanFeng508	108.89	91.07	141.68	63.27	80.36	58.84
FuYou10	258.33	43.48	57.72	38.71	56.52	46.49
ZhengDan958	79.59	96.30	162.04	48.72	92.59	69.18
ZhongDi77	75.68	82.69	140.04	28.57	44.23	43.87
PingAn185	200.00	73.58	74.57	75.00	84.91	67.08
DongDan213	416.67	87.23	52.60	32.00	53.19	48.41
HeYu3	366.67	89.13	65.49	69.70	89.13	71.47
JunDan6	56.76	171.88	245.26	14.29	34.38	31.53
LiNong24	341.67	107.41	75.05	9.76	29.63	22.29
ChuangShi998	50.00	123.91	122.39	155.56	58.70	59.95
XH84	46.67	78.57	92.98	142.86	53.57	52.78
DaYuM737	48.28	107.14	191.27	167.86	96.43	86.33
LvYu9915	90.70	101.92	159.14	53.85	78.85	60.46
JiNongDa885	97.83	98.21	155.75	55.56	94.64	73.06
TieYan36	32.14	103.92	161.42	55.56	80.39	91.46
LvYu4117	116.67	20.00	27.95	0	12.50	23.35
ChangDan297	27.59	90.20	131.50	50.00	27.45	28.26
JiuDan100	81.40	108.00	155.23	45.71	72.00	49.67
YuLong9	95.35	94.34	140.62	12.20	67.92	46.75
JiDan33	106.25	106.90	103.31	35.29	20.69	26.89
JiDan35	107.69	98.31	124.81	33.93	76.27	51.24
JiDan47	100.00	100.00	171.43	78.33	93.33	72.11
JiDan50	123.81	51.02	76.06	53.85	38.77	40.48
JiDan87	109.26	93.33	126.31	57.63	88.33	63.45
JiDan137	92.11	74.07	106.17	25.71	29.63	27.20
JiDan550	101.72	98.33	149.58	52.54	90.00	62.87
JiDan558	101.96	91.07	130.06	32.69	82.14	56.30
JiDan631	106.00	100.00	139.18	39.62	74.55	50.15
XianYu335	192.59	62.50	88.02	17.31	28.57	22.01
XianYu508	162.50	94.55	101.70	5.77	16.36	11.91
LiangYu88	255.56	45.65	52.88	4.35	8.70	5.48
YuanDan68	89.13	100.00	160.76	53.66	72.55	53.95
YinHe32	120.59	89.58	128.21	14.63	50.00	34.32
MeiHeDa529	240.00	57.41	65.85	8.33	33.33	26.03
LeiAo150	130.00	92.86	124.68	1.92	8.93	5.80
LiangYu99	162.50	97.30	110.75	0	2.70	2.35
LiMin33	90.53	120.00	137.86	31.82	94.00	60.51
YinHe33	88.89	100.00	165.08	17.50	39.62	29.38
LiangYu66	100.00	87.50	111.11	3.33	4.17	7.65

 Table S1
 Corn germination parameters at different temperatures.

GE, germination energy; GP, germination percentage; and GI, germination index

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Variety	15 °C			10 °C		
	MF for GE	MF for GP	MF for GI	MF for GE	MF for GP	MF for GI
ChangDan297	0.46	0	0.48	0.25	0.32	0.29
ChuangShi998	0.68	0.06	0.43	0.56	1.00	0.64
DaYuM737	0.57	0.05	0.75	0.93	1.08	0.94
DeDan1002	0.50	0.20	0.43	0.58	0.20	0.58
DongDan213	0.44	1.00	0.11	0.50	0.21	0.51
FuYou10	0.15	0.59	0.14	0.03	0.25	0.49
HengYu709	0.56	0.06	0.83	1.00	1.18	1.00
HeYu3	0.46	0.87	0.17	0.86	0.45	0.77
HW217	0.58	0.03	0.68	0.86	0.34	0.80
JiDan137	0.36	0.17	0.36	0.27	0.17	0.28
JiDan33	0.57	0.20	0.35	0.18	0.23	0.27
JiDan35	0.52	0.21	0.45	0.73	0.22	0.55
JiDan47	0.53	0.19	0.66	0.90	0.50	0.78
JiDan50	0.20	0.25	0.22	0.36	0.35	0.43
JiDan550	0.52	0.19	0.56	0.87	0.34	0.68
JiDan558	0.47	0.19	0.47	0.79	0.21	0.60
JiDan631	0.53	0.20	0.51	0.71	0.25	0.53
JiDan87	0.48	0.21	0.45	0.85	0.37	0.68
JiNongDa885	0.51	0.18	0.59	0.91	0.36	0.79
JiSheng385	0.54	0.19	0.50	0.67	0.33	0.57
JiuDan100	0.58	0.14	0.59	0.69	0.29	0.53
JunDan6	1.00	0.07	1.00	0.31	0.09	0.33
LeiAo150	0.48	0.26	0.45	0.06	0.01	0.04
LiangYu66	0.44	0.19	0.38	0.01	0.02	0.06
LiangYu88	0.17	0.59	0.11	0.06	0.03	0.03
LiangYu99	0.51	0.35	0.38	0	0	0
LiMin33	0.66	0.16	0.51	0.91	0.2.	0.65
LiNong24	0.58	0.81	0.22	0.27	0.06	0.22
LvYu4117	0	0.23	0	0.10	0	0.23
LvYu9915	0.54	0.16	0.60	0.76	0.35	0.65
MeiHeDa529	0.25	0.55	0.17	0.30	0.05	0.26
PingAn185	0.35	0.44	0.21	0.82	0.48	0.72
TieYan36	0.55	0.01	0.61	0.77	0.36	1.00
W1521	0.22	0.70	0.12	0.84	0.25	0.81
WeiKe3757	0.32	0.28	0.24	0.37	0.12	0.33
XH84	0.39	0.05	0.30	0.50	0.92	0.56
XianYu335	0.28	0.42	0.28	0.26	0.11	0.22
XianYu508	0.49	0.35	0.34	0.14	0.04	0.11
YanFeng508	0.47	0.21	0.52	0.77	0.41	0.63
YinHe32	0.46	0.24	0.46	0.47	0.09	0.36
YinHe33	0.53	0.16	0.63	0.37	0.11	0.30
YuanDan68	0.53	0.16	0.61	0.69	0.34	0.58
YuLong9	0.49	0.17	0.52	0.65	0.08	0.50
ZhengDan958	0.50	0.13	0.62	0.89	0.31	0.75
ZhongDi77	0.41	0.12	0.52	0.41	0.18	0.46

 Table S2
 Membership function (MF) evaluation of corn germination parameters at different temperatures.

GE, germination energy; GP, germination percentage; and GI, germination index