

Effect of KCN and Temperature on Sesame Germination

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ABSTRACT

Cyanide-resistant respiration is a usual pathway in plants. If plants do this pathway, it decreases energy producing and finally decreases growth and yield. For increasing growth and yield in plants one of ways is inhibiting of cyanide-resistance respiration. This research investigates cyanide-resistant respiration in *Sesamum indicum* on germination stage. An experiment was carried out in order to study the effect of different cyanide-resistance on sesame in germinating and seeding stage in a completely randomized design with three replications. Experiment was carried out separately at 15°C and 20°C. Cyanide levels applied were zero (control), 0.005, 0.001, 0.0005 and 0.0001 M. The following measurements were carried out: germination percentage, germination index, seedling dry weight, germination rate, 5% germination rate (D5), 50% germination rate (D50), 90% germination rate (D90) and KCN resistant index. Result showed there was no significant difference in traits between different concentrations of KCN at 20°C. But there was significant difference between KCN levels at 15°C. According to results at 15°C the highest amounts of germination rate, germination percentage, KCN resistant index, germination index and seedling dry weight were related to control and the lowest related to 0.005 M. About D5, D50 and D90 the highest amounts were related to 0.005 M and the lowest related to control at 15°C. It is concluded KCN decreases germination and seedling growth in sesame at 15°C and with increasing temperature, it shows resistant against KCN.

Key words: cyanide-resistant respiration, sesame, *Sesamum indicum*, KCN

Introduction

If cyanide (1mM) be added to the tissues of animals, cytochrome oxidase will be stopped and respiratory rate will reach to less than one percent of its basic value. Most of plant tissues resist on cyanide, so that in the presence of cyanide, respiration rate reaches to 25% and sometimes to 100% than normal respiration rate. Responsible enzyme for oxygen absorption in these cases is known as cyanide-resistant oxidase in respiratory chain of plant mitochondria[8]. In this pathway, electrons from main respiratory chain deviate to alternative pathway. Since electrons deviate from ubiquinone (Q) to alternative pathway, therefore, at least two sources of generating energy (2ATP) will

not be considered. If the electrons flow in the alternative pathway, the produced energy assumed for producing ATP, will be wasted as heat. Cyanide-resistant oxidase will be inhibited by special compounds such as SHAM. In some plants, such as *Araceae*, the alternative pathway will be increased before pollination, which this increasing of respiration rate causes the inflorescence temperature increases 14°C than environmental temperature[7]. It should be mentioned, that in most plants the cyanide-resistant respiration rate is too low that they do not produce noticeable heat. Lambers[3] believed that this alternative pathway acts as an overflow and it oxidize respiratory substrates which are more than what is needed for growth. From this perspective, electrons will be entered into the alternative pathway only after

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saturation of the capacity of the main pathway. Such a thing, in vitro, will occur after the addition of cyanide. Ferguson *et al.*[2] considered the effect of low temperature and respiratory inhibitors on calcium flux in mitochondria of avocado fruits. Unlike many studies that show KCN decrease the produced energy and also inhibit Ca^{++} uptake (such as studies about corn, mung bean and potato[1,5,7] in Ferguson's study, KCN clearly stimulate Ca^{++} uptake. Also, comparing to 25°C, 5°C partially increases Ca^{++} uptake.

According to different results of using cyanide in plants, this study was done to consider the resistance or sensitivity of *Sesamum indicum* in germination stages. According to previous results that temperature can affect sensitivity or resistance to cyanide, in this study effect of different temperature (15°C and 20°C) on germination was considered.

Materials and methods

This Research was performed in laboratory of Islamic Azad University Maybod Branch-Iran in 2010. This experiment was carried out on *Sesamum indicum* in germination and seedling stages in a completely randomized design with three replications. Treatments were included KCN levels (0, 0.0001, 0.0005, 0.001 and 0.005 M). After preparing different concentrations of KCN, pH of all solutions with HCL (1N) and NH_4OH (1N) reached to 7 (such as distilled water). Seeds were germinated on filter papers placed within 10 cm diameter plastic dishes. Seeds were sterile with NaOH 5% for 10 min and then washed carefully. Germination dishes (20 seeds per dish) were placed within controlled chambers. Experiment was carried out separately at 15°C and 20°C. Seeds were considered germinated when radicles were ≥ 3 mm long. Germinated seeds were counted daily until seven days.

The following measurements was carried out: germination rate (was calculated by the equation of Maguire[4] germination percent, seedling dry weight, cyanide resistant index, germination index, 5% germination rate (the time it takes 5% of seeds to germinate), 50% germination rate (the time it takes 50% of seeds to germinate) and 90% germination rate (the time it takes 90% of seeds to germinate). The germination data were subjected to statistical analysis using the SAS system. All percentage data were modified according in arcsine for analysis. Finally, Duncan multiple range test was applied to compare the means at $p < 0.05$.

Results and discussion

Effect of KCN on seedling dry weight:

Based on statistical analysis, there was

significant difference in the seedling dry weight between concentrations of KCN at 15°C (table2). The maximum seedling dry weight was detected in control group (1.7 mg) and the minimum was observed in 0.005M KCN (0.25 mg)(figure1). So, it is clear that KCN causes reduction of sesame seedling dry weight in low temperature. This is related to the reduction of ATP production due to the increasing of cyanide-resistant pathway. There was no significant difference in the seedling dry weight between different concentrations of KCN at 20°C (table1). Consequently, with increasing temperature, sesame shows resistant against KCN.

Effect of KCN on cyanide- resistant index:

Analysis of variance showed that KCN has no role in cyanide-resistant index at 20°C (table1). But KCN has a significant role in cyanide-resistant index at 15°C (table2). So that the cyanide-resistant index has the lowest weight (2.935g) related to the 0.005M of KCN and has the maximum weight (5.739g) in control group. Therefore, increasing the concentration of KCN reduced cyanide-resistance index in low temperature, meanwhile increasing the temperature leads to the increasing of cyanide-resistant index so that the highest concentration of KCN had more resistance to cyanide in accordance with control.

Effect of KCN on germination percentage:

Results showed that the germination percentage was significant at 15°C (table2). According to table of comparing of means the highest percentage related to the control (56.03%) and the lowest related to the 0.005M (25.36%). There was no significant difference in germination percentage at concentrations of KCN at 20°C (table1). So KCN reduces germination of sesame at low temperature.

Effect of KCN on the 90% germination rate:

The result of ANOVA showed the significant effect of KCN on 90% germination rate at 15°C between different group ($P < 0.005$) (table2). The highest average rate in concentration of 0.005 M (164) has a significant different with other groups and the lowest average was related to the control (160.55). The reason of this 90% germination rate increasing is related to this matter that cyanide stops respiration, so by decreasing the respiration rate, the produced energy will decreases, consequently the seeds germination later. But the results at 20°C do not show the same results, and the different between groups were not significant (table1). But the KCN treatment (88) was higher control group with regard to the 90% germination rate.

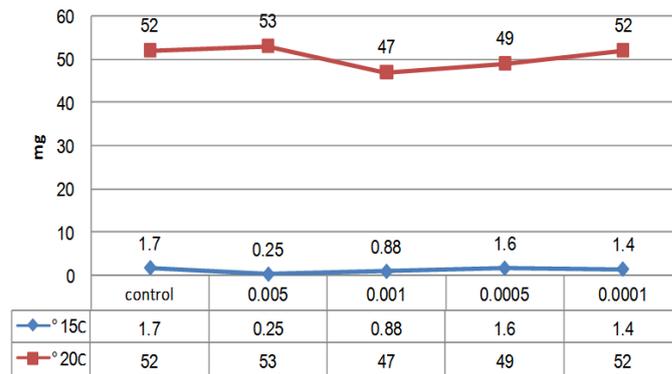


Fig. 1: Effect of different temperature on seedling dry weight in KCN levels

Table 1: Effect of KCN on traits of sesame germination at 20°C

treatment	Germination rate	Germination percentage	KCN resistant index	D5(h)	D50(h)	D90(h)	Germination index	Dry weight(mg)
Control	47.36 A	83.85 A	0.572 A	25.26 A	36.65 A	46.78 A	13.8 A	52 A
0.005	42.57 A	90 A	0.579 A	26.36 A	45 A	69.33 A	14.28 A	53 A
0.001	44.19 A	85.69 A	0.54 A	25.57 A	39.78 A	88 A	14.04 A	47 A
0.0005	44.22 A	77.07 A	0.55 A	25.42 A	38.25 A	73.60 A	13.57 A	49 A
0.0001	48.05 A	90 A	0.575 A	25.30 A	37.09 A	55.58 A	14.28 A	52 A

Table 2: Effect of KCN on traits of sesame germination at 15°C

treatment	Germination rate	Germination percentage	KCN resistant index	D5(h)	D50(h)	D90(h)	Germination index	Dry weight(mg)
Control	12.39 A	56.03 A	5.739 A	76.96 A	121.55 C	160.55 C	8.285 B	1.7 A
0.005	3.698 C	25.36B	2.935 B	107.4 A	148.0 A	164.0 A	1.523 D	0.25 C
0.001	7.29 BC	41.10 AB	4.532 A	82.67 A	137.11 AB	162.51 AB	5.00 C	0.88 B
0.0005	11.80 A	55.37 A	5.615 A	97.37 A	143.2 A	163.35 AB	11.019 A	1.6 A
0.0001	10.31 AB	49.8 A	5.35 A	78.45 A	128.94 BC	161.5 BC	8.381 B	1.45 A

Effect of KCN on the 5% germination rate:

5% germination rate is one of the qualities that can be used for comparing the rate of initial germination of plants. The plant which can germinate faster and is more vigor, it has a better chance too. This study showed KCN has no effect on 5% germination rate, there were no significant different between groups. So it can be concluded that the effective time of using KCN for 5% germination rate of seed is not short enough to decrease the germination rate. Although there was no significant different between treatments in this regard, the highest amount was evident in KCN concentration of 0.001M and the lowest amount in the control group. When the cell faces with the KCN, the respiration rate increase in the early stages and after passing a time it returns to its original rate. This can be the result of this increasing rate of 5% germination rate.

Effect of KCN on germination rate:

The result of the germination rate at 15°C showed there was a significant different between groups using KCN concentration (table2). The highest rate of germination was related to the control group (12.39), and the lowest one related to the 0.005 M (3.69) concentration. The result of ANOVA

at 20°C showed that KCN treatment had no effect on sesame germination rate and this is not a significant factor (table1). But it should be mentioned that the rate of germination was higher in 0.0001 M (48.05) comparing to control group (47.36). In sesame by increasing the KCN concentration, the rate of germination decrease. The higher the temperature, the more the rate of germination would be sesame. And this was true for those seeds treated by KCN and it was more than usual in some cases. So it can be said that by increasing the temperature, the KCN resistant pathway decreases. For this reason the rate of germination did not decrease by increasing the temperature.

Effect of KCN on the 50% germination rate:

There were significant different between groups using different concentrations of KCN at 15°C (table2). The highest rate was related to the concentration of 0.005M (148) and the lowest rate was related to the control (121.55). There was no significant different between concentrations of KCN at 20°C (table1).

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