

## Study of the Effect of Calcium Ion in dark-induced senescence leaves of peony

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**Abstract.** The effect of calcium ion on peony aging was studied. Different four calcium ion concentrations (0mmol/L,100mmol/L,250mmol/L, 500mmol/L) was setted. Leaf of the peony Roufuring on same growth period was cultured in vitro, Chlorophyll concentration was measured per four days. The results revealed that calcium ion concentrations at 100mmol/L gave the slowest aging speed among four samples, and when calcium ion concentrations was 500mmol/L, peony was aging fastest.

### Introduction

Calcium, as a large number of elements in plant, which can be combined with calmodulin, playing an important role in signal transduction. Meanwhile, Calcium can be integrated with the negatively charged cell membrane as the divalent cation, playing a role in stabilizing cell membranes. Poovaiah and their coworkers found 0.1-100mM CaCl<sub>2</sub> can delay aging speed from leaves of corn and the sorrel, which remained chlorophyll at a high level, the performance of the membrane free space decreased membrane permeability decreased, increased hydration balance[15], but high concentrations of calcium plant will produce stress effect, and reduce the flow of the film, intensify peony aging. In order to verify the effect of calcium on the Luoyang Peony aging, 100mmol/l, 250mmol/l and 500mmol/l of CaCl<sub>2</sub> solution were used to culture peony blades in vitro, chlorophyll content was used as the index to study the leaf aging law.

### Materials and Methods

#### Source material

April 10, 2015 with the scissors clipping west of Luoyang Normal University Library of Small garden with Roufuring on the same strain of 12 small sticks, cleaned with water in lab.

#### Reagents and Instruments

Reagents: CaCl<sub>2</sub>; 95% ethanol; quartz sand.

Instruments: mortar; 25ml graduated cylinder; small funnel; filter paper; absorbent paper; lens paper; pipette; spectrophotometer; electronic balance; cuvettes; centrifuge; tube; 500ml brown reagent bottle.

#### Test Method

##### Materials Handling

Four peony leaf samples obtained were placed in four brown reagent bottle, four brown vial contain 100ml of liquid, these liquids are: water, 100mmol/l of CaCl<sub>2</sub> solution, 250mmol/l of CaCl<sub>2</sub> solution,

500mmol/l of CaCl<sub>2</sub> solution. Then sampled at day 0,4 days,8 days,12 days were measured in each sample chlorophyll content.

### Determination of chlorophyll content

The method of chlorophyll as References[1,9]:

### Data Processing

Using WPS tables and SPSS data processing software for the recording of data processing,use of biometrics in the univariate analysis of variance variation of the mathematical model of the sample content of chlorophyll content and SOD activity with the calcium ion concentration.

## Results and analysis

After four times measured in 12 days, chlorophyll content in four samples were determined as follows:

**Table 1 The chlorophyll content of Roufufurong leaves by treating CaCl<sub>2</sub> during dark-induced senescence**

	A (0mmol/l)	B (100mmol)	C (250mmol/l)	D (500mmol/l)
The chlorophyll concentration				
	16.531	13.7021	12.8147	15.2339
One day	16.1625	13.13.6129	12.6129	15.3945
	16.289	13.5692	12.6921	15.0142
Standards	16.32751±0.	13.62806±0.1	12.70655±0.122	15.21253±0.2197
error	242	329	6	
	16.4981	12.6321	11.0196	14.0179
4 days	16.5073	12.5916	11.0831	14.0263
	16.0278	12.4982	10.8946	13.8941
Standards	16.34439±0.	12.57397±0.1	10.99912±0.125	13.97943±0.1238
error	4702	339		
	12.1972	10.9865	9.9201	12.5603
8 days	11.8236	10.7299	10.0106	12.5213
	11.8406	10.8019	9.6549	12.4287
Standards	11.9538±0.3	10.83943±0.1	9.86185±0.2652	12.50344±0.1316
error	566	846		
	11.0384	10.8424	8.6343	11.1209
12 days	11.1081	10.7973	8.5712	11.2133
	10.8527	10.658	8.471	10.9832
Standards	10.99973±0.	10.76589±0.1	8.55883±0.1633	11.10581±0.1377
error	1857	844		

### The relationship between changes of concentration of calcium ion concentration and chlorophyll content as follows

After data analysis of variance, Table 2 shows the number row after analysis of variance calcium ion concentration and chlorophyll content changes of significantly the relationship, because P=0.001, which was lower than 0.05. The results showed that calcium concentration of tree peony leaves senescence rate was significant relationship, that chlorophyll content and calcium ion concentration.

**Table 2 The test of calcium ions and peony leaf chlorophyll content of the relationship.**

Source	Type Sum Of Squares	df	Mean Square	F	Sig.
Corrected Model	69.757a	6	11.626	19.469	.000
Intercept	2459.177	1	2459.177	4118.032	.000
Days	43.351	3	14.450	24.198	.000
Serial number	26.406	3	8.802	14.739	.001
Error	5.375	9	.597		
Total Corrected Total	2534.308	16			
	75.131	15			

a.R Squared=.928(Adjusted R Squared=.881)

In Table 3. numbers represent of each sample, subset not in the same representation column there were significant difference from the data analysis results: A sample (0mmol/ l) and D (500mmol/ l) like chlorophyll concentration change of calcium ion concentration relation was not significant, That is because the relationship between water and leaf senescence maximum calcium concentration was not significant. And the remaining samples of the rate of aging peony significant differences, that different calcium concentration of tree peony aging at different speeds, no significant relationship between the A sample and the sample D, there are two possible scenarios: First, water and a high calcium the concentration of the same make peony rate of aging, from water to 100mmol / l lower leaf senescence rate, higher than after 100mmol / l leaf senescence rate increased; Second, there is the experimental error of the A sample and the sample D was not significant one reason.

**Table 3 Multiple comparisons of each sample**

Serial number	N	Subset		
		1	2	3
Student-Newman-Keuls <sup>a,b</sup>	4	10.5316		
3.00	4		11.9518	
2.00	4			13.2003
1.00	4			13.9064
	Sig	1.000	1.000	.229

Means for groups in homogeneous subsets are displayed. Based on Type Sum of Squares The error term is Mean Square(Error)=0.597. a. Uses Harmonic Mean Sample Size=4.000 b. Alpha=0.5.

The study has proven that content change of calcium ion concentration and chlorophyll concerned had a different impact on different calcium concentration peony leaf senescence rate, Fig, 1 shows that as the calcium ion concentration increased, chlorophyll was degradated more faster, when the calcium ion concentration was 100mmol / l, chlorophyll degradation more least,while the calcium ion concentration was 500mmol / l, chlorophyll degradation more faster. A sample group of degradation appear abnormal change phenomena, which may be measured according to laboratory fluorescent light emitted decomposition of chlorophyll.

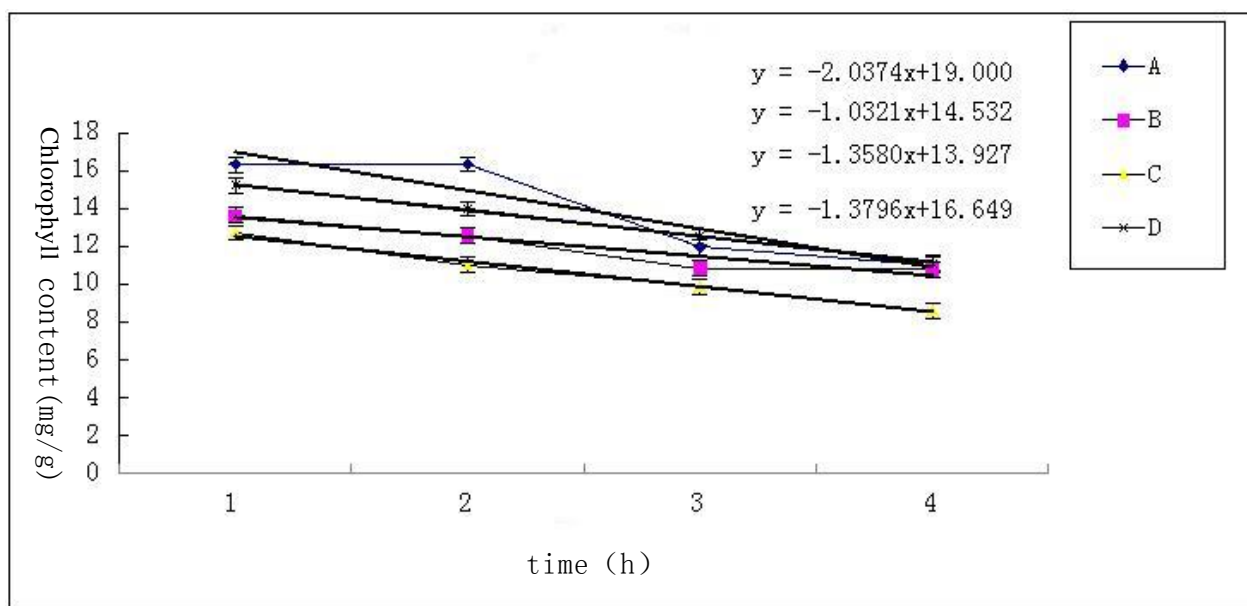


Fig. 1 Changes chlorophyll content of each sample Line charts and trend lines  
A: water, B: 100mmol/l of CaCl<sub>2</sub>, C:250mmol/l CaCl<sub>2</sub>, D: 500mmol/l CaCl<sub>2</sub>

## Discussion

The experimental result showed that: Calcium not only affects the speed of aging peony, but also different calcium concentration have different effects on the aging rate for the peony. The optimal concentration of calcium ion is 100mmol/L during leaf senescence slowest, the result was according with Li Donglin, et al<sup>[10-15]</sup>.

Because the test sample is too small capacity, lack of repetition, limited experimental conditions, is not enough to fully and accurately the impact of calcium on senescence of peony, still need further study in the future by increasing the sample volume, increase the number of repetitions and increase the measurement frequency.

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