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The Relationship between Environmental Factors during the Rainy Season and Un-opened Floret Yellowing in *Dendrobium* Sonia 'Ear-Sakul'

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ABSTRACT

The pre-harvest yellowing in the un-opened florets of *Dendrobium* Sonia 'Ear-Sakul' grown under 50% shade in a saran-house was studied. In the rainy season, the most sensitive period for yellowing in un-opened florets was found to be the ready-to-bloom stage. The highest percentage of inflorescence with un-opened floret yellowing was obtained from those with no open florets. The percentage of inflorescence with yellowed un-opened florets could be predicted (R^2= 0.613) using an environmental model consisting of: 1) cumulative hours of daytime with 0 μmol $m^{-2}s^{-1}$ light intensity; 2) average rainfall during 1000-1400 hours; 3) cumulative hours of daytime with the temperature less than 25°C; and 4) cumulative hours when the relative humidity (RH) was in the range 75-90% in the period 3 d before un-opened floret yellowing. The developmental stage of inflorescence did not affect un-opened floret yellowing. The simulated stress environment of the rainy season had no effect on the sucrose, glucose and fructose contents in the first- and second-largest un-opened florets, nor on the calcium and boron contents in the inflorescence and leaves. Under the simulated stress environment of the rainy season in a growth chamber, spraying 1% sucrose solution might be useful to reduce yellowing in un-opened florets.

Keywords: *Dendrobium,* flower bud, wilting, dropping, light intensity, rainfall, temperature, relative humidity

INTRODUCTION

Thailand is the largest producer of cut flowers of tropical orchids in the world. In 2009, the export value of cut orchid-flowers was 2,366.4 million baht (Office of Agricultural Economics, 2009). Cut orchid-flowers are grown on a large scale around Bangkok. The most popular genus of orchid for commercial production in Thailand is *Dendrobium*, which has accounted for approximately 80% of the total production (Bureau of Agricultural Economics Research, 2003).

One of the critical problems in the commercial production of *Dendrobium* cut-flowers is pre-harvest yellowing of un-opened florets. The problem can be observed at any time of the year. As a result, any inflorescence exhibiting dropping of un-opened florets does not meet export quality. Many environmental factors have been shown to affect flower abortion, such as low light intensity and low temperature. In general, flowers were more easily dropped when

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grown under light stress, because they accumulated less soluble sugar (Aloni *et al*., 1996). In Thailand, the control of temperature cannot be managed under field production, even though it has strong implications for flower quality. Sawa (1991) found that the wilting of un-opened florets of *Dendrobium* Sonia surged as the growth temperature plunged. In addition, Sithichai (2005) reported that Thai orchid growers had observed a drastic climate change caused abnormal florets of *Dendrobium* Sonia 'Joe-Daeng'. To understand better the cause of the un-opened floret yellowing, the present study aimed to determine the relationship between environmental factors and un-opened floret yellowing, as well as the composition of some endogenous chemicals in *Dendrobium* Sonia 'Ear-Sakul' during the rainy season.

MATERIALS AND METHODS

Plant material

Two-year-old *Dendrobium* Sonia 'Ear-Sakul' plants were grown in saran-houses under approximately 50% sunlight. The experiments were conducted at the Orchid 2002 Farm, Nakhon Pathom province and at the Uttaradit Rajaphat University, Uttaradit province.

Monitoring of un-opened floret yellowing

Monitoring of un-opened floret yellowing in four flowering stages of inflorescence was carried out three times a week. The four stages were: 1) without any open florets; 2) with two open florets; 3) with four open florets; and 4) with six open florets per inflorescence. For each stage, 20 plants were evaluated by counting the number of inflorescence with un-opened florets that had yellowed. The weather conditions (light intensity, relative humidity, air temperature and rainfall) were recorded by a data logger (Model 450, Watch Dog, USA) at 30-minute intervals. The relationship between the environmental parameters and the percentage of inflorescence with yellow un-opened florets was determined using correlation and stepwise multiple regression analysis. The statistical analysis was performed using the SPSS V.11.5 program (SPSS Inc., USA).

Determination of sugar, calcium and boron contents under stress condition

To evaluate the yellowing in un-opened florets and to determine the endogenous chemical contents, plants were moved to a growth chamber when the inflorescence had one open floret. For induction to the stress conditions in the monitored experiment, the growth chamber was set at 24°C day/25°C night, 86% day/90% night relative humidity (RH) and 7 h photoperiod of 150 mmol $m^{-2}s^{-1}$ light for 3 d, followed by transfer to normal conditions for 3 d, while another growth chamber was set for the normal condition at 30°C day/25°C night, 71% day/90% night RH with 13 h photoperiod of 150 μmol $m^{-2}s^{-1}$ light for 6 d. Two experiments were set up to investigate un-opened floret yellowing and to determine the endogenous chemical contents. The first experiment investigated the effects of a normal and a stress environment on the yellowing in the stages of inflorescence (with 0, 1 and 2 open florets per inflorescence). Yellowing in un-opened florets was observed on the third day under the simulated environment conditions and on the third day after transfer under the normal environment. In the second experiment before subjecting the plants to the same stress environment as in the first experiment, the effects were determined of normal and stress conditions and also spraying with 0.5% glucose solution on the endogenous sucrose, glucose, fructose, calcium and boron contents in the first and the second largest un-opened florets after being subjected to the treatment for 1, 2, 3 and 4 d, using 3×4 factorials in a completely randomized design with three replications (three plants per replication). Calculations of significant differences at the 5% level were carried out by analysis of variance. Samples of plant material (first and second un-opened florets for determination of the sugar contents; inflorescence and third leaves for determination of the calcium and boron quantities) were harvested 1-4 d after the treatments.

Samples were extracted two times with 10 mL of 80% ethanol at 80°C. The combined extracts were evaporated to dryness and redissolved in 3 mL distilled water. The sucrose, glucose and fructose contents were characterized and quantified by high-performance liquid chromatography (HPLC) with a refractive index (RI) detector, using an APS-2 Hypersil column at 35°C. Sucrose, glucose and fructose were identified by their retention times and quantified by integrating peak areas versus an internal standard (Kawamata *et al*., 2002).

Determination of the calcium and boron contents was performed using the method of Attanandana and Chanchareonsook (1999). The samples were dried at 70°C for 3 d in a hot-air oven. The calcium content was ascertained by nitric-perchloric digestion. Dried samples were digested in 5:2 nitric acid and perchloric acid at 200°C and diluted to a final volume of 50 mL, then filtered using Whatman No. 42 filter paper. A sample of 10 mL was mixed with 2 mL of 2.5% strontium chloride. This solution was subjected to atomic absorption spectroscopy at 422 nm wavelength. The boron content was determined using the azomethine H spectrophotometric method. One gram of the dried sample was dissolved in 5 mL NaOH. The solution was ashed for 90 min at 470°C. After attaining, the ash was kept at room temperature for 3 d and was dissolved later with 20 mL of 0.36 N H_2SO_4. The contents were filtered through Whatman No. 42 filter paper and diluted to a final volume of 25 mL. Then, 3 mL of the extracted solution was mixed with 2 mL of a buffer solution (220 g of ammonium acetate and 15 g of NaEDTA in 400 mL distilled water) and 1 mL azomethine H solution. Finally, the volume of the solution was brought to 25 mL with distilled water. The boron concentration was determined using a spectrophotometer at 430 nm wavelength.

Reduction of pre-harvest un-opened floret yellowing by sugar spray

A sucrose, glucose or fructose solution (each at 0, 0.5, 1 and 2%) was sprayed on the inflorescence before subjecting the plant material to the stress conditions of the rainy season in a growth chamber. Monitoring of un-opened floret yellowing was carried out twice after the treatment (on the third day under the simulated environment and on the third day after transfer under the normal environment). The experiments were set out using a completely randomized design (CRD) with three replications, with three plants for each replication.

RESULTS AND DISCUSSION

Un-opened floret yellowing behavior

In *Dendrobium* Sonia 'Ear-Sakul', the most sensitive stage for yellowing of un-opened flosWts occurred in the ready to bloom un-opened florets (the first largest un-opened floret). This agreed with the results of Thampitakorn (1993) and Prayuravong (1999), who found that the largest un-opened floret of *Dendrobium* was the most likely to drop under postharvest conditions. Based on the percentage of inflorescence with yellow un-opened florets at different growth stage of inflorescence, the inflorescence with no open florets was the most sensitive (Figure 1). Flower retention depended on assimilation of supplies to the reproductive organ, according to Karunsatitchai (2004), who reported that the inflorescence of *Dendrobium* Pompadour with more open florets had less un-opened florets drop due to the greater amount of sugar that was translocated from the opened florets to the un-opened florets under postharvest conditions. In addition, the hormonal status affects flower

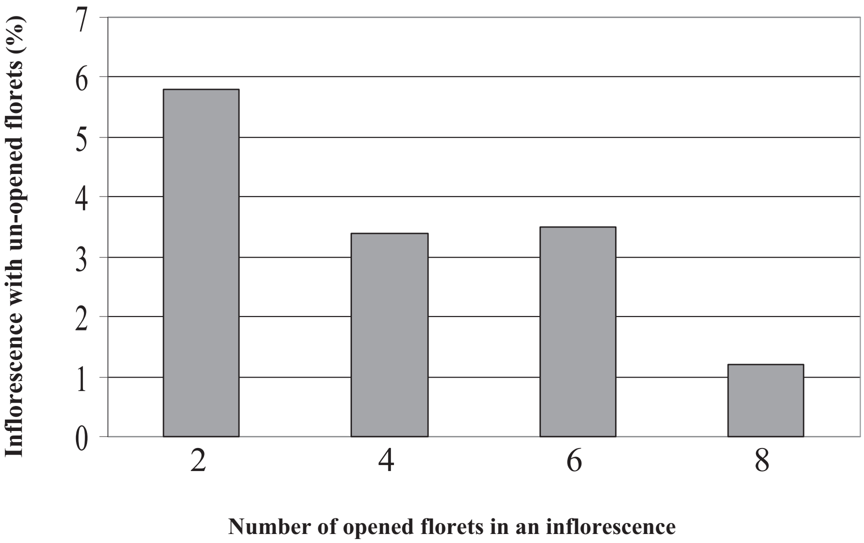

Figure 1 Un-opened floret yellowing of *Dendrobium* Sonia 'Ear-Sakul' growth in the 50% shade saran-houses at different stages of inflorescence.

retention. The ethylene production of *Dendrobium* Pompadour, *Dendrobium* Sonia 'Bom 28', as well as of diploids and tetraploids of *Dendrobium* Caesar, was higher in the un-opened florets than in the opened ones. As a result, inflorescence with no opened florets was the most sensitive to the un-opened floret yellowing.

Multiple regression was employed to establish a relationship between the percentage of inflorescence with yellow un-opened florets and the rainy season environmental parameters.

The independent variables, consisting of light intensity, relative humidity, air temperature and rainfall, had a greater effect during the 3 d before yellowing of the un-opened florets than during the periods of 5 and 7 d before yellowing of un-opened florets. It was also found that, many environmental parameters from 3 d before yellowing of un-opened florets had high correlation with un-opened floret yellowing (Table 1), for example, the decrease in average light intensity, the decrease in cumulative hours of daytime with no light (0 μmol $m^{-2}s^{-1}$ light intensity), the decrease in average temperature and the increase in average daytime RH and rainfall.

Thus, stepwise multiple regression analysis used only four environmental conditions from 3 d before un-opened floret yellowing. The percentage of inflorescence with yellowed un-opened florets (Y) was found to be correlated with: 1) cumulative hours of daytime with 0 μmol m^{-2} s^{-1} light intensity (X_1); 2) average rainfall during 1000-1400 hours (X_2); 3) cumulative hours of daytime with the temperature less than 25°C (X_3); and 4) cumulative hours of RH in the range 75-90 % (X_4). The regression equation produced was $Y = - 14.992 + 5.481 X_1 - 81.664 X_2 + 2.308 X_3 + 0.799 X_4$, with $R^2 = 0.613$. Chang *et al*. (2004) reported that young buds and flowers of *Phalaenopsis amabilis* var. 'Formosa Shimadzu' were sensitive to darkness. Sawa (1991) found that when the growth temperature dropped from high (35°C/day-30°C/night) to low (20°C/day-15°C/night), the wilting of *Dendrobium* Sonia flower buds increased. According to Monteiro *et al*. (2001), high production temperatures (29°C /day-24°C/night) compared to low production temperatures (24°C/day-18°C/night) increased postproduction flower longevity and decreased postproduction bud drop in 'Meirutral' and 'Meidanclar' miniature roses.

Table 1 Correlation coefficients between some rainy season environmental parameters from 3, 5 and 7 d before un-opened floret yellowing and the percentage of *Dendrobium* Sonia 'Ear-Sakul' inflorescence with yellow un-opened florets.

No.	Environmental parameter	Correlation coefficient (R)		
		3 d before un-opened floret yellowing	5 d before un-opened floret yellowing	7 d before un-opened floret yellowing
1	Average light intensity	-0.423**	-0.369**	-0.289*
2	Hours of light intensity = 0μmol $m^{-2}s^{-1}$	0.558**	0.415**	0.367**
3	Average temperature	-0.453**	-0.344**	-0.287**
4	Average daytime temperature	-0.447**	-0.338**	-0.266**
5	Average nighttime temperature	-0.413**	-0.326**	-0.298**
6	Average humidity	0.465**	0.395**	0.341**
7	Average daytime humidity	0.479**	0.404**	0.347**
8	Average nighttime humidity	0.312*	0.245	0.220
9	Average rainfall	0.133	0.082	0.092
10	Average daytime rainfall	0.334*	0.167	0.168
11	Average nighttime rainfall	-0.085	-0.126	-0.071

*, ** = significant correlation at 95% and 99% levels, respectively.

Sugar, calcium and boron contents under stress condition

Growing the *Dendrobium* Sonia 'Ear-Sakul' under a simulated stress environment of the rainy season in a growth chamber did increase yellowing of un-opened florets. In general, un-opened floret yellowing was significantly higher in the inflorescence of stressed plants than in normal ones. The developmental stage of inflorescence did not affect un-opened floret yellowing. Nevertheless, due to the high %CV, inflorescence with no opened florets (stage 1) tended to have more yellow un-opened florets than that with opened florets, regardless of the statistical result (Table 2). According to Van der Meulen-Muisers *et al*. (1995), the increase in flower longevity of the remaining flowers after bud removal was mainly attributed to a decrease in sink strength within inflorescence. Thus, the more the un-opened florets, the higher the number of yellow un-opened floret resulted.

The glucose content in the un-opened florets was higher than the sucrose and fructose content (Tables 3-5). However, the content of the three sugars was generally unaffected by the treatments and by the time after being subjected to the treated environments. However, due to the high CV percentage, the raw data may be taken into consideration. The simulated stress environment tended to decrease the sucrose, glucose and fructose contents in the first and second largest un-opened florets of the inflorescence, regardless of the statistical result (Tables 3-5). Only the fructose content in the second largest un-opened floret of inflorescence, which slightly increased with time, decreased on the fourth day, when the plants were sprayed with 0.5% glucose prior to the simulated stress treatment (Table 5). A similar effect has been found in some other flower species. According to Nayyar *et al*. (2004), cold stress during reproductive growth caused flower abortion in a warm season crop resulting in a decrease of 40, 25 and 23%, respectively, in the sucrose, glucose and fructose content. Chang *et al*. (2004) indicated that young buds of *Phalaenopsis amabilis* var.

Formosa Shimadzu were sensitive to darkness due to carbohydrate deficiency. Moreover, the simulated stress environment had no effect on the calcium and boron content in the inflorescence and leaves (Tables 6-7).

Reduction of pre-harvest yellowing of un-opened florets by sugar spray

Under the simulated stress environment of the rainy season in a growth chamber, spraying of 1 or 2% sucrose decreased the yellowing percentage within 3 d after transferring to the normal environment, when compared to the normal conditions. The spraying of other sugars showed no effect on the yellowing. However, due to the high initial variation of the plant materials (high % CV), the raw data may be taken into consideration. The spraying of 1% sucrose or 0,

Table 2 Un-opened floret yellowing of *Dendrobium* Sonia 'Ear-Sakul' as affected by simulated environmental stress in rainy season.

Simulated environment	% inflorescence with yellow un-opened florets							
	3 d under simulated environment				3 d after simulated environment			
	Stage[1/]				Stage[1/]			
	1	2	3	Mean	1	2	3	Mean
Normal	0	0	0	0 b[2/3/]	7.41	7.41	0	4.94 y[2/3/]
Stress	16.67	5.56	5.56	9.26 a	29.63	11.11	14.82	18.52 x
Mean	8.33	2.78	2.78		18.52	9.26	7.41	
Environment (A)				*				*
Stage (B)				ns				ns
A × B				ns				ns
CV(%)				81.59				76.47

[1] Stage 1= no open floret; Stage 2 =1 open floret; Stage 3 =2 open florets.
[2] Data transformed by √(X+1) before statistical analysis.
[3] Means within a column followed by the same letter are not significantly different at the 0.05% level using Duncan's multiple range test.
* = significant difference at $P \leq 0.05$; ns = non-significant.

Table 3 Sucrose content (mg per gFW) in un-opened florets of *Dendrobium* Sonia 'Ear-Sakul' under different conditions.

Condition	First un-opened floret					Second un-opened floret				
	Time (d)					Time (d)				
	1	2	3	4	Mean	1	2	3	4	Mean
Normal	1.08	0.93	1.17	0.98	1.04	0.48	1.28	0.82	1.44	1.01
Stress	1.07	0.91	1.01	0.62	0.9	0.66	0.69	0.61	0.78	0.68
Stress with 0.5 % glucose	0.87	1.11	1.14	0.26	1.09	0.23	0.65	0.7	0.55	0.53
Mean	1.01	0.98	1.11	0.95		0.46	0.87	0.71	0.92	
Condition (A)					ns					ns
Time (B)					ns					ns
A × B					ns					ns
CV(%)					54.59					71.73

ns = non-significant.

0.5 and 2% fructose resulted in no yellowing of un-opened florets, even after stress, regardless of the statistical result (Tables 8-10). The beneficial effects of spraying twice with 8% sucrose solution after bud emergence on flower longevity have been demonstrated previously in rose cv. 'Alexander' (Mehran *et al.*, 2008). Sucrose apparently inhibits ethylene production by inhibiting 1-aminocyclopropane-1-carboxylic acid oxidase (ACO) activity, thus extending vase life (Pun *et al.*, 2005).

CONCLUSION

In the rainy season, the ready-to-bloom florets of *Dendrobium* Sonia 'Ear-Sakul' were the most sensitive to yellowing. The inflorescence with no opened florets showed the highest percentage of inflorescence with yellow un-opened florets. Un-opened floret yellowing was influenced by many environmental stimuli. It was found that higher cumulative hours of daytime without photosynthetic photon flux, low

Table 4 Glucose content (mg per gFW) in un-opened florets of *Dendrobium* Sonia 'Ear-Sakul' under different conditions.

Condition	First un-opened floret					Second un-opened floret				
	Time (d)					Time (d)				
	1	2	3	4	Mean	1	2	3	4	Mean
Normal	4.91	3.74	3.8	4.6	4.26	3.33	3.81	4.12	5.13	4.1
Stress	3.98	3.52	4	3.08	3.64	3.85	3.73	3.92	3.51	3.75
Stress with 0.5 % glucose	3.82	3.92	4.52	3.29	3.89	3.57	3.19	4.25	3.11	3.53
Mean	4.24	3.73	4.11	3.66		3.58	3.55	4.1	3.92	
Condition (A)					ns					ns
Time (B)					ns					ns
A × B					ns					ns
CV(%)					15.16					17.38

ns = non-significant.

Table 5 Fructose content (mg per gFW) in un-opened florets of *Dendrobium* Sonia 'Ear-Sakul' under different conditions.

Condition	First un-opened floret					Second un-opened floret				
	Time (d)					Time (d)				
	1	2	3	4	Mean	1	2	3	4	Mean
Normal	0.83	0.89	0.91	0.87	0.87	0.72 bc[2/]	1.01 ab	1.16 a	1.12 ab	1.01
Stress	0.94	0.85	0.89	0.7	0.84	1.00 ab	1.03 ab	0.90 ab	0.82 abc	0.94
Stress with 0.5 % glucose	0.92	0.91	0.94	0.76	0.88	0.98 ab	0.80 abc	0.98 ab	0.45 c	0.8
Mean	0.9	0.88	0.91	0.78		0.9	0.95	1.02	0.8	
Condition (A)					ns					ns
Time (B)					ns					ns
A × B					ns					*
CV(%)					17.23					4.78

* = significant difference at $P \leq 0.05$; ns = non-significant.

temperature, high moisture and rainfall from 3 d before un-opened floret yellowing were the major environment factors. The simulated stress environment had no effect on the content of sugars in the un-opened florets, nor on the calcium and boron contents in the inflorescence and leaves. Sugar spraying produced no significant reduction of the yellowing.

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Table 6 Calcium content (%) in inflorescence and leaves of *Dendrobium* Sonia 'Ear-Sakul' under different conditions.

Condition	Inflorescence					Leaves				
	Time (d)					Time (d)				
	1	2	3	4	Mean	1	2	3	4	Mean
Normal	0.29	0.48	0.43	0.34	0.38	1.28	1.71	1.79	1.61	1.60
Stress	0.41	0.41	0.35	0.41	0.39	1.39	1.71	1.71	1.82	1.66
Stress with 0.5 % glucose	0.42	0.41	0.33	0.43	0.40	1.68	1.81	1.89	1.84	1.81
Mean	0.37	0.43	0.37	0.39		1.45	1.74	1.80	1.75	
Condition (A)					ns					ns
Time (B)					ns					ns
A × B					ns					ns
CV(%)					38.01					46.85

ns = non-significant

Table 7 Boron content (ppm) in inflorescence and leaves of *Dendrobium* Sonia 'Ear-Sakul' under different conditions.

Condition	Inflorescence					Leaves				
	Time (d)					Time (d)				
	1	2	3	4	Mean	1	2	3	4	Mean
Normal	7.40	7.49	7.39	7.17	7.36	23.23	15.33	16.56	20.32	18.86
Stress	7.78	6.20	12.12	6.25	8.09	14.89	17.94	23.93	16.87	18.41
Stress with 0.5 % glucose	6.67	7.01	10.73	6.98	7.85	24.01	20.71	22.86	29.66	24.31
Mean	7.28	6.90	10.08	6.80		20.71	18.00	21.12	22.28	
Condition (A)					ns					ns
Time (B)					ns					ns
A × B					ns					ns
CV(%)					14.23					16.69

ns = non-significant

Table 8 Un-opened floret yellowing of *Dendrobium* Sonia 'Ear-Sakul' sprayed with sucrose solution prior to growing under the simulated stress conditions of the rainy season.

Environments /sucrose	Inflorescence with yellow un-opened florets (%)	
	3 d under simulated environment	3 d after simulated environment
Normal/ 0%	27.778 a [1, 2]	18.517[2]
Stress/ 0%	11.111 ab	18.517
Stress/ 0.5%	16.667 ab	7.407
Stress/ 1%	0.000 b	0.000
Stress/ 2%	0.000 b	7.407
P-value[3/]	0.036	0.289
CV(%)	56.23	66.31

[1] Means within a column followed by the same letter are not significantly different at the 0.05% level using Duncan's multiple range test.
[2] Data transformed by √(X+1) before statistical analysis.
[3] *P*-value > 0.05 = non-significant.

Table 9 Un-opened floret yellowing of *Dendrobium* Sonia 'Ear-Sakul' sprayed with glucose solution prior to growing under the simulated stress conditions of the rainy season.

Environment/glucose	Inflorescence with yellow un-opened florets (%)	
	3 d under simulated environment	3 d after simulated environment
Normal/ 0%	25.926[1]	0[1]
Stress/ 0%	44.444	0
Stress/ 0.5%	48.148	0
Stress/ 1%	51.852	0
Stress/ 2%	37.037	0
P-value[2/]	0.166	-
CV(%)	30.15	-

[1] Data transformed by √(X+1) before statistical analysis.
[2] *P*-value > 0.05 = non-significant.

Table 10 Un-opened floret yellowing of *Dendrobium* Sonia 'Ear-Sakul' sprayed with fructose solution prior to growing under the simulated stress conditions of the rainy season.

Environments /fructose	Inflorescence with yellow un-opened florets (%)	
	3 d under simulated environment	3 d after simulated environment
Normal/ 0%	0[1]	0.000[1]
Stress/ 0%	0	0.000
Stress/ 0.5%	0	0.000
Stress/ 1%	0	7.407
Stress/ 2%	0	0.000
P-value[2]	-	0.452
CV(%)	-	70.62

[1] Data transformed by √(X+1) before statistical analysis.
[2] *P*-value > 0.05 = non-significant.

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