Application of quadratic rotation-orthogonal composite experimental design to Assess the Relationship between Growth Environment and Ultraweak Luminescence of Osmanthus Tree Seedings

Yong Yu[1], Yao Zhan[1], Yi Lin[1], Chunfang Wang[1], Jianping Li*

[1] *College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou, Zhejiang, 310058, China*

* *Corresponding author, address: College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou, Zhejiang, 310058, China, Tel:-86-571-88981211, Email: jpli@zju.edu.cn*

Abstract. Quadratic rotation-orthogonal composite experimental design was applied in this experiment to analyze the ultraweak luminescence property of osmanthus tree seedings affected by multilateral growth environmental factors. The influence models of four factors on luminescence were established including illumination, temperature, humidity, and moisture. The ANOVA (analysis of variance) analysis showed that different factors and their interaction had different effect on luminescence property, among which the linear effects of four variables, the quadratic effects of illumination, relative humidity and moisture, and the interaction effect of illumination × moisture and temperature × relative humidity all have significant effect on the luminous value of osmanthus ($p \leq 0.01$). The maximum luminous value was obtained when ten stick of lamp tube (intensity of illumination value 4229 Lx) was used, mean temperature, humidity and water were 35 ℃, 85% and 90 mL/d respectively. On the base of that, linear effects and interactions that affected ultraweak luminescence were further studied.

Key words: Osmanthus tree seedings; Growth environment; Ultraweak luminescence

Introduction

Ultraweak luminescence is a kind of low level biological photon emission which was first discovered by a former Soviet Union scientist G. Gurwitsh in his famous onion experiment in 1923. Although the mechanism is not revealed completely up to now, it has been demonstrated that ultraweak luminescence is a dynamic indicator that reflects the metabolism, function mediation and information exchange inner and outer the cells in organisms [1-2]. The discovery of the intimate positive correlation between plant luminescence and vitality is one of the most basic achievements and is one of the most important detecting techniques applied in modern farming management [3].

Researches showed that viability and luminescence of soybeans were improved significantly after electromagnetic radiation. The viability of Mung bean was brought down by using metabolic inhibitor, and the same as the luminescence [4]. Similar consequences were obtained in previous studies with rice, peanut leaf and spinach [5].

On the basis of that, many researches aiming at viability support and detection were done to study rules and factors that influence the bio-photon emission. Plant's viability and luminescence are affected by some physical (oxygen, temperature, irradiation, etc.) [6-9], chemical (oxidant, metabolic inhibitor, etc.) factors [10], hormone and environmental stress [11-13]. Benefited from the development of electronic and information technology, the alteration of plant's luminescence can be identified ahead of the change of viability. It effectively facilitates the application of ultraweak luminescence technology in the fields of industrial seeding nursery, intelligent cultivation and breed.

However, the study of plant's ultraweak luminescence is not complete, especially the luminescence properties affected by multilateral growth environment factors, and the effect of interaction of different factors is not clear to plant luminescence, which significantly restricts the application in the fields mentioned before.

In this article, quadratic rotation-orthogonal composite experimental design was used to study the luminescence properties of osmanthus tree seedings that affected by multilateral growth environment factors, and the relationship between luminescence properties and different factors and their interaction was explored. Correlative knowledge was prepared to promote the application of ultraweak luminescence testing technology in modern farming management.

1 Materials and methods

1.1 Materials and processing

45 bonsai of osmanthus tree seedings were supplied by Zhongtai nursery stock base of Hangzhou New Blue-Sky Landscape Group, seedings grew in a greenhouse with identical environment and nutrient conditions for 30 days, and the degrees of dense foliage were kept similar. Seedings were put into an artificial climate incubator (Artificial Climate Box, RXZ) after collected, kept in 30℃ and 90% in humidity and exposed in light before use. The experiment was carried out on three replicates.

1.2 Range of environment factors

The environment factors of osmanthus tree seedings include illumination, temperature, relative humidity and moisture. These factors were controlled by RXZ except for moisture that artificially controlled by using a injector with scales. Ranges of these four factors were formulated as follows:

Illumination: according to the average illumination of the greenhouse, illuminance was regulated by ten 40 W fluorescent tubes, intensity was controlled by opening different numbers of tubes (intensity of one 40 W fluorescent tube is 422.9±10.1 Lx).

Temperature: the temperature range was automatically controlled by RXZ at 15 ℃ ~ 35 ℃, accuracy was ±0.5 ℃.

Humidity: the relative humidity was controlled by RXZ at 65%~85%, accuracy is ±1%.

Moisture: according to the moisture range (10~90 mL/d) obtained from preliminary test, moisture was controlled artificially by using a injector with scales at the same time of everyday.

1.3 Measurement of ultraweak luminescence value

The optimum detect condition of luminescence was obtained from pre-experiment. Osmanthus seedings were cultivated in given conditions for 3 days before test, 2 g of seedings (leaves) were detected under 35 ℃ to get the maximum luminescence value.

Photo-induced delayed luminescence was observed at the beginning of test, emission of seeding leaves in constant time interval decreased rapidly and waved evidently. After 3 ~ 5 min, delayed luminescence disappeared gradually, emission tended to be steady. Hence, the whole data acquisition time of each experiment was 10 min, and data of last 5 min was adopted (detection cup without samples as control).

1.4 Experimental design and data analysis

Quadratic rotation-orthogonal composite experimental design was used to analyze the effect of illumination, temperature, relative humidity, moisture, and interaction to the luminescence of samples. Quadratic rotation-orthogonal composite experimental design in coded and actual level of variables and the luminous value of each sample were detected (data not shown). 36 experiments were carried out with 12 replicates at the center point.

The result was analyzed to build the model of environmental factors to the luminescence by using Data Processing System (DPS), the optimum values of environment variables were obtained when luminous value was maximum, and the effect of influential factors and interaction on luminescence was further studied.

2 Results and analysis

2.1 Regression analysis and optimal parameter analysis

Polynomial regression equation with linear, quadratic and interaction terms was obtained by using DPS, analysis of variance (ANOVA) of each effect was shown in Tab.1.

Tab.1 showed that linear effects of 4 environment factors significantly ($P \leq 0.01$) affected the luminous value of leaves, according to P-value, the impact of each linear effect followed the order: moisture > humidity > temperature > illumination and the impact of nonlinear effect followed the order: moisture > humidity > illumination > temperature. Hence, moisture, humidity, illumination, temperature were all significant factors that influenced the growth of seeding leaves, and should be paid attention in modern farming management.

Table 1 Model and condensed ANOVA (analysis of variance) table in coded level of variables

Source of variations	Coefficient of polynomial	F-Value
Constant	4.3258	—
x_1	1.4379	21.02781***
x_2	1.6296	27.00721***
x_3	1.5571	24.65757***

x_4	3.2204	105.47528***
x_1^2	1.0128	13.90988***
x_2^2	0.7103	6.84170**
x_3^2	0.9153	11.36067***
x_4^2	1.3853	26.02321***
$x_1 x_2$	0.7056	3.37585*
$x_1 x_3$	0.5469	2.02774 NS
$x_1 x_4$	1.3894	13.08803***
$x_2 x_3$	-1.1431	8.85978***
$x_2 x_4$	1.0469	7.43062**
$x_3 x_4$	0.9381	5.96701**
r	0.976	—

Note: Variables: x_1—light, x_2—temperature, x_3—relative humidity, x_4—moisture. Non-significant at $p > 0.10$; * Significant at $p \le 0.10$; ** Significant at $p \le 0.05$; *** Significant at $p \le 0.01$.

Besides, interactions of four environment factors had different influence on the luminous value of seeding leaves. It was revealed that moisture was a crucial factor which affected the growth of seedings, its importance was reflected not only in it as a single factor, but also in the strong interaction between it and the other three factors. Nevertheless, illumination had no strong interaction with temperature or humidity, therefore, regulation of moisture and illumination should be attached great importance in modern farming management of osmanthus tree seedings.

Tab.1 also showed that regression equation of response function had a high correlation coefficient(r=0.993), can be used to forecast luminous value of seedings in trial stretch.

To determine the value of four factors including illumination, temperature, moisture and humidity required by osmanthus tree seedings [3], optimal calculation of luminous value was carried out based on the intimate positive correlation between luminous value and viability of plants. Results showed that the maximum luminous value (48.11 photon/s) was acquired when 10 lamps were used (illumination value was 4 229 Lx), temperature, humidity and moisture were 35 ℃, 65% and 90 mL/d respectively. Experiments showed that luminous value was 45.76 photon/s under this optimum condition, slightly lower than the predicted value, but higher than that under other experimental conditions.

2.2 Analysis of effect of each factor and interaction on ultraweak luminous value

In order to facilitate further analysis of single factors and interaction effects on luminous value, the regression model shown in Tab.1 was corrected. First, non-significant terms were rejected, the rest of terms (coded level) constitute the relational model with the luminous value of osmanthus (Eq.1).

$$Y=4.3258+1.4379x1+1.6296x2+1.5571x3+3.2204x4+1.0128x12+0.7103x22+0.9153x32 +1.3853x42+0.7056x1x2+1.3894x1x4-1.1431x2x3+1.0469x2x4+0.9381x3x4 \quad (1)$$

The correlation coefficient was further elevated (r=0.983).

2.2.1 Effect of each factor on ultraweak luminous value of osmanthus tree seedings

Fig.1 and Fig.2 showed that luminous value of seedings first decrease then increase with four single factors when the other factors were zero. The maximum luminous value was obtained when factors reached maximum respectively, and because of the positive correlation between ultraweak luminescence and viability of plants.

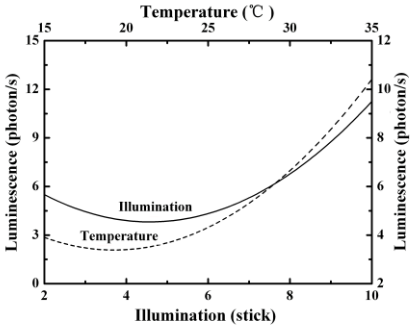


Fig. 1. Effect of illumination and temperature on the bio-illumination of osmanthus tree seedings sample

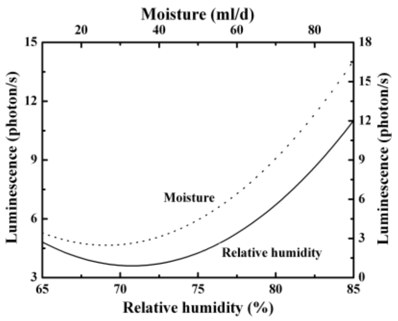
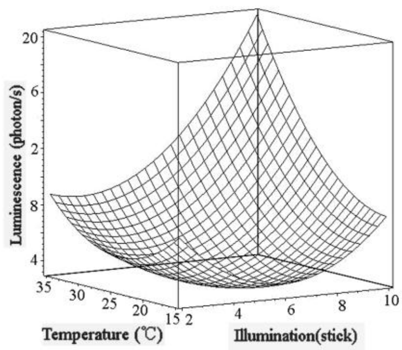


Fig. 2. Effect of relative humidity and moisture on the bio-illumination of osmanthus tree seedings sample

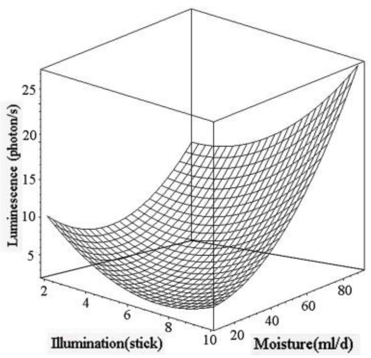
Luminous value (viability) was larger while illumination increased, that is because light is energy source of photosynthesis, and is the essential growth condition for chloroplast development and chlorophyll synthesis. Light can also regulate the activity of some enzymes in carbon cycle of photosynthesis. Furthermore, as the main external factor that affects transpiration, illumination facilitates stoma open and reduces stomatal resistance. In addition, illumination increased vapor pressure gradient and transpiration rate by enhancing air temperature and leaf temperature. To osmanthus seedings, illumination of 6 ~ 8 h is required. Under this illumination condition, luminous value of seedings was elevated with increased illumination. Meanwhile, luminous value increased with increasing of temperature, humidity and moisture.

2.2.2 Interaction effect of four factors on ultraweak luminous value of osmanthus tree seedings

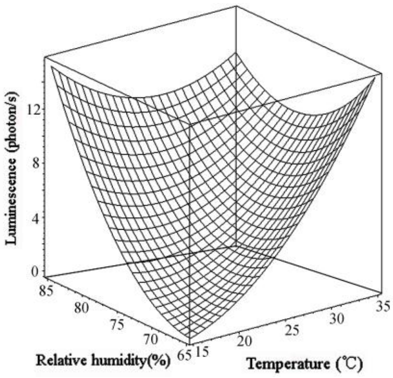
Fig.3(a) showed that, with the increase of environmental humidity, the variation trend of luminous value remained unchanged with varied illumination, luminous value first decreased then increased with the increase of illumination. Nevertheless, the increasing rate of luminous value was enhanced significantly when illumination increased at a high humidity. Likewise, with the increase of illumination, luminescence was firstly decreased then increased when humidity increased. When illumination was high, the increasing rate of luminescence was elevated significantly with the increase of humidity. This fully demonstrated the interaction between humidity and illumination.



(a) Temperature and illumination

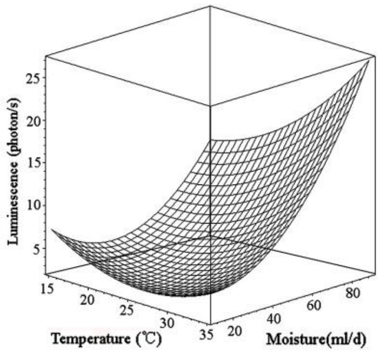


(b) Illumination and moisture

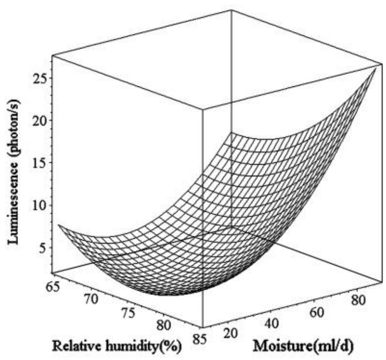


(c) Relative humidity

and temperature



(d) Temperature and moisture



(e) Relative humidity and moisture

Fig.3. Effect of different factors on the bio-illumination of osmanthus tree seedings sample

Compared with Fig.3(a), the other interactions had similar effects on luminescence of seedings. When illumination, temperature, moisture and humidity were in high level, the variation rate of luminous value increased with variation of factors which significantly correlated with luminescence. Luminous value that higher than maximum luminescence was obtained, and the same as the viability of osmanthus tree seedings.

Different from effects of other interactions, interaction of temperature and humidity was unique to luminescence of osmanthus. Almost linear correlation was observed between temperature and luminous value when osmanthus tree seedings were in low humidity, with the increase of humidity, luminous value became initially decreased then increased. Furthermore, the maximum luminous value appeared at the maximum temperature in the condition of low humidity, in contrast the maximum luminescence showed at the minimum temperature when it was in high humidity. Meanwhile, the minimum luminescence in high humidity was much higher than that was in low humidity. The same trend was observed in humidity.

So it should be noticed in modern farming management of osmanthus tree seedings that the influence of humidity on viability reduced while in high temperature, and when it was in high humidity, effect of temperature decreased in the same way. Hence, energy could be saved by controlling one of the two factors.

3 Discussion

Fig.1-Fig.3 showed that no matter single factor response curve or two-factor response surface, there was a minimum luminous value of seedings influenced by illumination, temperature, humidity and moisture. Luminescence increased when variables were higher or lower than that corresponding to the minimum luminous value. It could not explained that growth of osmanthus tree seedings was promoted when four variables were higher or lower than certain critical values.

It can be explained by Liu [14] who demonstrated the theory of coherent states and its application in physical stimulation of acupoint [15-16]. According to the theory, acupoint is commonly existed in organisms, and it affects bio-photon emission. Generally, most normal cells in organisms like osmanthus tree seedings are in a sub-change state, and photons can be easily stored in this state. In other words, osmanthus seedings are in the most steady state when photon emission is lowest.

When environmental variables are higher or lower, the growth status is destroyed and then pathological phenomena such as the increase of luminescence are observed. For instance, oversized and undersized cells both indicate pathological changes in human body. However, this pathological phenomenon is sometimes profitable when it comes to sensory demanded plants and animal products. For example, when environmental variables are higher than the most stable level, osmanthus tree may be taller, having larger leaf area and more buds, though it is a pathological phenomenon strictly. These can be explained by the physiological resistance of bio-systems when the environment condition deviates the most stable state. For modern farming management, it is to take advantage of this bio-resistance to get products that meet these sensory needs.

4 Conclusion

Single factors and interactions of illumination, temperature, humidity and moisture have effect on the luminous character of osmanthus tree seeding leaves. This is explained by the variation of acupoint state when the environment factors change. For single factors, moisture and humidity are more significant than illumination and temperature. For interactions, the interactions of illumination and moisture, temperature and humidity have the most significant influence on bio-emission, and the interaction of temperature and humidity has particular effect comparing with others. In addition, the mathematical model established in this study effectively reflects the impacts of various environmental factors on ultraweak luminous properties of osmanthus tree seedlings.

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