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Effect of Seed Coating with Fluorescent Compound on Quality and Fluorescence of Cucumber seeds

Thiphinkong, D. and Sikhao, P.*

Department of Plant Production Technology, Faculty of Agricultural Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand.

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Abstract The quality and fluorescence of seed after coating with fluorescent compound on cucumber was investigated. The results showed that the coated seed with all types of fluorescent compound had a fluorescence when examined by using a portable ultraviolet light projector. The coated seed with coating substance mixed with Rhodamine had the clearest fluorescence. All the coated seed with coating substance mixed with fluorescence compound were specifically emissions spectra observed from a spectrofluorophotometer. In addition, there were also not affected germination and germination index of seed. In summary, coating with coating substance mixed with Rhodamine is an effective and convenient to mark any cucumber seed lot for provide identity and other qualifications as well as preventing counterfeiting of seeds.

Keywords: Seed coating, Fluorescent compound, Seed quality

Introduction

Seeds are a key fundamental factor influencing quality and quantity of agriculture products. From an economic point of view, seed production can generate 45 million US dollars in international trade worldwide (International Seed Federation, 2021). The seed industry in Thailand has been continuously growing due to the suitable climate for producing seeds. Thailand is also an agricultural country and Thai farmers have experienced and expertised in cultivation. According to statistical data, the export revenues of controlled seeds from Thailand has increased every year. In 2020, the amount of controlled seed exports were 1,742,813.44 tons, which was equal to 261,318,057.85 million baht. However, the amount import of controlled seed was only 203,280.67 tons or equal to 46,624,878.87 million baht (Office of Agricultural Regulation, 2021). In today's seed trade, there are exploitations without regard to values, morality, and ethics. The cases are selling poor, fake,

***Corresponding Author**: Sikhao, P.; **Email:** potjana.si@kmitl.ac.th

or imitation seeds, stealing seeds from other companies' field plots, and stealing parent breeds (Guan *et al.*, 2013) which negative effects for good seed producers. Although, the use of fluorescent coating imposes the high cost of seed production that can prevent seed counterfeiting. This technology is not only applied a thin and uniform coating on the seed surface without changing seed’s shape, but also increased seed’s adhesion to active ingredients which helps for protecting seeds, absorbing active substances, and promoting growth (Siri, 2015). The seed coating techniques and tools have been developed from the pharmaceutical industry using polymers with an appropriate level of stickiness and a combination of useful active ingredients to improve seed quality. Increasing percentage of germination can be done by coating the seeds with plant nutrients, growth regulators, plant hormones and pesticides. Preventing adulteration can be done by coating them with distinctive colors. Nowadays, the coloring technique is just for giving identities about seeds or owners, which is not sufficiently specific to prevent counterfeit or imitation. Farmers still have risks to buy seeds with poor quality from fake lots resulting in low yield in crop production. The success of crop production relies on the quality of the seeds used for planting, so coating the seeds with a more specific substance might solve a problem of seed forgeries. As in the experimental work of Guan *et al.* (2013) they used Rhodamine B with different concentrations to apply as a coating on tobacco seeds. The seeds were then examined for fluorescence property at a wavelength of 530-560 nm. They found that the seeds with a coating of Rhodamine B showed a red fluorescence and the seeds without a coating of Rhodamine B did not show red fluorescence. This research showed that coating and additional markers on the seed surface using various fluorescent substances can be helpful. The fluorescence must be examined for emission spectra under a spectrofluorophotometer, it provides a unique hidden identity about seeds and ownership to prevent counterfeits.

Materials and methods

Preparation of seed coating substance

Seed coating substance was prepared by using a ready-made coating (Ceres International Co., Ltd.) mixed with various fluorescent substances such as safranine (PanReac AppliChem ITW Reagents), Riboflavin (Loba Chemie Pvt. Ltd.), Rhodamine (Loba Chemie Pvt. Ltd.), Curcumin (SK Herb Co., Ltd.) and Chlorophyll from in-lab preparation. Briefly, chlorophyll was obtained by cutting 150 g of mango leaves into small pieces, grinding with 80% acetone as a solvent, adjusting the volume to 100 ml, and putting it into a beaker covered with aluminum foil, respectively. The beaker was stored in the dark room immediately at room temperature for 24 hours or until the green color from the leaves had completely dissolved.

Cucumber seed coating

The experimental design was done using a Completely Randomized Design (CRD). Different types of fluorescent coatings were used for coating hybrid cucumber seeds (TSA Co., Ltd.). The coatings and other mixing substances for fluorescent properties were prepared in 7 different ways including 1) coating seeds with only a coating substance (T1), 2) coating seeds with a combination of coating substance and 0.03% safranine (T2), 3) coating seeds with 0.5% riboflavin (T3), 4) coating seeds with 1% chlorophyll (T4), 5) coating seeds with 0.3% rhodamine (T5), and 6) coating seeds with 0.2% curcumin (T6). The uncoated seed was used as a control (T0). The coating process was performed by using a rotating disk seed coating machine (model RRC150) using 140 ml of coating substances per 1 kg of seed (Tian *et al.*, 2013). After that, the coated seeds were dehumidified by a hot air oven at 35 ℃ to reduce the moisture content to close to the level before coating. The fluorescence property was then examined on the surface of the seeds with a portable ultraviolet light projector at a wavelength of 254 nm. and the emission spectra were observed with a SHIMADZU spectrofluorophotometer (model RF-6000. The properties of seed including moisture content, germination and germination index were also examined.

Fluorescence examination on seed surface

10 coated and uncoated seeds were observed for fluorescence on the surface of the seeds with a portable ultraviolet light projector at a wavelength of 254 nm. and for the emission spectra with a SHIMADZU spectrofluorophotometer (model RF-6000) at an excitation wavelength of 300-600 nm. All coated and uncoated seeds were randomized into the solid sample holder, spread evenly, measured their emission spectrum, and then recorded the results as fluorescence intensity values at different wavelengths.

Seed moisture content

Seed moisture contents were checked by the RHINO RR Moisture machine (model HC2-AW-USB-SW) which calculated the seed moisture by measuring Water Activity. The coated and uncoated seeds were loaded into the probes. Then, the probe sets were placed into the Sample Holder and the water activity values from the machine were reported. The Water Activity values were converted by the software application namely Seed Viability to the seed moisture content in percentage and recorded as results.

Germination percentage of seeds in laboratory conditions

The percentages of germination of coated and uncoated cucumber seeds were checked by the between paper method with a number of samples of 50 seeds in quadruplicate. Briefly, seeds were put on a stack of two damp planting papers and placed in an incubator where its temperature was controlled at 20-30 ℃. The germinations were evaluated by counting normal germinated seedlings at 4 days old (as a first count) and 8 days (as a final count) (ISTA, 2019).

$$\text{seed germination (\%)} = \left[\frac{\text{number of normal seedlings}}{\text{number of seeds}} \right] \times 100$$

Germination index

All coated and uncoated cucumber seeds were cultivated according to the method of seed germination examination in the laboratory. Counting the number of normal germinated seeds was performed on day 4 (as the first count) and day 8 (as the final count) (ISTA, 2019).

$$\text{seeds germination index} = \left[\frac{\text{daily seedlings}}{\text{number of days after cultivation}} \right]$$

Results

Effect of different fluorescent substances on the quality of cucumber seeds after coating

When inspecting the quality of coated and uncoated cucumber seeds, it was found that the percentages of moisture content of cucumber seeds were not statistically different in different coatings. A combination of coating substance with curcumin on seeds (T6) showed the highest moisture content (12.50%) followed by only a coating substance on seeds (T1), a combination of coating substance with chlorophyll (T4), uncoated seeds (T10), a combination of coating substance with riboflavin (T3), rhodamine (T5), and safranine (T2), respectively. The effects on germination of seeds grown in laboratory conditions were not statistical difference. Seeds coated with chlorophyll (T4) had the highest percentage of germination (94.50%), followed by seed coated with safranine (T2), which was 93.75 percent, corresponding to seed germination index. There were not statistical differences between all coating treatments. The effects on germination index showed that seeds co-coated with chlorophyll had the highest germination index (14.68), followed by uncoated seeds (T0), seeds co-coated with riboflavin (T3), safranine (T2), curcumin (T6), only a coating substance (T1) and rhodamine (T5), respectively (Table 1).

Table 1. Seed moisture content, seed germination and germination index of uncoated and coated seed with coating substance and coating substance mixed with different type of fluorescent compound after coating

Coating substance[1/]	**Moisture (%) Content**	**Germination (%)**	**Germination index**
T0	12.18	91.00	14.12
T1	12.42	86.50	13.00
T2	11.73	93.75	13.34
T3	12.17	92.00	14.06
T4	12.22	94.50	14.68
T5	12.15	91.00	12.75
T6	12.50	91.50	13.31
F-test	ns	ns	ns
C.V. (%)	4.52	5.85	7.80

ns = non statistical significant difference.
[1/]T0: control, T1: Seed coating with coating substance only, T2: Seed coating with coating substance mixed with Safranin, T3: Seed coating with coating substance mixed with Riboflavin, T4: Seed coating with coating substance mixed with Chlorophyll, T5: Seed coating with coating substance mixed with Rhodamine, T6: Seed coating with coating substance mixed with Curcumin.

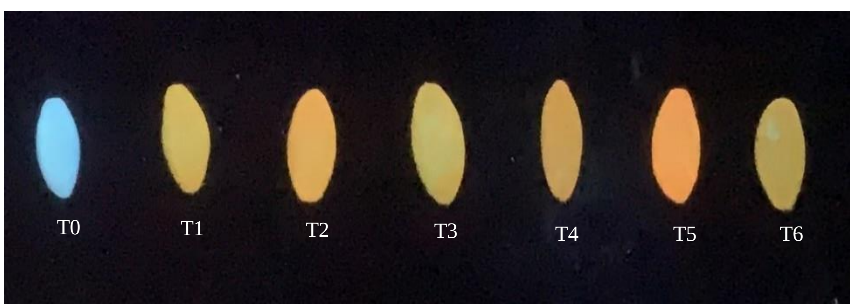

Figure 1. The fluorescent of cucumber seed after coated with ultraviolet light, T0: control, T1: Seed coating with coating substance only, T2, T3, T4, T5 and T6: Seed coating with coating substance mixed with Safranin, Riboflavin, Chlorophyll, Rhodamine and Curcumin, respectively

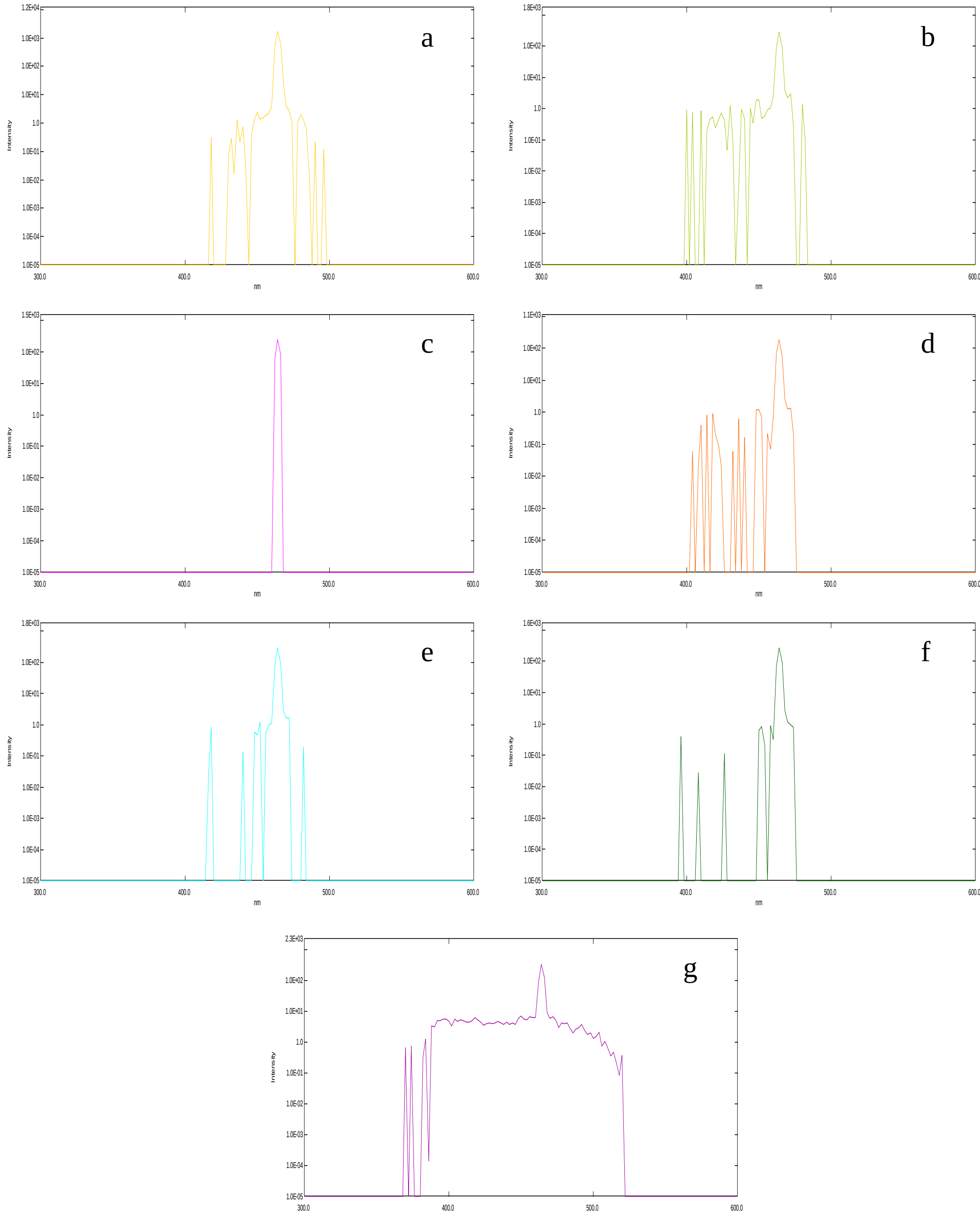

Figure 2. The fluorescence emission spectra from a spectrofluoro photometer of uncoated and coated cucumber seeds. The X axis is a fluorescence intensity (a.u.) and the Y axis is a wavelength (nm). The letters a: control (T0), b: Seed coating with coating substance only (T1), c, d, e, f and g: Seed coating with coating substance mixed with Safranin (T2), Riboflavin (T3), Chlorophyll (T4), Rhodamine (T5) and Curcumin (T6), respectively

The fluorescence property of cucumber seeds after coating with different types of fluorescent substances

The coated and uncoated seeds were examined for fluorescence by using a portable ultraviolet light projector. Under ultraviolet light, the uncoated seed (T0) emitted a blue fluorescence and the seeds coated with only a coating substance (T1) showed no fluorescence. Other seeds were coated with various types of fluorescent substances emitted lights differently. Seeds that co-coated with safranine (T2), riboflavin (T3), chlorophyll (T4), rhodamine (T5) and curcumin (T6) were emitted fluorescence as orange, greenish yellow, bright yellow, orange and bright yellow, respectively (Figure 1). The cucumber seeds were examined for the emission spectra by using a spectrofluorophotometer at the stimulating wavelength of 300-600 nm, the uncoated and coated seeds with coating substance only were emited lights at similar wavelengths. All the coated seed with coating substance mixed with fluorescence compound were specifically observed emissions at these wavelengths in groups of uncoated seeds and only coated with a coating substance (Figure 2).

Discussion

The research was investigated the effects of coating on cucumber seeds using a coating substance and several combinations of a coating substance and fluorescent substances, including safranine, riboflavin, chlorophyll, rhodamine and curcumin, which provide identity and other qualifications. From the inspection after coating, it was found that coated seeds with fluorescent substances did not affect moisture content, germination in laboratory conditions, and germination index when compared to germination at 93-94% of uncoated seeds and seeds that coated with a coating substance. It is possibly due to the film from combinations of a coating with active ingredients could dissolve quickly and did not block oxygen absorption during germination (Kim and Taylor, 2004). Also, each fluorescence substance is non-toxic and needed in small quantities, therefore it is safe for seeds. Chaiwicha (2016) reported that tomato seed coatings with 0.20% rhodamine B did not show any effects on seed quality when examined in greenhouse conditions. Rhodamine B is not toxic to seeds, as it was tested to use as an oral rabies biomarker and vaccine for wildlife (Mitchanthae and Thepsumethanont, 2001). Siri and Simakhon (2013) studied seed coatings with a fluorescent substance, riboflavin, at various concentrations, they reported similar findings that the coating did not affect germination and germination rate. Sikhao *et al.* (2014) found that coating cucumber seeds with different concentrations of riboflavin had no effects on seed quality. Riboflavin is extracted from vitamin B which is a nutrient that can be found in nature (Ampro, 2017). Therefore, it can be assumed it is absolutely harmless to the plant. This finding is also observed in tobacco seedlings coating with safranine T (Guan *et al.,* 2013) which is a biological substance used in cytology. Safranine is used in cell staining protocols to dye the nucleus to be red and as a redox indicator for chemical analysis. Thanomkwan *et al.* (2018) said that co-coated cucumber seeds with curcumin had a higher tendency and a higher speed of germination compared to seeds coated with auramine O. This might be because curcumin is an extract of beneficial turmeric which is a natural product that is used for protecting and treating various diseases, such as cancer (Onsurathum *et al.,* 2012). The seeds were coated with chlorophyll extract showed a positive effect on seed germination. Chlorophyll is a green organic compound that plants synthesize for absorbing solar energy and transmitting to receptors in chain reaction to generate molecules of energy like carbohydrate and water (Croft and Chen, 2017). Furthermore, Sikhao *et al.* (2014) reported that the coating substance of choice had not obscured the fluorescence under the ultraviolet light. The experiment showed that when seeds were coated with a coating in combination with safranine (T2), riboflavin (T3), chlorophyll (T4), rhodamine (T5) and curcumin (T6), fluorescence was observed in orange, greenish yellow, bright yellow, orange and bright yellow, respectively. This is consistent with the experimental work of Tian *et al.* (2013) using rhodamine B and safranine T to coat green pea seeds for preventing seed counterfeiting. Coating of green pea seeds with fluorescent substance had no effect on germination of seeds and seedlings. Later, Chaiwicha (2016) studied the coating of tomato seeds using rhodamine B and found to be a clear orange fluorescence. Rhodamine B was used in biology as a fluorescent dye and in biotechnology for many applications. The study from Sikhao *et al.* (2015) is consistent with research by Sellei (1941), Guan *et al.* (2011) and Guan *et al.* (2013) on experiments in tobacco and marigold seeds. They showed that rhodamine B emitted orange lights was stimulated by green fluorescence. Sikhao *et al.* (2015) coated the seeds with polymer together with riboflavin and examined under ultraviolet light. They found that only coated cucumber seeds with a combination of polymer and riboflavin emitted orange. Riboflavin is a fluorescent substance which extracted from vitamin B can be found naturally and does not showed any negative effects on seed quality.

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