Seed coating with DNA for anti-counterfeiting of cucumber seeds

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ABSTRACT: The objective of this experiment was to create seed identity by DNA coating to protect counterfeiting or imitate of inbred line. The experiment was conducted at Transgenic Plant Technology and Biosensor Laboratory, Department of Botany, Faculty of Science, Chulalongkorn University. Cucumber seed were coated with isolation of shrimp virus plasmid DNA as gelatin polymer. Identification of seed was performed by immersing coated seed in 10 μL deionized water for 10 min with gentle agitation and 2 μL of DNA identification. Detection of DNA identifier was carried out based on basic PCR principle. The result was shown that banding pattern of coated seed sample appear and same to banding pattern of plasmid DNA. This method could be created the cucumber seed identity. The cucumber seed quality was assessed by seed germination and speed of germination. The result was shown that seed coating with DNA did not affect on seed quality.
Keywords: cucumber seed, seed identity, DNA coating, seed counterfeiting

Introduction

The quality of seed is very important factor of agriculture product. In the last 10 years, both government and private companies have developed many kinds of plants and new plant varieties. Nevertheless, there are problems such as counterfeit of the seeds, that have been found and still could be found in the seed market of developing contries (Guan et al., 2013), which was cause crop yield and seed quality to be in low, include has effect on farmer's interest (Guan et al., 2011). At present, there are many researches about anti-counterfeiting technologies such as, using Audio MarQ for seed's ownership (Kasetsart University, 2008), coated tomato and cucumber seed with DNA for identity seed (Chaumpluk, 2009; Srikaow et al., 2010), soaked tobacco seed by using safranine T to fluorescent labeling for an-counterfeiting (Guan et al., 2011) which was similarly to Guan et al. (2013) used rhodamine B, coated pea seed with rhodamine B and safranine T for falsification-preventing (Tian et al., 2013). In addition, Guan et al. (2013) pelleted tobacco seed with fluorescent compound and magnetic powder simultaneously in the same pellet that can be improved anti-counterfeiting technology and enhanced the seed security. Nevertheless, most of anti-counterfeiting technologies were not easy to preparing and detection process. Nowadays, seed coating method has been used worldwide because chemical coating reagent attaches uniformly on the seed surface to provide unique characteristics and quality assurance. The main formula of seed coating consists of polymer, solvent and additive, the additive used in this research is DNA that is used for anti-counterfeit seed.

Deoxyribonucleic acid (DNA) is a molecule that encodes the genetic instructions used in the

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development and functioning of all known living organisms and many viruses. Gel electrophoresis is a method for separation and analysis of macromolecules (DNA, RNA and proteins) and their fragments, based on their size and charge. Therefore it is possible to use DNA as an additive in seed coating compound to provide a means of seed authentication.

Materials and methods

Seed material

Hybrid cucumber seeds CU023 (*Cucumis sativus L.*) were obtained from AG Universal Company, Khon Kaen, Thailand.

Coating process

Gelatin was dissolved in water at 45±1 °C. Then, 5 µL plasmid DNA were added at room temperature. The cucumber seeds were coated by centri coater (model SKK10) with application rate of 150 ml kg^{-1} seed. After that, the coated seeds were dried by seed dryer machine at 35 °C for 3 hours. Then, the coated seeds were tested the seed quality and soaked in deionized water, the soaked water was detected by gel electrophoresis.

Seed measurement

Seed germination under laboratory condition

Three replicates of 100 seeds from each treatment were incubated on moist paper towels in germinator at 20-30 °C. The first count was carried out after 4 days and the second one after 8 days following the rules of cucumber seed germination protocol according to International Seed Testing Association (ISTA, 2008). Percentage of seed germination was calculated by following formula:

$$\text{Percentage of seed germination} = \left(\frac{\text{No. of normal seedling}}{\text{Total of seed used in germination}} \right) \times 100$$

Speed of germination

The speed of germination as following to seed germination under laboratory condition was calculated as described in the International Seed Testing Association (ISTA, 2008) by following formula:

$$\text{Speed of germination} = \frac{\text{No. of germinated seed}}{\text{Days of first count}} \; \ldots + \ldots \; \frac{\text{No. of germinated seed}}{\text{Days of final count}}$$

Gel electrophoresis detection

The coated seeds were detected by using gel electrophoresis method at 63 °C for 60 minute. DNA were divided into three part. The first part was tested DNA product on 3% agarose gel in 100 volt 1x TAE at room temperature then dyed with 0.5 µg/ml ethidium bromide, there were detected under ultraviolet light by UV transilluminator. The second part was detected DNA fluorescent by cyber green.

Experimental design

The design was randomized complete block with 3 replications. Data were statistically analyzed by ANOVA and the difference between seed coating treatment was tested by Duncan's new multiple range test (DMRT).

Results and Discussion

DNA detection on cucumber seed

According to **Figure 1**, the first band is negative control (NTC), distilled water was used as a negative control. The second band is positive control (PTC), plasmid DNA was used as a positive control. The third band is experiment control (T_0). The fourth band is DNA sample from the coated seed (T_1). The coated seeds were detected by using gel electrophoresis method then tested DNA product on 3% agarose gel in 100 volt 1x TAE at room temperature then dyed with 0.5 µg/ml ethidium bromide, there were detected under ultraviolet light by UV transilluminator. The result shown that the positive control (PTC) band and the DNA sample from the coated seed (T_1) had the same DNA product, which is the same as DNA fluorescent **(Figure 2)**. So that, seed coating with DNA can be identity seed same as Chaumpluk (2009) who found that seed coating with DNA can be identity by gene sensor. In addition, Audio MarQ was used to machine for detect seed's ownership (Kasetsart University, 2008).

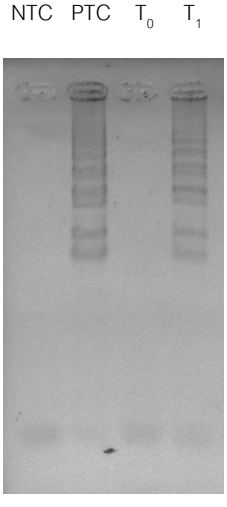

Figure 1 Gel electrophoresis of DNA coated on cucumber seed.(NTC: negative control, PTC: positive control, T_0: control, T_1: sample)

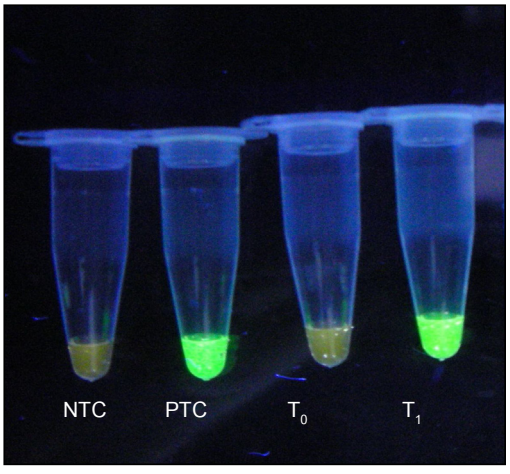

Figure 2 Fluorescence of DNA coated on cucumber seed. (NTC: negative control, PTC: positive control, T_0: control, T_1: sample)

Cucumber seed quality after coating

The coated seed with gelatin and gelatin with DNA were not significantly different on seed germination. The coated seed with DNA had increase germination but there were not significantly different. The speed of germination were not significantly different the same as seed germination **(Table 1)**. Ming et al. (2004) found that seed coating did not affect germination and seedling quality of cucumber seed, Similarly Saengpeng and Chulaka (2009) found that the polymer coating had no effect on germination and mean germination time of cucumber seeds. Almeida et al. (2005) found that polymer coating with hydroxylethy cellulose (HEC) did not affect germination and speed of germination after coated and accelerated aging on broccoli seeds.

Table 1 Seed germination (%) and speed of germination under laboratory conditions of cucumber seed after DNA coated.

Treatment	Seed germination (%)	Speed of germination
Non-coated	70.00	17.41
Gelatin coated	64.66	15.41
Gelatinwith DNA coated	73.33	18.08
F-test	ns	ns
CV (%)	13.69	14.43

ns =non significant

Conclusions

Seed coating with DNA can be seed used for seed authentication and anti-counterfeit seed, especially inbred line. Seed coating with DNA did not affect seed germination and speed of germination.

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