



Flower bagging affects seed quality and dormancy period in Na-Oogst tobacco (*Nicotiana tabacum* L. var H-382)

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ABSTRACT

Seeds of tobacco plants are unable to germinate immediately after harvest because they have a dormancy period. The seed dormancy period may be shortened by bagging methods to affect the hormone content in the seeds. The bagging flower makes the dark conditions in the process of flowering and seed development, affecting the content of the hormone's auxin and ABA. This study aimed to determine the effects of different bag colors on the dormancy period of tobacco seeds as evidenced by their vigor and viability. The research was conducted from March to August 2022 at PT. Dwi Light Tobacco Green House, Lombok Wetan, Bondowoso, Indonesia. Four crepe colors of paper were used as bagging treatments (black, red, yellow, blue) and control (without bagging) and replicated four times. The results showed that the bagging treatment significantly affected one hundred-seed weight, seed germination, seed growth rate, and seedling height. Black crepe paper resulted in the highest seed germination percentage (98.5%) and seed growth rate (11.68).

Keywords: bag color, seed germination, viability, vigor

INTRODUCTION

Tobacco is one of the plants that sexually produce seeds for reproduction. A common problem in large-scale tobacco propagation is seed dormancy caused by the use of seeds directly after harvest. Therefore, seeds with a shorter dormancy period are needed. In general, mature tobacco seeds lack germination ability because they experience a period of seed dormancy after harvest (Leubner-Metzger, 2005). Seed dormancy occurs because seed ripening increases growth-inhibiting hormones such as ABA, while there is a decrease in the auxin hormone. The hormonal imbalance in the fruit ripening process results from dormancy in tobacco seeds due to the high ABA hormone contained. The bagging method aims to reduce the intensity of light received during the fruit ripening process which may cause etiolation symptoms and increase the auxin hormone in the seeds. The auxin hormone plays a role in plant cell division, enlargement, and differentiation in plants.

The bagging color can be determined and adjusted to the color spectrum emitted by sunlight. The intensity and quality of the light spectrum can affect the synthesis of auxin in plants. The light spectrum emitted can be received by plants through various photoreceptors. Photoreceptors from UV-A and blue light are Phytochromes, Cryptochromes, and UVR8, while the photoreceptors from red light are Phytochromes (Christiaens et al., 2019). The red-light spectrum received by phytochromes with various responses can control the expression of auxin biosynthesis. Some phytochromes genes expressed in plants imply their essential roles in mediating de-etiolation and photomorphogenesis at the seedling stage (Zhao et al., 2022). In addition, phytochromes that absorb red light affect the available active auxin through excess conjugation of IAA

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transcribed by the GH3 gene. The gene plays the role of encoding an enzyme to catalyze the conjugation of IAA into amino acids. Proteins expressed by GH3 will mark IAA for storage or degradation process (Christianese et al., 2019).

Reducing a plant's sunlight intensity can slow down the performance of the auxin hormone as a plant growth promotor (Wendi et al., 2014). The bagging treatment on tobacco flowers can reduce the dormancy period of the seeds due to changes in hormone levels where the levels of auxin (IAA-Asp) increase two times higher compared to the ABA hormone (Farci et al., 2020). The dark conditions in bagging treatment caused the production of auxin hormone and distributed quickly and actively in the fruit (Hidayat et al., 2020). High levels of auxin hormone in seeds play a role in the germination period in accelerating the enlargement and elongation of cells in roots and stems (Küpers et al., 2023). Research on bagging treatment for local Besuki cigar tobacco plants has not been studied recently. This study aimed to determine the effects of different bag colors of crepe paper bagging on the dormancy period of tobacco seeds as evidenced by their vigor and viability.

MATERIALS AND METHODS

This research was conducted from March to August 2022 at PT. Dwi Cahaya Tobacco, Wonosari, Bondowoso Regency, East Java. The materials used tobacco plants in the flowering phase, tobacco seeds var. H-382, crepe paper in black, red, yellow, and blue, filter paper, water, soil, and polybags. The experiment used a completely randomized design with five different crepe paper colors (control, black, red, yellow, and blue paper) as treatments with four replications, resulting in 20 experimental units. The Duncan's Multiple Range Test (DMRT) at the 5% level was used if the F value showed significant differences within the observed variables.

Tobacco plants were selected during the third harvest around 50 days after planting. Bagging treatment was done at the early flowering stage before pollination occurs. Flower was bagged with 3 layers of crepe paper. The flowers from the selected tobacco plants were then covered with crepe paper up to the flower stalks. The crepe paper was tied using a plastic rope to prevent the paper separated from the plant. The covered flowers were maintained and supervised until the seeds harvest. Then the harvested seeds were germinated in a petri dish containing wet filter paper with a total of 50 seeds for each treatment. Observations were done at 7 days after sowing, including seed morphology, 100-seed weight (HSW), germination rate (%) calculating normal seeding to total seed, and seed growth rate (%/etmal).

Seedling's morphology was observed by planting tobacco seeds in polybags using soil media and observed after the seedlings were 42 days old after being planted. Seedlings morphological observations were conducted including seedling height (cm), leaf number, and root length (cm). The seed growth rate was determined by calculating normal seedlings' percentage per etmal (24 h) until the final count period.

$$SGR = \frac{G_1}{D_1} + \frac{G_2}{D_2} + \dots + \frac{G_n}{D_n}$$

SGR = Seed growth rate (%/etmal)

G = The number of seeds was germinated on a particular day

D = Adjusted time depended on G

n = Number of days in the final count

RESULTS AND DISCUSSION

The bagging treatment on tobacco fruit using different colored crepe paper reduced the intensity of light received by the fruit, therefore the fruit development was in dark conditions. The light intensity was able to manipulate plant growth and development in response to changes that occur in the external environment. Light intensity affects plants' physiological processes, which can be seen through their morphological conditions (Novák et al., 2021). Moreover, plants grown at low light intensity were smaller and thinner (Wassenaar et al., 2022). Figure 1 shows the morphological differences in the

color of tobacco fruit due to bagging treatment. This condition was influenced by bagging treatment, which can reduce the intensity of sunlight received by the fruit and the chlorophyll content within the fruit.

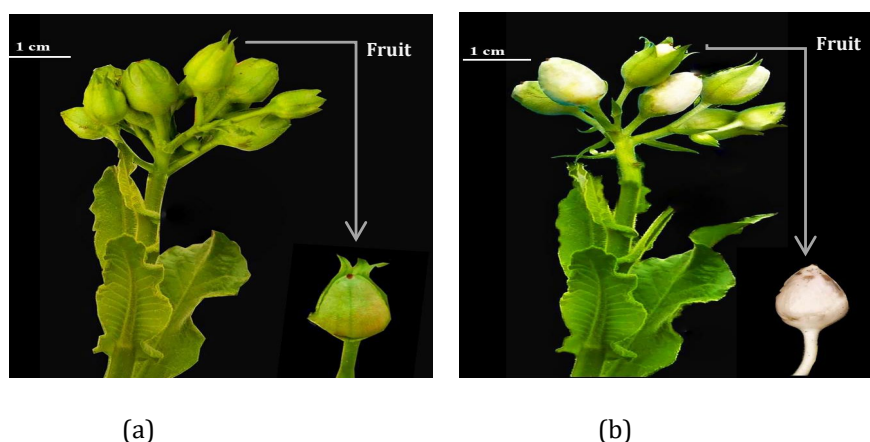


Figure 1. Tobacco fruit color from different bagging treatments. (a) Green fruit color without bagging treatment (control), (b) white fruit color of bagging treatment (black bag). Tobacco seeds are located inside the fruit.

The part of the plant that is not exposed to sunlight will be pale in color due to its incapability to form chlorophyll. Chlorophyll is a plant's photosynthetic pigment, which measures the plant's ability to use light as a material in the plant's metabolism processes (Zhang et al., 2016). The flower bagging treatment on tobacco fruit was able to degrade chlorophyll as indicated by the change in the green color of the fruit to pale white. The color changing indicates etiolation that occurs during the development of tobacco fruit and seeds.

One hundred-seed weight (HSW)

This research carried out the bagging on the process of forming tobacco fruit so that it allowed the development of the fruit in dark conditions. The bagging treatment using black-colored crepe paper significantly affected the weight of tobacco seeds as well as its average compared to other treatments. The black-colored crepe paper resulted in the highest increase of one hundred-seed weight (10.89%), higher than the red-colored crepe paper treatment (5.16%), blue (4.21%) and yellow (0.38%) compared to the control treatment (Table 1). The high average value of the black treatment correlates with the temperature factor formed in the bagging phase.

Table 1. Effect of bagging treatment on one hundred-seed weight, seed growth rate, and germination percentage.

Bag color	One hundred-seed weight (mg)	Seed growth rate (%/etmal)	Seed germination percentage (%)
Control	5.23b	3.36d	26.0c
Black	5.80a	11.86a	98.5a
Red	5.50b	10.78b	92.0a
Yellow	5.25b	6.68c	57.5b
Blue	5.45b	6.69c	60.0b

Note: Numbers followed by the same letter in the same column are not significantly different based on DMRT at the 5% level.

Black-colored crepe paper resulted in the highest fruit weight compared to other treatments since the black color could absorb the entire spectrum of white light without reflecting it, so this black paper can heat up faster and reach the highest temperature (Fajar & Rohmah, 2019) (Figure 2).

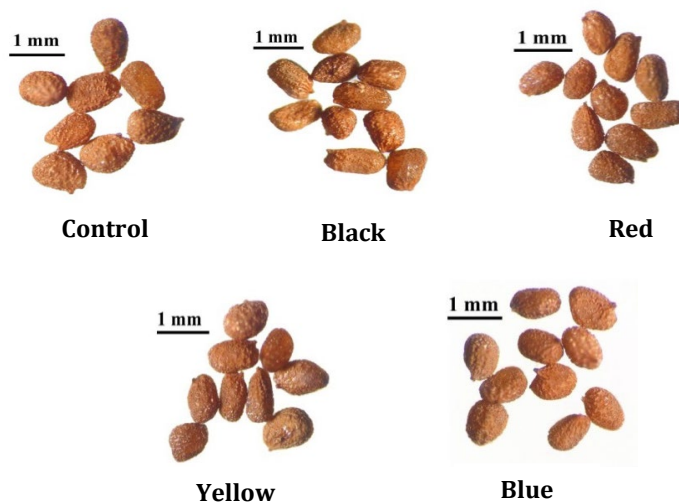


Figure 2. Tobacco seed morphology in different bagging treatments using different color bags.

Environmental factors such as high temperatures can accelerate the rate of assimilation and photosynthate translocation from leaves to grain or seed. Increasing temperature can increase sink strength in fruit such as assimilate translocation and fruit development rate, thus increasing fruit and seed size (Romalasari et al., 2017). The sink strength and capacity in plants is the maximum volume available to accommodate the results of bio-photosynthesis which later can be expressed in the number, size, and weight of seeds (Mastur, 2016).

Seed growth rate

The seed growth rate is one of the methods that can be used to determine the rapidity of seed growth and give an indication of seed vigor level. Seed growth rates are based on the principle that vigorous seeds grow faster than poor vigorous seeds even under favorable environments. The seed vigor index is an indicator that can be used to determine the speed and uniformity of germination of seed (Fatikhasari et al., 2022). High seed vigor will affect the strength of seed growth and storability.

The bagging treatment using black crepe paper significantly affected the seed growth rate compared to other treatments. The bagging treatment using black crepe paper significantly increased the average seed growth rate (126%), higher than the red (106%), yellow (33.46%), and blue (27.91%) compared to the control treatment (Table 1). Normal sun exposure has a light intensity of around 10,000-100,000 lux and can change according to weather conditions. The black bagging treatment reduced the light intensity value to 1,939 lux. It was suspected that this treatment could etiolate the tobacco fruit better because the light intensity received by the fruit was lower when compared to other treatments. This will encourage a higher increase in the auxin hormone since the auxin hormone will be activated in dark conditions. The increase in the hormones auxin and gibberellin in the black bagging treatment stimulated the rate of imbibition, respiration, and seed metabolism during germination. An increase in the auxin hormone will affect the increase in osmotic pressure, which determines the amount of water that enters the seed and accelerates the imbibition process in the seed. The occurrence of imbibition will spur physiological processes in cells thereby accelerating the germination process.

Germination percentage

The value of seed germination is influenced by the number of seeds that can germinate either normally or abnormally (Taghvir et al., 2018). The effect of the bagging treatment from black crepe paper resulted in the highest increase in average germination percentage (278%), significantly higher than the red (253%), yellow (221%), blue (130%), and the control treatment (Table 1). The highest seed germination was due to an increase in the auxin hormone during seed development (Figure 3). These results inline with the research of Farci et al. (2020) who stated that the use of black crepe paper increase the levels of the hormones auxin and cytokinin in seeds with concentrations 2 times higher than without bagging treatment. Auxin and cytokinin are required to promote bud outgrowth from dormancy which steps to sustained outgrowth so that the seed germination develops well (Ferguson & Beveridge, 2009).

The bagging treatment reduces the ABA hormone with a concentration of 1.6 times lower due to the dominance of the auxin hormone in the seeds (Farci et al., 2020). The auxin hormone in the bagging treatment caused a high growth rate of seeds and reduced their dormancy period.

Using black-colored crepe paper can etiolate tobacco fruit, reducing the ABA hormone and accelerating the seed dormancy period. The hormone ethylene regulates the fruit ripening process, but the seed dormancy phase is regulated by the balance between the action of ABA hormones and auxin or gibberellins (Farci et al., 2020). Auxin and gibberellins play an important role in seed development, formation, maturation, and germination (Figueiredo & Köhler, 2018; Sreenivasulu, 2017). The bagging treatment using black crepe paper can increase the concentration of the auxin hormone which plays a role in encouraging cell division in roots and stems to elongate and enlarge, which can support embryo development and germination (Robert, 2019).

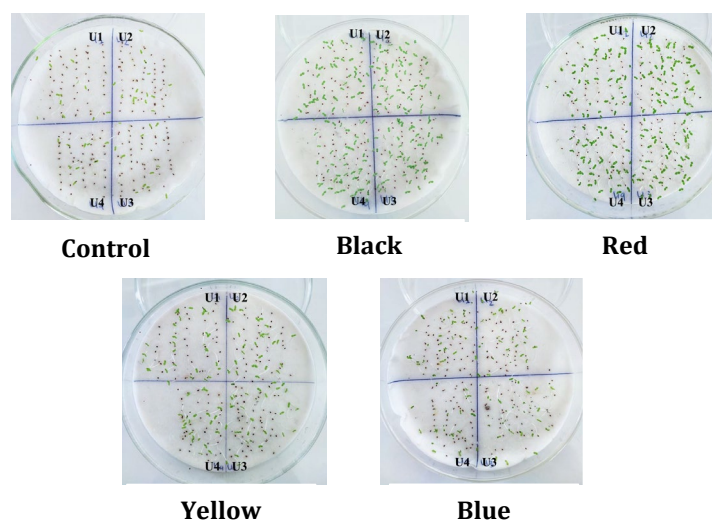


Figure 3. Observation of tobacco seed growth rate 7 days after sowing in different bagging treatments.

Seedling morphology

The bagging treatment using different colored crepe paper significantly affected seedling height, but did not significantly affect leaf number and root length. The black-, red-, yellow-, and blue-crepe paper increased seedling height by 14.49%, 5.00%, 4.83%, and 3.96%, respectively, compared to the control treatment (Table 2).

Table 2. Effect of bagging treatment using different color bagging on seedling morphology at 42 days after planting.

Bag color	Seedling height (cm)	Leaf number	Root length (cm)
Control	11.38b	4.7	5.50
Black	13.03a	5.0	7.25
Red	11.95b	5.0	7.15
Yellow	11.93b	4.7	6.05
Blue	11.85b	4.5	6.90

Note: Numbers followed by the same letter in the same column are not significantly different based on DMRT at the 5% level.

Plant height is an indicator of growth due to cell division and elongation which is influenced by plant auxin (Anni et al., 2013). The bagging treatment using black crepe paper increased the height of the tobacco plant seedlings compared to other treatments (Figure 4). This is because the seeds that develop in the dark with bagging treatment can significantly increase the auxin hormone compared to controls (Farci et al., 2020). Auxin plays an important role in plant growth which can regulate the enlargement and elongation of cells at the back of the meristem tips (Mutryarny & Lidar, 2018). Differences in hormone concentrations formed during seed development are suspected to be factors that influence significantly different yields on the height of tobacco plant seedlings.

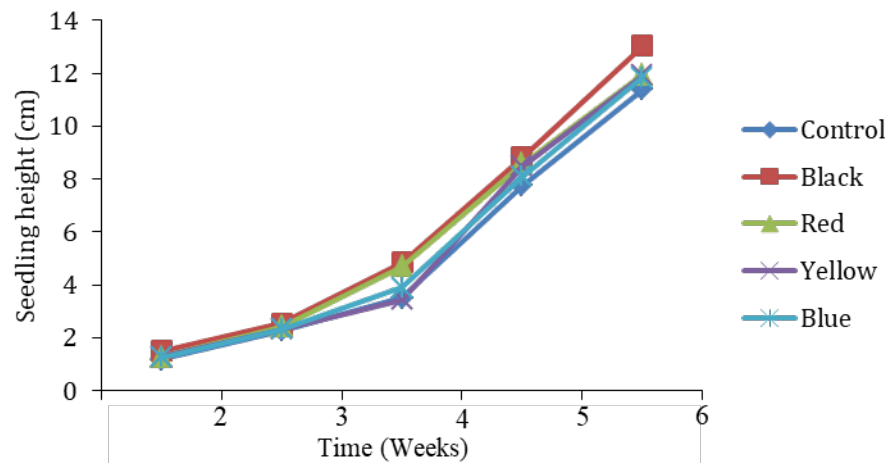


Figure 4. Weekly tobacco seedling height from seeds produced by bagging treatment using different color bagging 42 days after planting.

The leaf numbers, between 4-5, did not differ among treatments. (Table 2). This condition is due to the other factors that affect leaf number besides auxin and gibberellin which is the plant height factor. The increase in leaf number on plants is in line with the height of the plant seeds (Mutryarny & Lidar, 2018). Based on observations, the bagging treatment only gives a difference of 2 cm between the lowest value and the highest value of plant height. There is no significant difference in plant height, therefore the nodes produced in each treatment are not much different.

The bagging treatment of tobacco seeds did not significantly affect root length due to environmental factors. Environmental factors that influence plant root systems include soil moisture, acidity, aeration, temperature, mechanical resistance, competition, and root interactions (Imansyah & Rodhiya, 2020). In the implementation of the research, the tobacco seeds were watered at an intensity of 2 times a day to meet the plant's water needs. Adequate water availability affects root length. Since plants' roots take advantage of surface water, it is effective for tobacco plants to be planted shallowly with sufficient water on the surface.

CONCLUSIONS

The bagging treatment using different colored crepe paper affected seed weight, germination, seed growth rate, and plant height. The use of black colored-crepe paper gave the best results as indicated by the highest seed growth rate of 98.5% and the vigor index of 11.86.

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