

A novel seed balling technology and its effect on cotton emergence, yield and fiber quality

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Highlights

- Propose a new way of seed treatment-seed balling treatment.
- A large amount of non-polluting, easily accessible fertilizers and other beneficial substances can be wrapped around the seeds and sown together.
- Balled treatment can wrap the seeds to form a relatively stable internal environment to resist biotic and abiotic stresses and improve the seedling emergence rate.
- Preliminarily solved part of the shortcomings of the traditional seed coating, after the seed germination and seedling emergence, the spherical material can continue to promote the growth and development of seedlings.

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Abstract

Seed coating is the most important type of pretreatment. Since cotton is an important economic crop, the cost of its cultivation and the resulting economic benefits are undoubtedly important aspects to be considered. In recent years, the high cost of coating materials and complex production processes have prevented the widespread application of cotton seed coating. Moreover, cotton plants emerge from cotyledons, and the coating material on the seed coat does not play a role after the seed emerges. Given the above shortcomings, to adapt to the mechanized direct seeding method and to include a large number of fertilizers and fungicides, insecticides can be used together with the seed direct seeding into the soil; at the same time, this will improve the cotton seedling emergence rate, the physiological qualities of cotton seedlings after the emergence of cotton seedlings, and the resilience of cotton seedlings in the early stage of resistance ability. In this study, we devised a technique for balling cotton seeds employing components such as cassava starch, bentonite, diatomite, attapulgite, and seedling substrate. The compositional ratios of the method were determined *via* a growth chamber trial, and we evaluated its effect throughout the cotton reproductive period using field trials. The results showed that the emergence and emergence hole rates of the balled cotton seeds increased by 34.42% and 28.84%, respectively, compared with the uncoated control. In terms of cotton yield, the seed balling treatment increased the number of bolls per plant and the overall cotton yield. Seed balling technology is different from traditional seed pelleting or seed coating techniques. It gathers one or more seeds in seed balls, enabling the simultaneous sowing of multiple seeds of the same variety or different varieties in the same crop. Additionally, seed balls can encourage seeds to carry fertilizer and pesticides into the soil, further weakening soil-borne diseases and abiotic stresses, form a relatively stable internal environment in the soil, and ensure the germination of cotton seeds. Our findings provide a reference point to improve cotton seedling emergence through the utilization of this novel technology.

Introduction

Cotton is a *Gossypium* plant of the Malvaceae family. It is an economic crop known as “white gold”, and the Yangtze River Basin is an important planting area in China. As global agriculture mechanizes, the direct seeding mode has gradually replaced the traditional seedling-transplantation mode in China. The new direct planting of cotton seedlings following rape or wheat harvest achieved initial success and has been widely applied in southern China (Xiao *et al.*, 2009; Zou *et al.*, 2021). However, there are still some problems with this method, such as the difficulty of growing a seedling out of each seed, resulting in low yield and poor fiber quality. Hunan Province, the main cotton production region in the Yangtze River Basin, suffers from cold spells every late spring that cause plant losses during cotton planting. Low temperatures and heavy rain lead to more cotton diseases and later seed emergence, severely restricting the yield of direct seeded cotton following rape harvest (Li *et al.*, 2013).

Ensuring that every seed develops into a strong seedling is the basis for high and stable yields of cotton (Dong, 2004). Cotton seed treatment is usually applied to improve the emergence rate and seedling quality. In recent years, representative techniques such as seed coating and pelleting have been applied to improve seed germination quality and early seedling development (Wang, 2019). There have been many studies on the pre-sowing treatment of cotton seed, but they all focus on seed pelleting (granulation) or coating treatment, characterized by the formation of a layer of seed coating and pelleting agent on the seed surface. The high cost of coating and the complex pelleting process increase the cost of cotton planting. Additionally, since cotton is a dicotyledonous plant, most of its seed shells with coating or balling agents remain in the topsoil following seed germination, and so they cannot continue to play a role in seed germination for later and more developed cotton seedlings (Zhang *et al.*, 2001). Furthermore, the current methods of coating and pelleting cotton seeds are based on single seeds and are unable to provide the “correct” seed form to meet the needs of different seed numbers during seeding and planting. Therefore, further research on cotton seed pre-planting treatment technology to improve seedling emergence rate, achieve seed with fertilizer and medicine into the soil, and prolong the holding time of fertilizer and medicine is very important for the realization of mechanization of cotton sowing and the safety and success of cotton planting.

We considered the possibility of applying a low-cost, non-hazardous fertilizer using an adhesive that treats cotton seeds in the form of a wrapper. Previous research has shown that diatomite, bentonite, and attapulgite can be used to enhance seed germination, seedling growth, and field cultivation and fertilization for various crops, including cotton (Hu, 1995; Huang *et al.*, 2017; Xiao *et al.*, 2018; Chekaev *et al.*, 2019; Liang *et al.*, 2019; Alsar *et al.*, 2020; Datta *et al.*, 2020; Gong *et al.*, 2021). We proposed a novel seed balling technique in cotton that adopts the special cotton seedling substrate as the balling substrate. This work confirmed the auxiliary fillers, screened the adhesives and finally determined the proportions of each component and the total balling efficiency. This work tentatively establishes the cotton seed balling technology and explores the potential effect of the new technology on cotton seed emergence, yield, and fiber quality.

Materials and Methods

Materials

The cotton seeds (age of seeds: 3 months) used in this experiment were the conventional early-maturing variety JX0010 provided by the Cotton Science Institute of Hunan Agricultural University. Before testing, the lint was removed, and the seeds were dried. Bad seeds, vacant seeds, and other impurities were manually removed. Cotton seeds that were relatively consistent in plumpness were selected as the experimental materials. The seedling substrate (the main ingredients of peat soil, horticultural perlite, horticultural vermiculite, washed coconut bran, carbonized rice husk, slow-release organic fertilizer, $\text{pH} \geq 6.6$) used in this experiment were produced by Hunan Xianghui Agriculture Technology Co. Ltd, Liuyang, China. Materials including diatomite, attapulgite, and bentonite, and 8 adhesion agents (xanthan gum, sodium carboxymethyl cellulose, starch acetate, dextrin, guar gum, hydroxyethyl cellulose, corn starch, and cassava starch) were purchased from Huayunda New Materials Co. Ltd., Shandong, China. The parameters of the light incubator (GDN-400E-4, Ningbo Ledian Instrument Manufacturing Co. Ltd., Ningbo, China) were as follows: the light cycle was 14h day/10h night, the temperature cycle was 35°C day/23°C night, the light intensity was 30,000 lux, and the relative humidity was 71%. Seedling trays with dimensions of 54×28×4.5 cm was used for the test. Diatomite, bentonite, and attapulgite were mixed evenly according to a mass ratio of 1:1:1 (hereinafter referred to as the mixed substrate) for the test.

Selection of adhesive

In March 2021, the balling reagent selection test was conducted at the Cotton Research Institute of Hunan Agricultural University. The adhesives (xanthan gum, sodium carboxymethyl cellulose, starch acetate, dextrin, guar gum, hydroxyethyl cellulose, corn starch, and cassava starch) were dissolved in ultrapure water (Wang, 2019). After the above 8 adhesives were fully dissolved, the seedling substrate suitable for the volume of adhesive solution was added, and the mixture was kneaded into a ball with a diameter of 3-4 cm. A total of 5 seeds were included in each adhesion seedling ball, and this was repeated 15 times. The non-balled seeds were used as the blank control group. The balled seeds and non-balled seeds were placed into their respective seedling trays in the light incubator. The emergence rate [the percentage of emerging seeds in the total number of seeds (Zhao *et al.*, 2021)] was recorded as the evaluation index, and the screening tests of different adhesives were carried out.

Selection of seed balling formula

The adhesives were set at 5 concentration ratios (0.5%, 0.8%, 1.0%, 1.5%, 2.0%, w/w), mixed with the substrate, kneaded into balls in the same manner, and repeated 5 times to determine the emergence rate and emergence velocity.

Field trials of balled cotton seed

In May 2021, field trials were conducted at Yunyuan Experimental Station of Hunan Agricultural University (N 28°11', E 113°04'). The soil had the following properties: pH 7.7, 22.1 g·kg⁻¹ organic matter, 1.1 g·kg⁻¹ total nitrogen (N), 2.5 g·kg⁻¹ Olsen phosphorus and 7.9 mg·kg⁻¹ exchangeable potassium. The soil properties test was based on samples taken from the upper 15 cm of the soil before sowing. Using the balling formula determined

from the above 2 steps, the cotton seeds were balled, and non-balled cotton seeds were used as the control group (Figure 1A). First of all, we carefully carried out a small-scale indoor germination test of cotton seed balling. The possibility of cotton seed balling for the field test was preliminarily verified (Figure 1 B-D). Then the balled seeds and the control group were placed in the holes dug in the field (the depth of each hole was about 4-5 cm) and then covered with loose soil. The test was designed as 3 repeat areas, with a total of 6 split plots with an area of 20 m² (5×4 m). The planting density of balled and non-balled seeds was 4500 plants·ha⁻¹, and the total fertilization amount was 600 kg urea, 75 kg P₂O₅ and 150 kg K₂O per ha, including 25% before sowing, 15% at seedling stage, 10% at bud stage, and 50% at flowering and boll stages. The irrigation was conducted in August with less precipitation, and the amount of irrigation was suitable when the soil surface was wet and there was no obvious ponding. Other field cultivation and management followed the local technical rules of Hunan Province (DB43/T 286-2006) (Hunan Provincial Bureau of Quality and Technology Supervision, 2006). The seedling emergence rate, cotton yield, and fiber quality were measured after harvest for the whole growth period of cotton.

Sampling, measurements, and calculations

Assessment of yield characteristics

Regarding the number of bolls per plant, at the peak of cotton

fluffing, the number of individual bolls was observed on cotton plants in each plot (excluding bad bolls, rotten bolls, *etc.*). Average per plant: to an accuracy of 0.01.

Regarding the single boll weight of cotton plants, in each plot, cotton plants were harvested from the lower part of the plant with fully flocculated bolls, for a total of 50 flocculated bolls per plot, dried and weighed. Boll weight, lint percentage, seed cotton yield, and lint yield were calculated (Guo, 2014).

Fiber quality test

After the opened cotton bolls were collected by sections, ginned, and sufficiently dried by sunlight, 20 g of cotton fiber from each section was properly packed and sealed in an envelope and sent to the Cotton Quality Supervision, Inspection and Testing Center of the Ministry of Agriculture and Rural Affairs of China (Institute of Cotton Research of CAAS, Anyang, Henan) for the testing of 5 high volume instrument indices (mean length, uniformity index, fiber strength, micronaire value, and elongation rate) according to GB/T 20392-2006 test method of properties of cotton fibers by high volume instruments.

Data analysis

The data were analyzed according to the analysis of variance [SPSS Statistics 23 (IBM, Armonk, NY, USA)]. Homogeneous groups between treatments were identified using a one-way analysis of variance with the least significant difference *post-hoc* test.



Figure 1. Germination test of balled cotton seed and control. **A)** Unballied cotton seed (left) and balled seed (right); **B)** days after planting (DAP) 0, balled cotton seed (left) and unballied cotton seed (right); **C)** DAP 3, balled cotton seed (left) and unballied cotton seed (right); **D)** DAP 6, balled cotton seed (left) and unballied cotton seed (right).

Results

Effect of balling treatments on the germination of cotton seeds across different adhesion agents

As shown in Figure 2, the emergence rates of cotton seeds balled with 8 different types of adhesion agents (xanthan gum, sodium carboxymethyl cellulose, starch acetate, dextrin, guar gum, hydroxyethyl cellulose, corn starch, and cassava starch) were reduced or increased to varying degrees when compared to the control group. Of the adhesion agents, it may be due to the high viscosity of xanthan gum and starch acetate, causing most seeds to be unable to break the seedling ball to emerge. Hydroxyethyl cellulose had high compactness, and dextrin could not be completely dissolved, meaning that a small amount of precipitation was left on the surface of the seedling ball. Both of these agents had an adverse effect on the emergence of the balled cotton seeds. Therefore, sodium carboxymethyl cellulose, guar gum, corn starch, and cassava starch were selected for further screening.

Concentration and ratio selection of adhesion agent

As shown in Figure 3, as the concentration of each adhesion agent increased, their emergence rate and emergence velocity showed a downward trend. Seedling balls adhered to cassava starch showed the best emergence rate, reaching 100% when the proportions with mixed soil were 1.0 and 1.5. Considering that in actual planting, the seeding balls may be damaged due to transportation, we finally selected the ratio of cassava starch to mixed soil of 1.5 as

the binder and formulation ratio for cotton seed balling. Despite the calculation, the optimal mass ratio of cotton seed balling was finally determined to be 13:8:8:8:93:171(cassava starch:diatomite:ben-tonite:attapulgit:seedling substrate:ultra-pure water).

Effect of seed balling treatment on cotton emergence

In the field plot test experiment (Figure 4), the emergence and emergence hole rates (the holes with at least one seedling) of the balled cotton seeds were 91.11% and 99.26%, respectively. This represented an increase of 34.42% and 28.84%, respectively, when compared with the rates of 67.78% and 77.04% in the control group. This showed that the cotton seed balling treatment significantly improved the emergence rate and emergence hole rate of cotton seeds.

Effect of seed balling treatment on cotton yield

Boll number per plant and single boll weight are important components of cotton yield (Isong *et al.*, 2017). As shown in Table 1, the seed balling treatment had a significant impact on the boll number per cotton plant. The mean number of bolls per plant was 35.9, a 33.96% increase when compared with an average of 26.8 bolls in the control group. There was no significant difference in single boll weight and lint percentage. The seed cotton yield and lint yield of the seed balling treatment were 6.36 t ha⁻¹ and 2.59 t ha⁻¹, respectively, compared with the control (4.39 t ha⁻¹ and 1.79 t ha⁻¹), indicating a significant increase of 44.90% and 44.58%, respectively. This showed that cotton seed balling treatment increased the yield of cotton by increasing the number of bolls per plant.

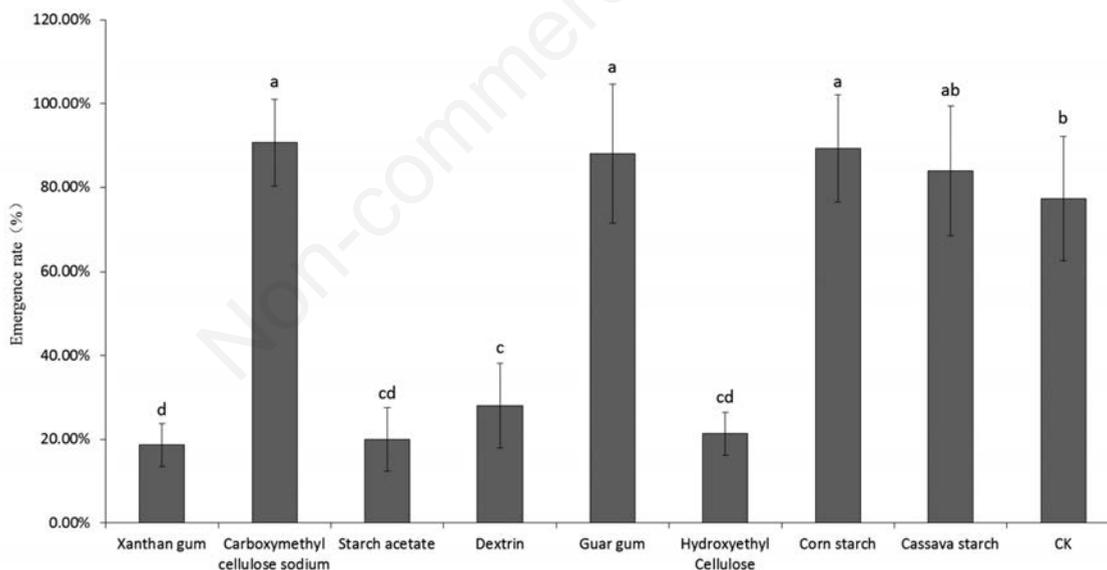


Figure 2. Effects of balling treatments on the germination of cotton seed across different adhesion agents. Different lowercase letters denote statistical differences between treatments according to Duncan's test (0.05).

Table 1. Effect of seed balling treatment on cotton yield.

	Boll number per plant, pieces	Boll weight, g	Lint percentage	Seed cotton yield, kg/ha	Lint yield, kg/ha
Balled seeds	35.9±0.75 ^a	4.63±0.04 ^a	40.66±0.39 ^a	6357.73±99.69 ^a	2584.92±46.54 ^a
Control	26.8±0.20 ^b	4.28±0.20 ^a	40.75±0.24 ^a	4387.79±179.31 ^b	1787.78±68.92 ^b

^{a,b}Different lowercase letters denote statistical differences between treatments according to Duncan's test (0.05).

Effect of seed balling treatment on cotton fiber quality

As shown in Table 2, among the 5 indexes reflecting cotton fiber quality, there were no significant differences between balled seeds and the control group in their mean length, uniformity index, fiber strength, micronaire value, and elongation rate. This showed that the cotton seed balling treatment had no significant effect on cotton fiber quality.

Discussion

Bentonite is commonly used as a chemical and physical natural soil conditioner due to its high cation exchange capacity (Davies and Jabeen, 2002; Genç and Dogan, 2015), which can improve soil fertility and promote plant growth (Bouabid *et al.*, 1991). Hu (1995) found that the germination rate of cotton seeds treated with

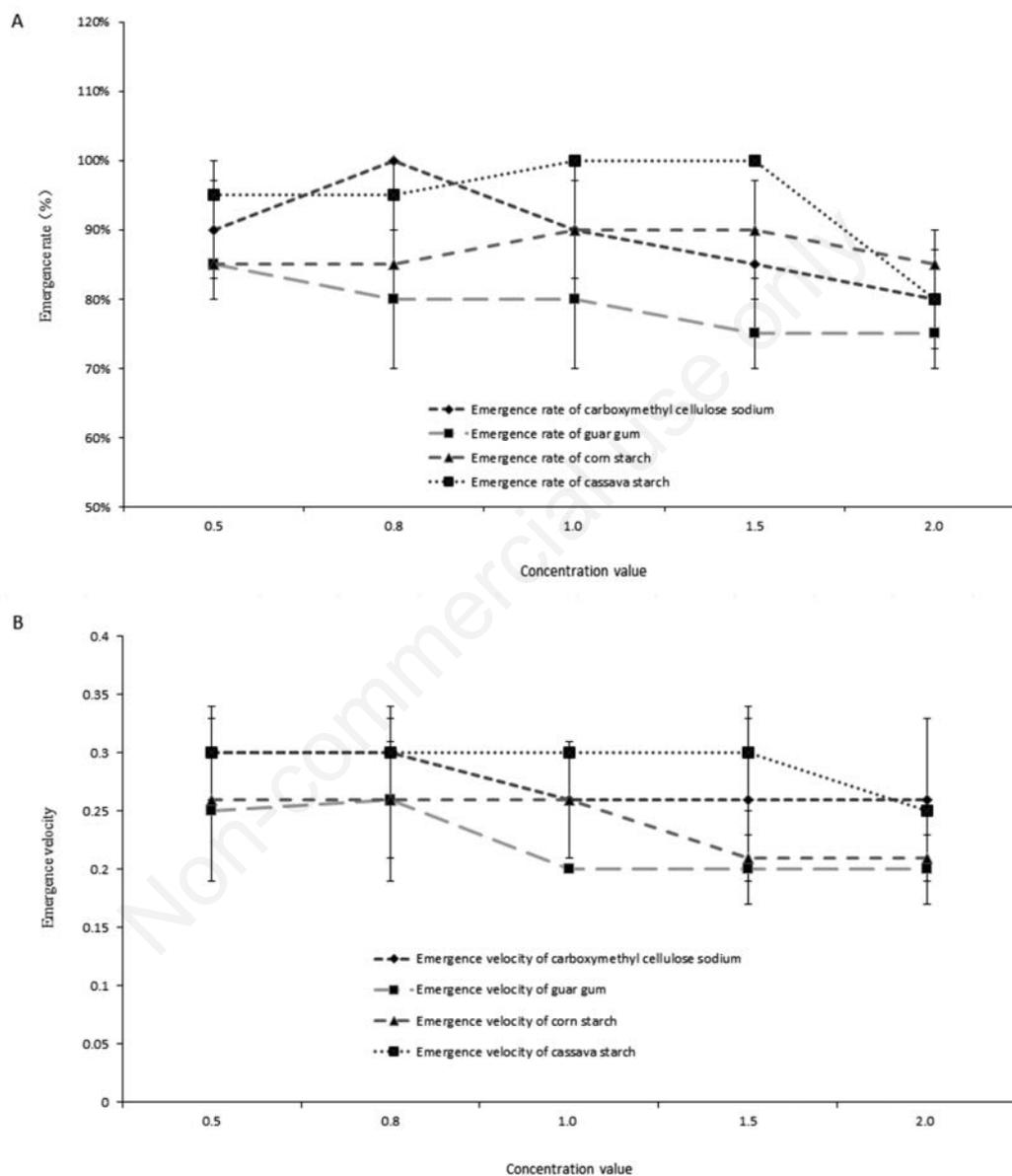


Figure 3. Effects of balling treatments on the germination of cotton seed across different adhesion agents. Different lowercase letters denote statistical differences between treatments according to Duncan's test (0.05).

Table 2. Effect of seed balling treatment on cotton fiber quality.

	Mean length, mm	Uniformity index, %	Fiber strength, cN/tex	Micronaire value	Elongation rate, %
Balled seeds	30.7±0.23	87.0±0.06	33.0±0.67	5.2±0.06	6.8±0.06
Control	30.1±0.49	87.1±0.72	31.8±0.74	5.2±0.06	6.7±0.06

bentonite was significantly higher than that of untreated seeds. The cotton yield increased by 4.8~1.5% after applying 0.5~1.5 tons of bentonite per hectare in a cotton field. Zhao *et al.* (2019) studied the effects of bentonite on the continuous cropping yield of peanut and rhizosphere soil fertility by using different application rates of bentonite. They found that the application of bentonite significantly improved enzyme activity as well as peanut yield.

Attapulgite has been shown to improve crop yields in combination with compound fertilizers because of its adsorption, suspension, and slow-release properties (Datta *et al.*, 2020). In addition, attapulgite is abundant, inexpensive, environmentally friendly, and can be used as a filler in compound fertilizers. Its application according to the nutritional needs of crops at different growth stages can help reduce production costs, reduce fertilizer waste, and improve crop yield (Guan *et al.*, 2014). Gong *et al.* (2021) found that attapulgite had a significant effect on the growth rate and germination of *Agaricus blazei* mycelium. The appearance, yield, and quality of *Agaricus blazei* were maximized when attapulgite at a dry weight ratio of 2% was mixed with soil. Xiao *et al.* (2018) found that the agronomic properties of tobacco were better than those of conventional fertilization after the application of attapulgite before ridging and trenching. The yield of flue-cured tobacco was the highest, and the chemical composition was best when attapulgite was applied before ridging and transplanting.

Diatomite is a siliceous sedimentary rock mainly composed of silicon dioxide. It has strong adsorption capacity, is easily accessible, and is an environmentally friendly compound with a porous structure, environmental benefits, and excellent mixing ability. Therefore, diatomite has been used as a compound fertilizer for crops (Huang *et al.*, 2012). Diatomite can also loosen soil, maintain soil moisture, prolong fertilizer effects, and promote the growth of crops. Sherif *et al.* (2018) studied the effect of diatomite on the growth and active compounds of *Moringa oleifera*. The results showed that growth and development-related parameters when irrigated with 2.5 g/L diatomite were significantly improved. Ilyasov *et al.* (2020) combined diatomite and organic fertilizers to improve the structural state of the crop, soften the tillage layer, and improve the crop yield.

The inorganic substrates were composed of perlite, vermi-

culite, and peat, and the organic substrates were composed of plant wastes such as bark, sawdust, rice straw, and animal excreta such as chicken and pig manure. The composted substrates formed after retting and fermentation are widely used during soilless cultivation and seedling strengthening in plants. The composition of the substrates employed in this experiment bore a resemblance to the aforementioned one, housing organic as well as inorganic constituents, which indicates a marked increase in the emergence of tomato and cucumber seeds (Ren *et al.*, 2013; Liang *et al.*, 2015).

Starch is a relatively cheap and renewable product that can be obtained from various plants. It is widely used as an additive and adhesion agent (Whistler and Paschall, 2009). Biopolymers derived from starch or starch blends have been the subject of many studies. They have shown promise as a seed coating material with multiple potential applications (Mali *et al.*, 2006; Sanchez *et al.*, 2010). Abidin *et al.* (2014) discovered the use of cassava starch for the copolymerization of superabsorbent polymer composites as a backbone, creating the possibility of further application in agriculture. Perotti *et al.* (2017) concluded that the nanomaterials modified from cassava starch quickly released fertilizers and nutrients into the soil and showed promising applications in promoting plant growth and controlling plant diseases.

The results showed that the rates of emergence and emergence holes for the seeds under balling treatment were 19.63-25% and 14.82-20.74% higher than those of direct seeding, respectively, and the differences were significant. Compared with the control, the balling treatment group's boll number per plant, seed cotton yield, and lint yield of cotton were significantly higher. Cotton fiber quality is an important factor used to determine the economic benefits of cotton production (Chen and Burke, 2015), and previous research has shown that it is mainly determined by genetic factors (Ruan *et al.*, 2004; Li *et al.*, 2018).

In this trial, water served as a catalyst for the complete dissolution and dispersion of attapulgite, bentonite, and diatomite within the substrate's larger particles. This resulted in the formation of balls that securely adhered to cotton seeds *via* cassava starch, providing them with a relatively stable "internal environment" to cope with various biotic and abiotic stresses. The ball in the attapulgite can adsorb the water around the soil and prevent the ball in the

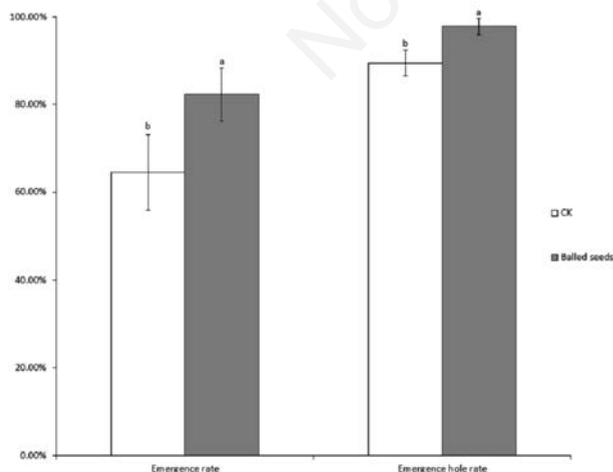


Figure 4. Effect of seed balling treatment on cotton emergence. Different lowercase letters denote statistical differences between treatments according to Duncan's test (0.05). CK, control group.

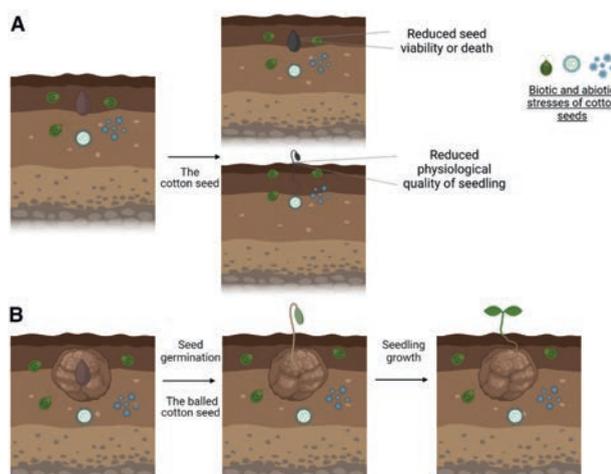


Figure 5. Schematic diagram of untreated and balled seeds. **A)** Untreated cotton seeds; **B)** balled cotton seeds.

moldy soil. Diatomite maintains the ball in the water, the seed absorbs water and swells to break the shell out of the seedling, and the substrate in the coconut husk, vermiculite, and other large inorganic materials are more loose to promote the cotton seedling hypocotyl “ball” elongation. The substrate was relatively loose with coconut husk and vermiculite, which promoted the elongation of hypocotyls. On the other hand, balled continued to be retained around the seedling root system and dispersed in the soil after splitting; bentonite-maintained soil fertility around the root system; diatomite prolonged the duration of fertilization in the substrate; and the viscosity of cassava starch declined over time and eventually harmlessly biodegraded to water and carbon dioxide. Therefore, the seed balling treatment technology in this experiment can promote the seed to improve the seedling emergence rate and, at the same time, improve the physiological quality of cotton seedlings after the seedling has laid a material foundation so that the seed has “high seedling emergence rate; the seedling also grows well and fast” (Figure 5). The seed balling treatment had no significant effect on the cotton fiber quality. This is consistent with previous research. Comprehensive analysis showed that cotton seed balling can significantly improve the emergence rate of cotton seeds and increase the yield of seed cotton and lint by increasing the boll number per plant.

Outlook for future applications of seed balled technology

In future practical applications, also taking into account the differences in cultivation conditions, farming systems, and water and fertilizer management in different regions and climatic conditions, a reasonable transformation of the balling technology and balling formula, fully exploiting the production potential of cotton seed balling treatment, and supporting the appropriate mechanized seeding technology can further tap the potential of cotton seed balling technology.

Conclusions

Seed balls are able to continue to play a role in the growth and development of seedlings after the ball is broken. The results obtained from this work will improve the emergence rate, effectively reduce soil-borne diseases during the seedling period and increase cotton yields. The results of our study will be of great significance for maximizing the idea of “one seed, one seedling” and for simplifying and mechanizing cotton planting.

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