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Effect of osmopriming and coating seed with captan and metalaxyl on the germination and seedling growth of field corn

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Abstract Priming of corn seeds with 0.4 g of KNO_3 did not affect germination percentage and speed of germination. On the contrary, it promotes better shoot length, root length and total seedling length than other methods. Priming with 0.4 g of KNO_3 followed by any coating methods, with or without fungicide, did not affect germination percentage and speed of germination but resulted in higher shoot length when tested under laboratory condition. However, the same seed treatments under the greenhouse condition, though did not affect germination percentage and speed of germination, but resulted in higher coleoptile emergence percentage when compared to control. In addition, coating corn seeds with 0.5 g.ai. of metalaxyl gave rise to the highest shoot length. Therefore, corn seed treatments which involved priming with 0.4 g of KNO_3 followed by coating with 0.5 g.ai. of metalaxyl was the most suitable for field corn.

Keywords: Seed enhancement, Seed priming, Fungicides

Introduction

During 2009–2013, field corn plantations covered a total area of 7.1–7.5 million rai (1,136,000–1.2 million hectares) of land in Thailand, and the yield was 4.6–5.1 million tons. However, such yield was insufficient for the demand of the animal feed industry. As a result, field corn was imported from neighboring and non-Association of Southeast Asian Nations (ASEAN) countries. Due to the tentative growth of the animal feed industry, approximately 7.27% per year, the demand for field corn seeds increased. Additionally, significant issues affecting the field corn yield were the lack of appropriate production technology, including genetic engineering technology and seed optimization before planting that caused the deterioration of the seed quality, diseases, pest infestation, and inappropriate conditions (Agricultural Research Development Agency, 2016).

At present, optimizing the seed efficiency before plantation is one of the key plantation methods (Pedrini *et al.*, 2017, Pedrini *et al.*, 2018), particularly seed

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priming, which accelerates a constant rapid germination and enhances seedling growth. The germination time of seed priming was 30% less than that of normal seeds, and the growth rate was satisfactory even under inappropriate conditions (Bradford *et al*., 1990; McDonald, 2000). Seed priming is a process of a physiological change that increases the energy of the seed via respiration, so it is ready to germinate. Moreover, seed priming upgrades the seed quality by applying osmopriming technology to support field corn seed by coating the seeds with a polymer, which is called the seed coating approach. This prevents the seed from experiencing inappropriate conditions (Taylor *et al*., 1998; Pedrini *et al*., 2017) that expedite seed deterioration, such as humidity and unsuitable temperature. Furthermore, seed coating can maximize the active ingredients to prevent diseases, such as captan and metalaxyl. Thus, fungicides are considered a key external factor guarding against the destruction by fungi (Flexer and Belnavis, 2000; Inglis *et al*., 2006), and protect the seed during the germination process. Consequently, the seedling is free from any disease infestation and can develop into a vigorous plant for high yield.

Therefore, the research aimed to determine the effect of the osmopriming process and coating seeds with captan and metalaxyl on the germination and seedling growth of field corn for the potential of upgrading the seed quality to increased yield.

Materials and methods

The field corn seeds var. 'MJU62-1' were used in the experiments received from the Agronomy Program, Faculty of Agricultural Production, Maejo University, Chiang Mai province, Thailand. The experiments were conducted at the Seed Technology Laboratory, Agronomy Program, Faculty of Agricultural Production, Maejo University from July to December 2019. The study was comprised of two experiments.

Osmopriming process

For the first experiment, the effect of osmopriming was conducted to determine the germination and seedling growth. Field corn seeds var. 'MJU62-1' were primed with KNO_3 and compared with hydro-priming and untreated seed.

To process the osmopriming, the field corn seeds were soaked in KNO_3 under different conditions: non-soaked seeds (T1), soaked seeds with H_2O only (T2), osmoprimed seeds at 0.2 g, 0.4 g and 0.6 g of KNO_3 (T3, T4, and T5, respectively), and osmoprimed seeds with 0.2 g, 0.4 g, or 0.6 g of KNO_3 in conjunction with polyethylene glycol 6000 (PEG), where the water potential difference was -1.5 MPa (T6, T7, and T8, respectively) at 25 ℃. After 24 hours, all seeds were washed with clean water for two minutes, the seed skin was dried, and the moisture was minimized at room temperature for 48 hours.

Seed coating process

The second experiment was used the seeds which obtained from the best condition for the osmopriming process to be coated with the different rates of fungicides. First, 0.1% of carboxymethyl cellulose (CMC) was prepared as the coating and mixed with captan or metalaxyl. The test conditions were non-soaked seeds (T1), seeds coated with polymer only (T2), seeds coated with 0.1 g.ai., 0.5 g.ai., 1.0 g.ai., or 1.5 g.ai. of captan (T3, T4, T5, and T6, respectively), and seeds coated with 0.1 g.ai., 0.5 g.ai., 1.0 g.ai., or 1.5 g.ai. of metalaxyl (T7, T8, T9, and T10, respectively). The field corn seeds were tested with each coating condition, and the moisture of the seed was minimized after coating at room temperature for 48 hours.

Seed measurement

Seed germination and vigor test

The seeds were randomized with three replications which were 50 seeds each and tested the germination by using Between Paper (BP). The seeds were put in the alternating temperature in germination incubator (at 30 ℃ for 8 hours and at 25 ℃ for 18 hours). The germination was counted on the fourth day after the first seeding (first count), and the seventh day after the second seeding (final count). The germination was measured using the standard of the International Seed Testing Association (ISTA, 2013). To monitor the speed of the germination, the seeds were randomized with three replications of 50 seeds each. The germinating seeds and number of germination days were counted from the first to the last day. For the radicle emergence, each condition had three replications, and counting was started when the radicle emergence was at 2 mm. The speed of the radicle emergence was monitored every day from the first to the third day of the three replications for each condition. For germination under the greenhouse conditions, the seeds were randomized with three replications of 50 seeds each to test the germination in a seed tray, in which peat moss was used for the seedling. The evaluation was conducted during the fourth to the seventh days, which was the same as the monitoring method under the laboratory conditions. To monitor the emergence, the coleoptile emergence of the corn seedling shoots from the seeding hole was randomly evaluated. Each condition had three replications. Finally, to monitor the speed of emergence, the coleoptile emergence of the field corn seedling shoots was randomized every day from the first to the third day after seeding. Then, the speed of the corn seedling emergence was calculated.

Seedling growth test

The shoot and root lengths were evaluated on the seventh day after seedling under the laboratory conditions. For the seedlings under the greenhouse conditions, only the seedling length was evaluated from the three replications of 10 seedlings each. Then, the randomized seedlings were measured with a ruler in millimeters.

Statistical analysis

The percentage of germination was arcsine-transformed to normalize the data before the statistical analysis. The percentages of the hard seeds and abnormal seedlings were transformed by the square root $\sqrt{x+0.5}$ before the statistical analysis. All data were analyzed by one-way ANOVA (Complete Randomized Design), and the difference between the treatments was tested by Duncan's Multiple Range Test (DMRT).

Results

The appropriate osmopriming process for germination and seedling growth

Radicle emergence and germination percentage

Priming with H_2O and 0.4 g of KNO_3 resulted in a high radicle emergence rate with a statistical difference when compared to the untreated seeds. However, there was no difference in the result of osmopriming. As can be seen in Figure 1, it showed that the germination of all the osmopriming seeds had improved the radicle extension more than the untreated seeds after 72 hours. Furthermore, the radicle emergence rate of all the osmoprimed seeds was quicker than the untreated seeds, which showed a statistical difference. However, the germination and speed of germination of all the osmoprimed seeds were not statistically different when tested under the laboratory conditions (Figure 2).

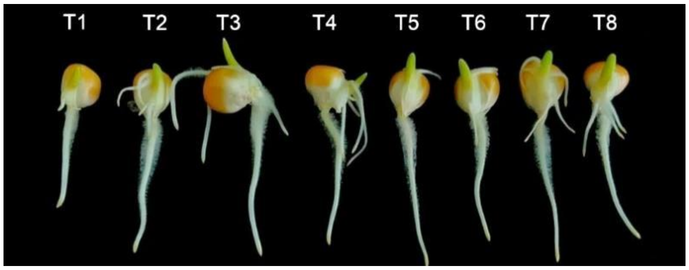

Figure 1. Effect of seed osmopriming with different rates of KNO_3; radicle emergence 72 hours after planting tested under laboratory conditions: T1 = control, T2 = priming + H_2O, T3 = priming + 0.2 g of KNO_3, T4 = priming + 0.4 g of KNO_3, T5 = priming + 0.6 g of KNO_3, T6 = -1.5 MPa PEG + 0.2 g of KNO_3, T7 = -1.5 MPa PEG + 0.4 g of KNO_3, and T8 = -1.5 MPa PEG + 0.6 g of KNO_3

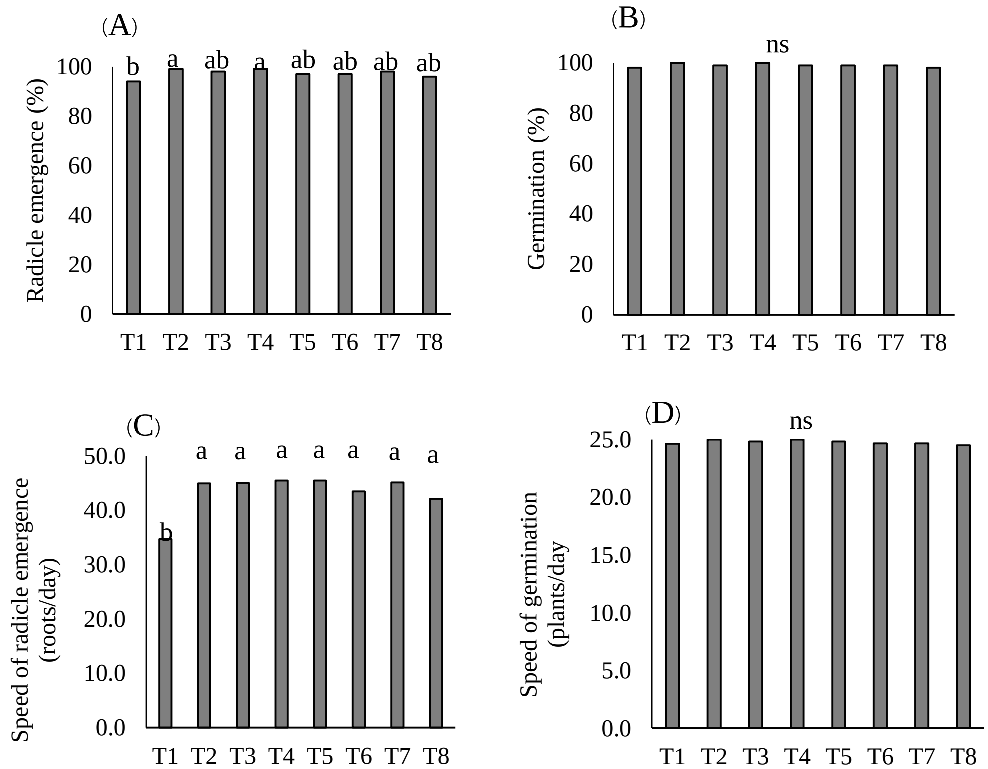

Figure 2. Radicle emergence percentage, germination percentage, the speed of radicle emergence and speed of germination of field corn seeds after seed priming and tested under laboratory conditions: T1 = control, T2 = priming + H_2O, T3 = priming + 0.2 g of KNO_3, T4 = priming + 0.4 g of KNO_3, T5 = priming + 0.6 g of KNO_3, T6 = priming + -1.5 MPa PEG + 0.2 g of KNO_3, T7 = priming + -1.5 MPa PEG + 0.4 g of KNO_3, and T8 = priming + -1.5 MPa PEG + 0.6 g of KNO_3

Seedling growth

From the laboratory, the length of all the osmoprimed seeds was longer than the untreated seeds, which showed some statistical difference. Seed osmopriming with 0.4 g of KNO_3 and -1.5 MPa PEG and 0.2 g of KNO_3 gave a longer radical emergence that displayed a statistical difference when compared to the untreated seeds.However, when considering the total seedlings, it showed that seed osmopriming with 0.4 g of KNO_3 and -1.5 MPa PEG with 0.2 g of KNO_3 had the best seedling growth, which showed a statistical difference when compared to the untreated seeds, where the different growth percentages of the seedlings and untreated seeds were 20% and 19%, respectively (Table 1).

Table 1. Shoot lengths, root lengths, and the total numbers of seedlings of field corn seeds after seed priming tested under laboratory conditions

Treatment[1]	Laboratory conditions					
	Shoot length		**Root length**		**Total seedlings**	
	(**mm**)	(%)[3]	(**mm**)	(%)	(**mm**)	(%)
T1	95.90 b[2]		143.13 c		239.03 c	
T2	109.33 a	(+14)	152.00 bc	(+6)	261.33 b	(+9)
T3	110.70 a	(+15)	170.76 ab	(+19)	281.46 ab	(+18)
T4	114.46 a	(+19)	173.06 a	(+21)	287.53 a	(+20)
T5	113.03 a	(+18)	169.86 ab	(+19)	282.90 ab	(+18)
T6	111.90 a	(+17)	172.83 a	(+21)	284.73 a	(+19)
T7	112.03 a	(+17)	160.36 a-c	(+12)	272.40 ab	(+14)
T8	108.63 a	(+13)	171.36 ab	(+20)	280.00 ab	(+17)
Mean	109.50		164.17		273.67	
F-test	*		*		**	
CV.(%)	5.56		6.44		4.20	

*, **: Significantly different at P ≤ 05.0and P ≤ 0.01, respectively.
[1] T1 = control, T2 = priming + H_2O, T3 = priming + 0.2 g of KNO_3, T4 = priming + 0.4 g of KNO_3, T5 = priming + 0.6 g of KNO_3, T6 = priming + -1.5 MPa PEG + 0.2 g of KNO_3, T7 = priming + -1.5 MPa PEG + 0.4 g of KNO_3, and T8 = priming + -1.5 MPa PEG + 0.6 g of KNO_3.
[2] Means within a column followed by the same letter are not significant at P ≤ 05.0 by DMRT.
[3] The number in the parenthesis refers to the percentage of the increase (+) compared to the control.

The effect of seed coating using captan and metalaxyl on germination and seedling growth

The most appropriate condition from the first experiment was selected, which was seed osmopriming with 0.4 g of KNO_3 (T4). Then, the seeds were coated with captan and metalaxyl of four rates each.

Radicle emergence and germination percentage

Seeds coated with 0.1 g.ai. of captan gave a good radicle emergence that displayed a statistical difference when compared to the untreated seeds. All the coated seeds gave the best speed of radicle emergence that had a statistical difference when compared to the untreated seeds. Seeds coated with fungicides had higher speeds of radicle emergence and longer root lengths than those of the untreated seeds (Figure 3). Moreover, seed coatings together with fungicides showed no difference in the germination and speed of germination after being monitored under laboratory conditions (Figure 4).

Figure 3. Effect of seed osmopriming with different rates of KNO_3, radicle emergence 72 hours after planting tested under laboratory conditions: T1 = control, T2 = coating + polymer, T3 = coating + 0.1 g.ai. of captan, T4 = coating + 0.5 g.ai. of captan, T5 = coating + 1.0 g.ai. of Captan, T6 = coating + 1.5 g.ai. of captan, T7 = coating + 0.1 g.ai. of metalaxyl, T8 = coating + 0.5 g.ai. of metalaxyl, T9 = coating + 1.0 g.ai. of metalaxyl, and T10 = coating + 1.5 g.ai. of metalaxyl

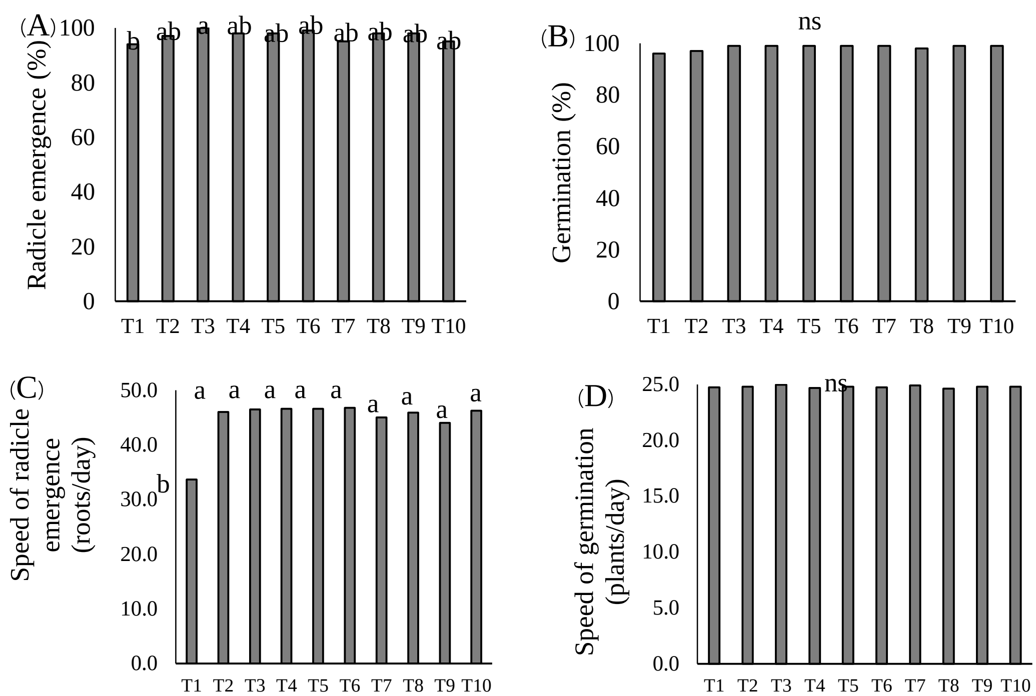

Figure 4. Radicle emergence percentage, germination percentage, the speed of radicle emergence and speed of germination of field corn seeds after coating with different types of fungicides and tested under laboratory conditions: T1 = control, T2 = coating + polymer, T3 = coating + 0.1 g.ai. of captan, T4 = coating + 0.5 g.ai. of captan, T5 = coating + 1.0 g.ai. of Captan, T6 = coating + 1.5 g.ai. of captan, T7 = coating + 0.1 g.ai. of metalaxyl, T8 = coating + 0.5 g.ai. of metalaxyl, T9 = coating + 1.0 g.ai. of metalaxyl, and T10 = coating + 1.5 g.ai. of metalaxyl

Seedling growth

All the coated seeds had the highest shoots, which showed a statistical difference when compared to the untreated seeds, but without any statistical difference in the root length. Nevertheless, when monitoring all the seedlings, it was found that all the coated seeds had the best seedling growth, which displayed a statistical difference when compared to the untreated seeds, except from the seed coating with 1.0 g.ai. of captan, which had no statistical difference when compared to the untreated seeds under the laboratory conditions (Figure 5). Monitoring under greenhouse conditions indicated that all the coated seeds made the highest change to the emergence that showed a statistical difference when compared to the untreated seeds. The germination percentage and speed of germination displayed no statistical difference. Furthermore, seed coating with 0.5 g.ai. of metalaxyl gave the longest shoot length of 192.50 mm, which had a statistical difference when compared to the other types (Table 2).

Table 2. Shoot lengths, root lengths, and total numbers of seedlings of field corn seeds after coating with different types of fungicides and tested under laboratory conditions

Treatment[1]	**Laboratory conditions**					**Greenhouse conditions**	
	Shoot length (mm)	(%)[3]	**Root length (mm)**	**Total seedlings (mm)**	(%)	**Shoot length (mm)**	(%)
T1	10.67 b[2]		17.95	28.62 b		117.39 b	
T2	12.96 a	(+21)	17.90	30.86 a	(+8)	131.10 b	(+12)
T3	13.36 a	(+25)	18.12	31.45 a	(+10)	138.03 b	(+18)
T4	12.66 a	(+19)	19.33	31.99 a	(+12)	144.27 b	(+23)
T5	13.50 a	(+26)	17.05	30.55 ab	(+7)	136.87 b	(+17)
T6	13.26 a	(+24)	18.44	31.70 a	(+11)	139.73 b	(+19)
T7	12.97 a	(+22)	18.35	31.32 a	(+9)	137.57 b	(+17)
T8	13.47 a	(+26)	18.44	31.90 a	(+11)	192.50 a	(+64)
T9	13.32 a	(+25)	18.40	31.76 a	(+11)	146.63 b	(+25)
T10	13.30 a	(+25)	18.04	31.34 a	(+9)	141.17 b	(+20)
Mean	12.95		18.20	31.15		142.53	
F-test	**		ns	*		*	
CV.(%)	3.86		6.39	4.00		18.77	

ns, *, **: Not significantly different; significantly different at P ≤ 05.0and P ≤ 0.01, respectively.

[1] T1 = control, T2 = coating + polymer, T3 = coating + 0.1 g.ai. of captan, T4 = coating + 0.5 g.ai. of captan, T5 = coating + 1.0 g.ai. of Captan, T6 = coating + 1.5 g.ai. of captan, T7 = coating + 0.1 g.ai. of metalaxyl, T8 = coating + 0.5 g.ai. of metalaxyl, T9 = coating + 1.0 g.ai. of metalaxyl, and T10 = coating + 1.5 g.ai. of metalaxyl.

[2] Means within a column followed by the same letter are not significant at P ≤ 05.0 by DMRT.

[3] The number in the parenthesis refers to the percentage of the increase (+) compared to the control.

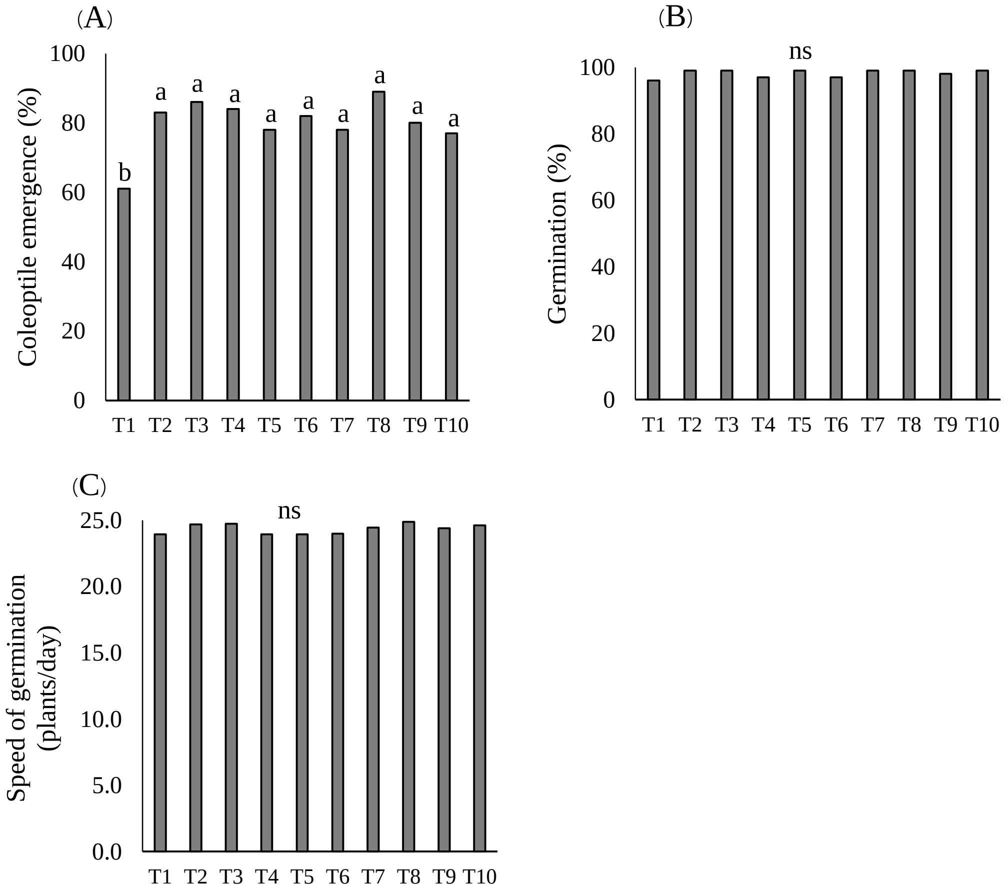

Figure 5. Coleoptile emergence percentage, germination percentage and the speed of germination of field corn seeds after coating with different types of fungicides and tested under greenhouse conditions: T1 = control, T2 = coating + polymer, T3 = coating + 0.1 g.ai. of captan, T4 = coating + 0.5 g.ai. of captan, T5 = coating + 1.0 g.ai. of Captan, T6 = coating + 1.5 g.ai. of captan, T7 = coating + 0.1 g.ai. of metalaxyl, T8 = coating + 0.5 g.ai. of metalaxyl, T9 = coating + 1.0 g.ai. of metalaxyl, and T10 = coating + 1.5 g.ai. of metalaxyl

Discussion

The results clearly illustrated that seed coating with fungicides had a positive effect on seed quality. Before seed coating, the seeds were processed through osmopriming with KNO_3, the germination was fast and constant. The test results showed that seed osmopriming with 0.4 g of KNO_3 had the highest germination and field corn seedling growth. Moreover, field corn seeds primed with

KNO_3 displayed the dissolution of KNO_3, which resulted in K^+ and NO_3^-. Nitrogen that was a component of the protoplasm and plant cell wall was in the form of NO^{3-}. The plant had to reduce NO^{3-} to NH_4^+ to generate amino acids. Nitrogen (N) was the composition of the biomolecule in the plant cells (Theerakulpisut, 1997; Bunnag, 1999, Barker and Pilbeam, 2007), there was higher protein synthesis in the seed when it absorbed nitrate. Consequently, this demonstrated better germination and growth than the non-osmoprimed seeds (Kaewnaree *et al.*, 2011; Krainart *et al.*, 2015).

When coating the osmopriming seeds with 0.4 g of KNO_3 and fungicides at different rates, the radicle emergence, speed of radicle emergence, shoot length, and total seedling showed a positive change, unlike the non-osmoprimed seeds under both laboratory and greenhouse conditions.

Seed coating with captan showed that there was no effect on the radicle emergence, speed of radicle emergence, and seedling growth, which was superior to the uncoated seeds. Captan is a phthalimide fungicide to prevent die contact or diseases. Thus, it restrained the growth and constructed fungi spores at the direct contact area of the captan. With direct contact action, this might affect the seed quality, if it was used at an inappropriate rate (Ek-Amnuay, 2007). The mechanism of the captan active component would also be efficient when dissolved in water; the active component was released in the form of tetrahydrophthalimide, tetrahydrophthalic acid, and three molecules of chloride (Cl^-), which might cause toxicity to the seed cells if a high concentration was absorbed (Van Iersel and Bugbee, 1996). Thus, 0.1 g.ai., 0.5 g.ai., 1.0 g.ai., or 1.5 g.ai. of captan could be used with the field corn seed coating without minimizing the radicle emergence, germination, speed of germination, seedling length, and root length when compared to the uncoated seeds. Moreover, there was more possibility of efficient growth than the untreated seeds.

Additionally, seed coating with metalaxyl showed that all rates did not minimize the quality of the seed. Metalaxyl is an absorbent fungicide, when the seed absorbed it, metalaxyl was transported to the various parts of the seedling (Ek-Amnuay, 2007). Therefore, the slow action of metalaxyl did not affect the germination and field corn seedling growth. However, a high rate of use might affect the seed quality, especially the deterioration of the cell membrane and the reduction of enzyme activities, which comprised glutamic decarboxylase, catalase, peroxidase amylase, and phosphatase (Copeland and McDonald, 1995). The results indicated that coating seeds with 0.5 g.ai. of metalaxyl promoted the highest seedling shoot growth.

However, it was only the initial step to select the osmopriming method and examine the type and appropriate rate of the fungicides for field corn seed coating. The next step should be to monitor the seed quality after being stored under different conditions to set the guideline for a quality upgrade and increasing yield.

The application of 0.4 g of KNO_3 did not show any significant difference in the germination and speed of germination when compared to the untreated seeds. Moreover, the seeds gave longer shoot and root lengths than other conditions when tested under laboratory conditions. Seed coating with 0.4 g of KNO_3 followed by seed coating with fungicides at all rates did not display any differentiation of the speed of germination under the laboratory conditions. However, seed coated with 0.5 g.ai. of metalaxyl gave the longest shoot length under greenhouse conditions.

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