

Improvement of lettuce germination and seedling growth by seed pelleting with ammonium nitrate compounds

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ABSTRACT: This experiment aimed to explore the appropriated ratio of plant nutrients for pelleting lettuce seeds, and followed the growth after pelleting the seeds with plant nutrients by measuring the germination, speed of germination, root length, shoot length, fresh weight and dry weight under the laboratory and greenhouse conditions. The experiment was conducted at Seed Quality Testing Laboratory, Seed Processing Plant, Faculty of Agriculture, Khon Kaen University. It was shown that pelleted seeds with nitrogen at the rate between 0.06 and 0.24 g did not affect the germination and the vigor of lettuce seeds both under laboratory and greenhouse conditions. For the growth of lettuce seeds after pelleting with ammonium nitrate (NH_4NO_3), it was found that pelleted seeds with NH_4NO_3 at the rate of 0.24 g had the best shoot length, root length, seedling fresh weight, and seedling dry weight under the laboratory and greenhouse conditions, followed by pelleting the seeds at the rate of 0.12 g. In addition, after accelerated ageing of the seeds, it was apparently shown that all methods of pelleted seeds had better germination than non-pelleted seeds clearly under laboratory and greenhouse conditions.

Keywords: nitrogen, plant nutrients, seed germination, seed encrusting

Introduction

Plant nutrients are vital to carry out activities within plant cells, or promote the metabolism of the plant to continue as usual, such as the use and energy storage, building and repair the wear and tear, etc. (Roy et al., 2006), especially nitrogen element. As nitrogen element is an important feature for fastly growth of plant seedlings and seedling establishment. Also the growth of leaves and stems increase of protein to the plant during seedling growth. However, nitrogen is easily dissolved, the seedling can uptake nitrogen in the higher rate than requirement. The nitrogen will losses and efficiency of nitrogen on seedling decreases. In addition, the nitrogen losses due to leaching, evaporating of ammonium nitrate, N_2O , NO and N_2 from the soil as well. At present, upgrading of

seed quality is essentially for planting system (Kaewkham et al., 2016). Especially, seed pelleting technology are widely used around the world because of increased seed size from small seeds, distorted and elongated shape, for example: tobacco, carrot, beetroot, bean and marigold seeds, etc. Lettuce seeds, small, flat and thin shape are pelleted for improving uniformity of germination (Kangsopa and Siri, 2015) In addition, lettuce seeds are also less food accumulation, the farmers or the farm producer organizations prefer to use coated or pelleted lettuce seeds with plant nutrients. Apart from the above mentioned problems, seed pelleting technology is the method to improve the condition of seeds for planting the seedlings to be most effective (Siri, 2015) as well as increases plant nutrients in the right amounts to stick the seed. Seedlings after germination can be used to plant

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immediately. The advantages of such method of Takahashi et al. (1991a) the soybean seeds had coated with urea fertilizer. It was found that urea fertilizer gradually released nitrogen without affected the operation of nodes of soybean root. It caused the growth of soybean improvement, stimulated the emergence of soybean roots, and helped the roots to absorb water and nutrients better (Takahashi et al., 1991b). It also reported when the seeds were coated with nitrogen, the dry weight of the roots changed, and the dry weight of the soybean increased (Kubota et al., 2008). Therefore, the aim of this study was to determine the appropriated rate of pelleted seed using NH_4NO_3 on germination and seedling growth of lettuce seeds.

Materials and methods

Seed pelleting process

Lettuce seeds (*Lactuca sativa* L.) varieties RUTLL 58-1 provided by the Faculty of Sciences and Agricultural Technology, Rajamangala University of Technology Lanna Lampang, Lampang, Thailand. The lettuce seeds were pelleted with calcium sulfate 250 g, which was the pelleted material approximately 0.3-0.4 mm. using Carboxymethyl cellulose (CMC; Sigma Aldrich) with 0.3% by weight as binder materials per 10 g of lettuce seeds. The process of studying was divided into 6 methods comprised of non-pelleted seeds (T1); pelleted seeds with calcium sulfate (T2); pelleted seeds with calcium sulfate mixed NH_4NO_3 at the rate of 0.06, 0.12, 0.24 and 0.48 g (T3, T4, T5 and T6, respectively). The lettuce seeds and pelleted materials were pelleted with a rotary drum (Model, SKK12, the

Seed Processing Plant, Khon Kaen University) and dehumidified pelleted seeds using the humidifier with hot air system, model SKK09 until the initiated moisture content of seeds (5-6%) and sampled the pelleted seeds to determine the quality of seeds.

Seed measurement

Seed germination and vigor test: In laboratory condition, germination by the top of paper method (TP) using random sampling for the seeds pelleting and non-pelleting for 4 replicates with 50 pelleted seeds was conducted. The seeds were placed in gearbox-type plastic boxes (110 mm L × 110 mm W × 30 mm H). Then, the seeds were placed in an alternating temperature germination chamber (8 hours, 30 °C and 16 hours, 20 °C), and the germination was counted at 4 days (first count) and 7 days after planting (final count) according to ISTA method (2013). In greenhouse condition, pelleted and non-pelleted seed were sown in the tray using peat moss as planting material for 4 replications with 50 pelleted seed. The germination was evaluated at 4 and 7 days after planting as well as the method under laboratory condition. The speed of germination was calculated from the number of normal seedling and the number of germinated days from first count to final count using ISTA (2013) in both under laboratory and greenhouse conditions.

Seedling growth test: The observations were evaluated at the length of shoot and root at 7, 14 and 30 days after planting, all were for 4 replicates with 10 plants using the growth pouch bag in the laboratory. Under greenhouse conditions in each trial, cut at the plant stem near planting materials and taken from random sampling to measure

using a ruler with millimeters, and monitored fresh and dry weight to test in the Temp-Humid chamber at 60 °C for 24 hours and then weighed on an analytical scale.

Accelerate ageing test: The pelleted and non-pelleted seeds were put in clothing bag (10x20 cm), placed on a sieve (2x2 mm) in the ageing-box (110 mm L x 110 mm W x 30 mm H). Inside the box had 100 ml of water below the sieve at 2 cm, closed the lid tightly and then put the ageing-box in the accelerated ageing chamber at 40 °C-100% RH for 48 hours. Germination test after accelerated ageing was conducted under the laboratory and greenhouse conditions.

Statistical analysis

Percentage of germination was arcsine-transformed to normalize data before statistical analyses. All data were analyzed by one-way ANOVA (complete randomized design) and the difference between treatments was tested by Duncan's multiple range test (DMRT).

Results and Discussion

Lettuce seed quality after pelleting process

Under laboratory and greenhouse conditions, it was found that the majority of non-pelleted seeds and pelleted seeds in all methods had high percentage of germination excepting pelleted seeds with 0.48 g. NH_4NO_3 (T6) which had the lowest percentage of germination. Speed of germination of pelleted seed with 0.48 g. NH_4NO_3 (T6) was slower and significantly different than other methods.

From this experiment, the filler material used calcium sulfate, was a fine powder, moderated

viscosity, and easily dissolved in water. The pH of soil was suitable for the growth of plants because there it had plant nutrients which could be applied to seedlings after germination (Saint-Gobain Formula, 2009). In addition, calcium sulfate can absorb moisture well, it made moisture and air can penetrate easily. Therefore, it was one of the factors for the germination and establishment of seedlings well (Peek et al., 2008). Furthermore, adding nutrients to stick to pelleted seed pieces that can also encourage seedlings to germinate well without hampering the germination of lettuce seeds. The results of the study found that non-pelleted seeds when had use of NH_4NO_3 with seeds at the rates of 0.06-0.24 g had not affected the germination percentage and germination speed of lettuce seeds, and pelleting methods with other plant nutrients.

As lettuce seedlings can use nitrogen in the appropriate amount of for germination, and seedlings can absorb to utilize both in the form of Ammonium ion (NH_4^+) and nitrate ions (NO_3^-) by plant roots (Osuna et al., 2015). From **Figure 1**, it was obvious that after two days after planting pelleted seeds with NH_4NO_3 at every rate. It tends to extended the of the roots length better than when compared to non-pelleted seeds. After planting four days, pelleting the seed with NH_4NO_3 all rates developed more long-roots, and the number of root hairs more than non-pelleted seeds clearly.

As nutrient content of pelleted seed increased, the germination decreased. The results showed that the higher rate of 0.24 g. NH_4NO_3 affected to decrease germination of pelleted seed. The high level of concentration of plant nutrient affects to decrease the amount of

plant hormone that stimulate the germination process. Especially, gibberellin (Copeland and McDonald, 1995) and cytokinin, which stimulate cell division and cell enlargement of seed germination of seeds (Kucera et al., 2005). In addition, NH_4^+ from NH_4NO_3 is a source of nitrogen that is absorbed during seed germination. The nitrogen plays an important role in respiration

process and nutrient degradation process in the seed. It makes faster seedling establishment, encourage to created protein to seedling and synthesize substances for cell division of seedlings (Marschner, 1995). Therefore, pelleted seed with appropriated rate of nitrogen had not affected germination and vigor of lettuce seeds.



Figure 1 Effect of seed pelleting with difference rate of nitrogen, radicle emergence 2 days (A) and 4 days (B) after planting tested under laboratory condition. Control, T1); Pelleted seed, T2); Seed pelleting mixed NH_4NO_3 rate 0.6 g., 0.12 g., 0.24 g. 0.48 g. T3), T4), T5) and T6) respectively.

Table 1 Germination percentage and speed of germination of lettuce tested under laboratory and greenhouse conditions after pelleting process.

Treatments ^{1/}	Laboratory condition		Greenhouse condition	
	Germination (%)	Speed of germination (seedling/day)	Germination (%)	Speed of germination (seedling/day)
T ₁	97 ab ^{2/3/}	24.49 a	97 a	23.95 a
T ₂	98 a	24.11 a	98 a	23.18 ab
T ₃	98 a	23.97 a	99 a	23.20 ab
T ₄	99 a	24.32 a	99 a	22.80 ab
T ₅	99 a	24.40 a	99 a	22.25 b
T ₆	95 b	22.96 b	94 b	20.79 c
F-test	*	**	*	**
CV (%)	1.37	2.26	1.75	3.28

* significantly different at $P \leq 0.05$, ** significantly different at $P \leq 0.01$.

^{1/}Control, T1); Pelleted seed, T2); Seed pelleting mixed NH_4NO_3 rate 0.6 g., 0.12 g., 0.24 g. 0.48 g. T3), T4), T5) and T6) respectively.

^{2/}Means within a column followed by the same letter are not significantly different at $P \leq 0.01$ by DMRT

^{3/}Data are transformed by the arcsine before statistical analysis and back transformed data are presented.

Many researchers had studied various types of pelleted seed using plant nutrients on germination and seedling growth. Pelleted sesame seeds with gypsum, ammonium molybdate, ZnSO_4 , MnSO_4 and borax had significantly increased germination and vigor (Balamarugan et al. (2003). Rathod et al. (2005) reported that pelleted seeds with plant nutrients using diammonium phosphate, borax, zinc sulfate and thiram at the rate of 3.0, 0.1, 0.3 and 3.0 g, respectively. It was found that had affected the germination, vigor, and dry weight of seedlings to be higher than non-pelleted seeds. In addition, Trachoo et al. (2016) found that pelleted tobacco seeds with 1 g of KCl had the highest seed germination (96 and 86%) and speed germination (12.84 and 9.97 plants/days) in both under laboratory and greenhouse conditions, respectively.

Seedling growth after pelleting process with nitrogen compound

After pelleted lettuce seeds with different nitrogen rates, the seedlings growth was evaluated. The results under laboratory condition showed that pelleted seed had the highest shoot length, especially, the pelleted seed with all rates of NH_4NO_3 , and significantly different to non-pelleted seed. The pelleted seeds with 0.24 g of NH_4NO_3 had the highest root length (152.25 mm) of lettuce seedlings and significantly different as compared with other methods. It also had affected the seedling fresh weight of lettuce seedlings was 0.442 g, followed by pelleted seeds with 0.12 g of NH_4NO_3 . For seedling dry weight, it was found that pelleted seeds with 0.12 g and 0.24 g of NH_4NO_3 had remained the best seedling dry weight and statistically significant differences as compared with non-pelleted seeds.

Table 2 Shoot length, root length, seedling fresh weight and seedling dry weight of lettuce tested under laboratory condition after pelleting process.

Treatments ^{1/}	Shoot length (mm)	Root length (mm)	Seedling fresh weight (g)	Seedling dry weight (g)
T ₁	32.72 b ^{2/}	133.05 c	0.231 c	0.0193 b
T ₂	33.62 b	146.52 b	0.272 c	0.0218 ab
T ₃	39.97 a	150.45 b	0.363 b	0.0211 ab
T ₄	39.92 a	150.70 b	0.404 ab	0.0229 a
T ₅	40.85 a	152.25 a	0.442 a	0.0237 a
T ₆	39.85 a	131.57 c	0.376 b	0.0212 ab
F-test	**	**	**	*
CV (%)	3.79	3.47	9.14	7.80

* significantly different at $P \leq 0.05$, ** significantly different at $P \leq 0.01$.

^{1/}Control, T1); Pelleted seed, T2); Seed pelleting mixed NH_4NO_3 rate 0.6 g., 0.12 g., 0.24 g. 0.48 g. T3), T4), T5) and T6) respectively.

^{2/}Means within a column followed by the same letter are not significantly different at $P \leq 0.01$ by DMRT

In this study, the lettuce seedlings were influenced by nitrogen for the growth. Therefore, the results found that all seed pelleting methods can enhance the growth of lettuce seedlings. Especially, the pelleting seeds with nitrogen, we have opinions that the filler materials that use to pelleted seed were the leader to carry the active ingredient to stick the seed. After seedling germinated, it can use nitrogen for the growth immediately. Moreover, calcium sulfate can dissolved easily, not toxic effect to the seeds, and help to digest nitrogen element for the plant to use more (Walworth, 2006). It can be combined with binders in order to bring nitrogen attached to the seed as well. After planting seedlings, it was demonstrated that the change of seedlings root of lettuce clearly using seed pelleting method with all rates of nitrogen to stimulate lettuce seedlings with a number of lateral root more than non-pelleted seeds and pelleted seed alone (**Figure 2A**). Other than the lettuce seedlings could be bring the nitrogen to use better through absorption by the roots in the form of sodium nitrate (NO_3^-) and ammonium nitrate (NH_4^+) that dissolved around the area of pelleted seed. The plant can grow faster, especially in the early stages of growth of the seedlings (Thompson et al., 1998; Zhang and Forde, 1998; Barker and Pilbeam, 2007). The nitrogen also helps to stimulate the work of auxin and cytokinin better to promote Nitric oxide (NO), which is an important molecule in the biochemical processes for stimulating the increase of root cells, stem cells of plants (Mi et al., 2008).

In greenhouse condition, it was found that seedlings developed the growth increasingly over time, especially all of seed pelleting methods, and it was obviously indicated that pelleting the seed with 0.24 g of NH_4NO_3 after 14 days. It was found that the shoot length was highest at 81.11 mm (**Figure 2B**), then monitored the growth of shoot length at the period of 30 days. The pelleted seeds at a rate of 0.24 g had the best of shoot length, and statistically significant differences when compared with non-pelleted seeds. Followed by pelleting the seed at a rate of 0.12 g. had the trend of shoot length was better than other method (**Figure 2C**). When examining shoot fresh weight, it was found that monitoring at 7, 14 and 30 days in pelleted seeds with 0.24 g of NH_4NO_3 had the best of seedling growth. After the examination at 30 days alone, it was found that the shoot fresh weight was up to 8.195 g, the shoot fresh weight was 4.284 g more than the non-pelleted seeds, and it was statistically significant differences when compared with other methods. In addition, after monitored the shoot dry weight at 30 days, it was found that pelleted seeds with 0.24 g of NH_4NO_3 , it still had the maximum of shoot dry weight, and statistically significant differences when compared with other methods. The pelleted seeds with a rate of 0.24 g had higher shoot dry weight (0.171 g.) than non-pelleted seeds approximately 2 times from original.

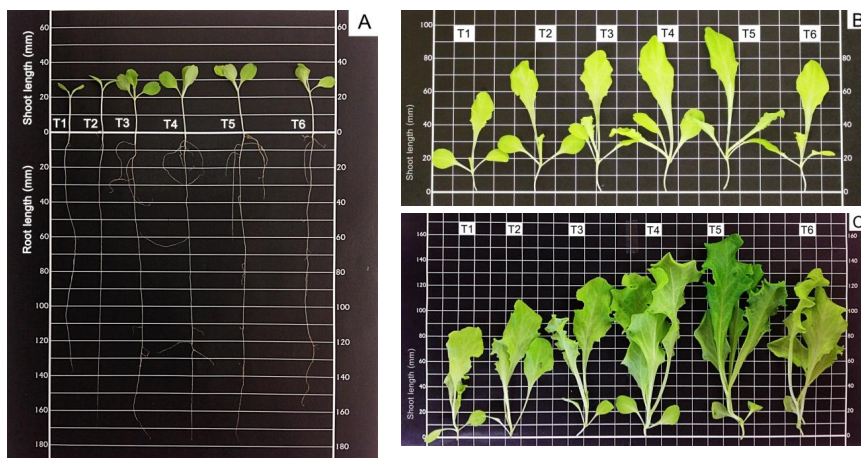


Figure 2 Show changes characteristic growth after seed pelleting with difference rate of nitrogen. Shoot and root length after planting 14 days (A) (laboratory condition), and shoot length after planting 14 days (B) and 30 days (C) (greenhouse condition). Control, T1); Pelleted seed, T2); Seed pelleting mixed with NH_4NO_3 of the rate 0.6 g., 0.12 g., 0.24 g. 0.48 g. T3), T4), T5) and T6) respectively.

Table 3 Shoot length, shoot fresh weight and shoot dry weight of lettuce tested under greenhouse condition after pelleting process.

Treatments ^{1/}	Shoot length (mm)			Shoot fresh weight (g)			Shoot dry weight (g)		
	Day after planting								
	7	14	30	7	14	30	7	14	30
T ₁	24.40 e ^{2/}	59.25 d	99.65 c	0.204 d	0.954 f	3.911 e	0.0165 b	0.0543 d	0.2147 d
T ₂	27.72 c	64.42 d	105.97 bc	0.211 d	1.226 e	5.194 d	0.0177 ab	0.0590 cd	0.2571 cd
T ₃	29.05 b	71.06 b	110.08 bc	0.245 c	1.521 c	5.363 d	0.0173 ab	0.0633 b-d	0.2917 bc
T ₄	33.47 a	73.16 b	114.96 ab	0.303 a	1.640 b	7.142 b	0.0180 a	0.0716 ab	0.3187 b
T ₅	33.70 a	81.11 a	124.93 a	0.310 a	1.835 a	8.195 a	0.0188 a	0.0788 a	0.3857 a
T ₆	26.37 d	68.83 c	111.26 b	0.276 b	1.419 d	6.181 c	0.162 b	0.0664 bc	0.3008 bc
F-test	**	**	**	**	**	**	*	**	**
CV (%)	2.13	3.18	6.16	6.54	4.54	4.75	6.97	10.93	11.16

* significantly different at $P \leq 0.05$, ** significantly different at $P \leq 0.01$.

^{1/}Control, T1); Pelleted seed, T2); Seed pelleting mixed NH_4NO_3 rate 0.6 g., 0.12 g., 0.24 g. 0.48 g. T3), T4), T5) and T6) respectively.

^{2/}Means within a column followed by the same letter are not significantly different at $P \leq 0.01$ by DMRT

For seedling growth, it was found that pelleted seed using nitrogen had higher seedling growth development than non-pelleted seed. In addition, pelleted seeds with the higher rate than 0.24 g affected to seedling growth of seedling. For 30 days after planting, the pelleted seeds at

the rate of 0.48 g. Decreased shoot length, shoot fresh weight and shoot dry weight as compared to the rate of 0.12 and 0.24 g. Therefore, the appropriated seed of nitrogen for pelleted seed had obviously higher shoot length than non-pelleted. Moreover, nitrogen can increase

amount of phosphorus because nitrogen adapted the soil pH to slightly acidic that phosphorus can dissolve in the form of HPO_4^{2-} as soil pH 6.8 to 7.2 and easily for seedling absorption.

Pelleted seed with nitrogen increase phosphorus absorption because nitrogen affect to root elongation and increase the area for uptake soil nutrient **Figure 2A** (Forde and Lorenzo, 2001; López-Bucio et al., 2003). Therefore, nitrogen affects to fastly seedling establishment, vigorous root system, good stem elongation and high seedling growth. Clearly, the seed pelleting with

plant nutrients have the growth better than non-pelleted seeds. In addition, the results found that the nitrogen at a higher rate than 0.24 g will be affected the restraint of seedling growth of lettuce. The seedlings uptaked nitrogen in high doses had directly affected on protein creating process, amino acid and enzymes of plant seedlings. Thus, the seedlings had risks hindering the growth or wither and die. (Brunner and Scheidegger, 1995; Monaco et al., 2003; Zhu et al., 2014).

Table 4 Germination percentages and speed of germination of lettuce tested under laboratory and greenhouse conditions after pelleting process and accelerated ageing.

Treatments ^{1/}	Laboratory condition		Greenhouse condition	
	Germination (%)	Speed of germination (seedling/day)	Germination (%)	Speed of germination (seedling/day)
T ₁	76 b ^{2/3/}	22.54 a	75 b	16.75 a
T ₂	84 a	18.75 c	77 b	14.21 b
T ₃	87 a	19.41 bc	81 ab	14.20 b
T ₄	89 a	19.48 bc	83 ab	14.71 ab
T ₅	88 a	20.82 abc	86 a	14.84 ab
T ₆	86 a	21.29 ab	79 b	13.92 b
F-test	*	**	*	*
CV (%)	6.23	6.41	6.45	9.75

* significantly different at $P \leq 0.05$, ** significantly different at $P \leq 0.01$.

^{1/}Control, T₁); Pelleted seed, T₂); Seed pelleting mixed NH_4NO_3 rate 0.6 g., 0.12 g., 0.24 g. 0.48 g. T₃), T₄), T₅) and T₆) respectively.

^{2/}Means within a column followed by the same letter are not significantly different at $P \leq 0.01$ by DMRT

^{3/}Data are transformed by the arcsine before statistical analysis and back transformed data are presented.

Lettuce seed quality after accelerated ageing

Accelerating ageing seeds is bringing the seed to place under conditions of high temperature and humidity to accelerate the process of seed respiration. Then, the seeds will release heat and moisture, the seeds deteriorate more quickly. The experimental results under laboratory conditions, all seed pelleting methods had percentage of germination better than and

statistically significant differences when compared with non-pelleted seeds. But non-pelleted seeds can germinate faster than all pelleted seed methods. However, the pelleted seeds with NH_4NO_3 at the rate of 0.24 and 0.48 g had the best of speed of germination when compared in the group of pelleted seeds. In greenhouse conditions, it was demonstrated that the pelleted seeds with 0.24 g of NH_4NO_3 had the highest of

percentage of germination was 86% and statistically significant differences when compared to other methods. Followed by pelleted seeds at the rate of 0.06 and 0.12 g, it was 81% and 83%, respectively. However, non-pelleted seeds had the highest of speed of germination.

Seed pelleting is a method of adding inert the fillers to wrap seeds. It likes the addition of insulation or armor to protect inappropriate environment such as heat and high humidity. As a result, it makes the seeds have slow respiration process when compared to non-pelleted seeds. In addition, pelleted seeds also have NH_4NO_3 stick to seeds. It makes seedlings after germination can promote germination, setting up of seedlings, and grows better than non-pelleted seeds. Peñaloza et al. (2005) reported that accelerating ageing of lettuce seeds had affected to seed quality decrease. In addition, seedlings were in disorder, as well as causing mold attached to the seeds. Buakaew and Siri (2016) found that after accelerating ageing of pelleted lettuce seed had percentage of germination better than non-pelleted seeds. Meanwhile, the report of pelleting tobacco seeds found that pelleted tobacco seeds had percentage of germination better than non-pelleted tobacco seeds (Kangsopa and Siri, 2014a; Kangsopa and Siri, 2014b).

Conclusions

Seed pelleting with nitrogen at the rates between 0.06 and 0.24 g had not affected the germination and vigor of lettuce seeds in both under the laboratory and greenhouse conditions. The growth of pelleted lettuce seeds with 0.24 g of NH_4NO_3 had the best of shoot length and root length, the seedling fresh weight and seedling dry

weight under laboratory and greenhouse conditions. In addition, all pelleted seed methods had good germination after accelerated ageing better than non-pelleted seeds explicitly.

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