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Original Research Article

Effect of KNO_3 Priming on Various Properties of Kenaf Seed under Non-Saline and Saline Conditions

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ABSTRACT The main objective of this study was to increase the germination percentage of kenaf seeds with less number of times under non-saline and saline conditions. Therefore, the first goal was to assess the response of kenaf seeds to NaCl. The second goal was to evaluate the effects of KNO_3 on kenaf seed germination. The germination percentage exhibited a decreasing tendency in germination rate. Plant dry weight was approximately 0.2 g in all treatments at 5 days after germination. As time passed, the electrical conductivity (EC) value of hydro-priming (HP) consistently increased by 8.7 mS/cm at 24 hours of immersion. However, seeds primed with KNO_3 showed no difference in EC values even as times passed. Regarding the priming effect, priming in 100 mM KNO_3 concentration for 12 hours increased germination up to 85% in H_20 solution and in 0 mM KNO_3 concentration upto 73.8% under 0.3% NaCl solution, compared to that of Control. Germination synchronization, shoot length, and leaf unfolding of primed seeds were greater than those of the Control. In addition, main root and hair roots appeared more rapidly in the treated seeds and were more abundant compared to that of the Control. The T50 (times to reach 50% of the final germination percentage) of the Control in both H_20 and 0.3% NaCl solutions was 18 and 22 hours, respectively. However, when treated KNO_3 priming (0 to 100 mM) in H_20 and 0.3% NaCl solution, 9 hours was sufficient to reach T50. Primed (hydro-priming and KNO_3) seeds had a lower MDG (mean days untill germination; 0.6-0.62) compared to that of the Control (1.13-1.31) in H_20 and 0.3% NaCl solutions. Regarding dry weight of plants after priming, an increasing tendency after the priming treatment in the H20 solution was observed. Furthermore, no significant difference in plant dry weight under 0.3% NaCl stress was observed between the Control and primed seeds. Taken together, the results suggest that 50-100 mM KNO_3 priming for 24 hours optimize seed germination rate in less number of times of exposure with great vigor. Therefore, it is recommended for kenaf seed invigoration before planting.

Keywords : kenaf, KNO_3, MDG, priming, salt, T50

Kenaf (*Hibscus cannabinus* L.) is a real jack of all trades due to a variety of utilization like fiber, forage and fuel. It is native to parts of Africa and closely related to cotton, okra, and hibiscus, kenaf is similar in appearance to hemp. Kenaf can be grown in a wider range of climates and soil types than any other commercial fiber crop (Danalatos & Archontoulis, 2010; Webber *et al.*, 2002). In developed countries such as USA and Japan, it has been grown for fiber production and forage. Kenaf was introduced in Korea in the 60s. Ever since, there has been a limitation of using kenaf in Korea. Of late years, its value in Korea has been increased in terms of forage and biomass production for fuel. Under conditioned storage at 20°C and 10% humidity, kenaf seed remained viable for about 8 months (Carberry *et al.*, 2012). However, under the hot and humid climates with average ambient temperature around 35°C and humidity above 60%, viability loss is faster (Daniel *et al*., 2012). There is a kenaf variety called 'Jangdae' released from KAERI (Korea Atomic Energy Research Institute). It has a low germination rate, causing a major limitation to commercially use in Korea.

The reclaimed tidal land of Korea is 135,000 ha which is about 9% of cultivation acreage (Lee *et al*., 2012). The various approaches have been carried out to increase an

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availability of the land and many plants suitable for the reclaimed tidal land were collected (Lee *et al*., 2000; Oh *et al.*, 2015). From the results, there were some problems containing a high C/N ratio posing nitrogen starvation and relatively low yield. However, kenaf has a relatively low C/N ratio of 30~60 and a higher yield of roughly 17~32 t/ha on the dry basis (Wang, 1994). Those merits of kenaf can overcome the drawbacks of the other plants all at once when applying kenaf at reclaimed tidal land.

Recent research on a range of crop species had shown that faster germination, early emergence and vigorous seedlings were achieved by controlled treatment of seeds by priming for certain number of hours followed by drying before sowing (Bradford, 1986). Several studies had supported the use of these methods for increasing germination and seed invigoration in various crops (Ella *et al*., 2011; Farooq *et al*., 2006; Mir-Mahmoodi *et al*., 2011). However, the secret to successful seed priming is ceasing the priming treatment at just the right time to allow re-drying, hence each species must be investigated for optimal priming treatments and treatment durations (Bradford, 1986). Seed priming has been used to accelerate the germination, uniform seedling emergence and improve a germination performance under the temperature or drought stresses (Janmohammadi *et al*., 2008; Jahangir *et al*., 2009).

The priming with nitrate solutions stimulates the germination, so Hendricks & Taylorson (1974) suggested that its mechanism could be trough a nitric oxide (NO) synthesis. NO breaks a seed dormancy trough the interaction with the phytochrome signaling pathways, the ethylene biosynthesis, and interplays with reactive oxygen species-ROS (Sírová *et al*., 2011). Nitrate-containing salts like KNO_3 were more efficient than ammonium salts in promoting germination (Hendricks & Taylorson, 1974).

Therefore, the objective of this trial was to assess the response of kenaf seed to NaCl and investigate the effects of KNO_3 on kenaf seed (Jangdae) germination rate and vigour in earlier times.

MATERIALS AND METHODS

Seed and other materials preparation

The kenaf variety was Jangdae released from KAERI (Korea Atomic Energy Research Institute). The experiment was conducted in the Laboratory of Agri-Food Processing, Agricultural Research and Extension Services, Iksan from February to April, 2017. Seeds (12 g) of each treatment were sterilized by soaking in NaOCl solution for 10 min and dried for 30 min. Containers named as brand TWIST 400 (10 × 7.5cm=diameter × height) were purchased at a store.

Measurement of seed water uptake and EC value

As to water uptake, three replicates of seeds (12 g) were soaked at water (150 ㎖) and kept seeds in an incubator (MULTI-ROOM CHAMBER HB-302S-4, HANBAEK SCIENTIFIC CO., Bu Cheon City, KOREA) of 25°C equal to germination temperature. A moisture content was measured in 1, 3, 6, 12 and 24 hours. About EC value, seeds were treated with various KNO_3 concentration (0, 50, 100, 150, 200, 250, 500 mM) for 24 hours at constant temperatures of 20°C the dark in an incubator (MULTI-ROOM CHAMBER HB-302S-4, HANBAEK SCIENTIFIC CO., Bu Cheon City, KOREA), and its value was measured in 1, 3, 6, 12 and 24 hours with EC measuring instrument (LAQUA act, D-74G, HORIBA, KYOTO, Japan).

Germination tests to NaCl and KNO_3

To confirm RD50, salt concentration causing 50% growth reduction, of kenaf seeds to salt, prepared seeds were inoculated in various NaCl (0, 0.1, 0.2, 0.3, 0.4, 0.5%) solutions for 72 hours. Three replicates of 20 seeds were placed in covered TWIST 400 container containing two filter paper with 7 ㎖ test solutions. Seeds were germinated at constant temperatures of 25°C the light in an incubator (MULTI-ROOM CHAMBER HB-302S-4, HANBAEK SCIENTIFIC CO., Bu Cheon City, KOREA). Germination was scored at same time daily. A seed was considered to be germinated as seed coat ruptured, plumule and radicle came out and were >2 mm long. Plant, three replicates of 10 plants, was dried at constant temperatures of 60°C for 2 days and dry weight was scored.

For KNO_3 test, which was used as osmopriming agent solution, seeds were primed with various KNO_3 concentration (0, 50, 100, 150, 200, 250, 500 mM) for 24 hours at constant temperatures of 20°C, which was used as suitable

temperature at many previous study (Bae *et al*., 2014; Kang *et al*., 2003), in dark condition of an incubator (MULTI-ROOM CHAMBER HB-302S-4, HANBAEK SCIENTIFIC CO., Bu Cheon City, KOREA). Three replicates of 20 seeds were placed in previous same container including two filter paper with 7 ㎖ test solutions, and germinated for 72 hours at constant temperatures of 25°C in light. The temperature (25°C) was used for crop germination (Shekari *et al*., 2015.). Germination was scored at same time daily. Plant, three replicates of 10 plants, was dried at constant temperatures of 60°C for 2 days and dry weight was scored.

Measurement of growth degree of shoot and root to salt

Growth degree of shoots and roots was evaluated in 5 days of germination beginning. All seedlings were taken from each treatment to measure growth degree of shoot and root to the naked eye. Root and shoot length of the seedlings were not measured due to severe distortion of shoot and root.

Germination performance measurements

At the end of experiment, germination rate, germination percentage, growth degree of shoots and roots in NaCl solutions, T50 (times to reach 50% of the final germination rate), MDG (mean no. of days to germination), dry weight were recorded to evaluate germination performance. Dry weight was measured by four digit balance and expressed in milligram (mg).

Statistics

Results were analyzed for analysis of variance (ANOVA) using the Statictical Analysis System (V. 9.1, SAS Institute, Cary, NC, USA). Means were compared at the 5% significance level using Duncan's multiple comparison.

RESULTS AND DISCUSSION

Response of kenaf to NaCl

The first aim of this study was to assess the response of kenaf seed at various NaCl levels. In this part of study, kenaf seeds only exposed to 0% NaCl was written as NaCl-Free (NF). The germination percentage of seed ranged from 11.3 to 58.8 after 24 hours and from 66.3 to 86.3 after 72 hours, respectively (Fig. 1). In 24 hours, there was a significant difference in germination percentage between seeds grown on solution with NF and 0.1% NaCl. However, we could not found some difference by 0.4% NaCl compared to NF in 72 hours. Only, a meaningful distinction in 72 hours was shown at 0.5% NaCl treatment (Fig. 1). Bases on this observation, it is concluded that kenaf was placed in the category of moderate salt tolerant non-halophytic crop under salinity. This result was in agreement with that of earlier report (Mass & Hoffman, 1977). Previous study showed that its germination percentage was declined with increasing salt concentration (Francois *et al.*, 1990). Curtis & Lauchli (1985) noticed that seed germination of kenaf cultivars was only slightly impaired by NaCl salinity up to 200 mM.

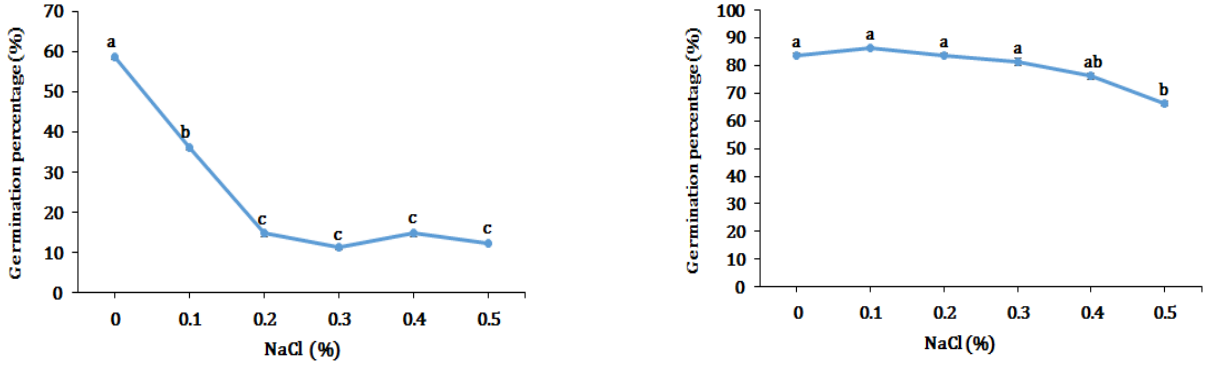

Fig. 1. Response in germination of kenaf seeds according to the difference in NaCl concentration after 24 (Left) and 72 (Right) hours of immersion.

Mean ± SE. Zero (NF, NaCl-free) indictes seeds grown in solution without NaCl. Different letters for each treatment indicate significant difference ($p < 0.01$) as determined by Duncan's multiple range test.

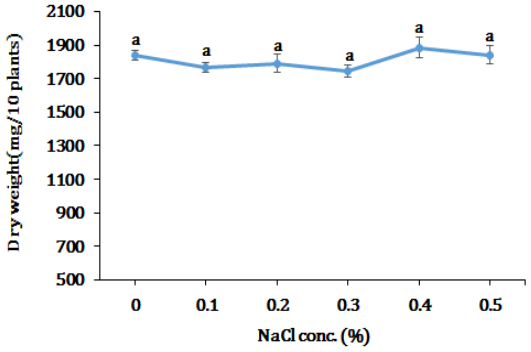

Fig. 2. Dry weight of kenaf plant at various NaCl concentrations at 5 days after germination. Dry weight was measured three times per repetition with 10 plants. Mean ± SE. The same letter for each treatment indicates no significant difference as determined by Duncan's multiple range test.

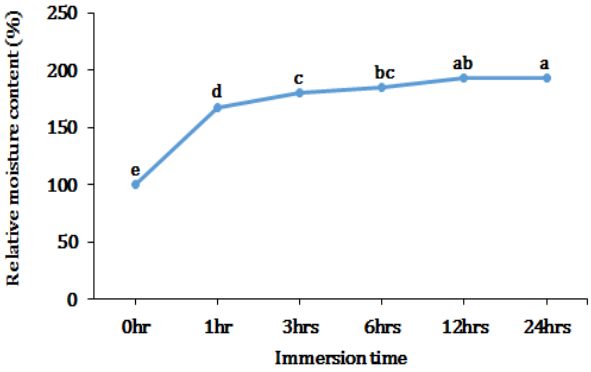

Fig. 3. Relative moisture content (%) of kenaf seeds according to different soaking times in water. Mean ± SE. Different letter for each treatment indicate significant difference as determined by Duncan's multiple range test.

Table 1. Growth differences of kenaf shoots and roots under various NaCl concentrations at 5 days after germination.

Tissues	NaCl (%)					
	0	0.1	0.2	0.3	0.4	0.5
Shoot	******	****	***	**	*	*
Root	*****	****	***	**	*	*

- Shoot : ****** (Excellent), **** (Good), *** (Medium), ** (Poor), * (Very poor).
- Root : ***** (Excellent), **** (Good), *** (Medium), ** (Poor), * (Very poor).
- Zero (NF, NaCl-free) indictes seeds grown in solution without NaCl.

There was a result on growth difference of kenaf shoots and roots in Table 1. Its vegetative development was decreased as increasing salt stress level. Based on both shoots and roots growth, a range of 0.2-0.3% NaCl was considered to be RD50 (salt concentration of causing 50% growth reduction). Curtis & Lauchli (1986) reported a threshold of about 0.21% NaCl for significant growth reduction of kenaf due to salinity. We took up 0.3% of NaCl as selection concentration for subsequent experiment. Leaf growth rate declined linearly with increasing salt stress (Curtis & Lauchli, 1985).

Plant dry weight was similar about 0.2 g under all salt treatments after 5 days of seed inoculation (Fig. 2). Based on this result, we thought that a kenaf dry weight was not given a negative impact upto 0.5% NaCl. It is concluded that a dry weight is not suitable criteria to estimate salt tolerance of kenaf upto 5 days. So, a long time of test is needed to use a dry weight as criteria to estimate salt tolerance of kenaf. Francois *et al.* (1992) reported that the dry weight of stem was only reduced 11.6% in soil salinity above 8.1 ds/m, which is comparable to 0.42% NaCl. When compared Fig. 1 and 2, we found that germination was more sensitive than dry weight.

Effect of various immersion times on seed water uptake

Kenaf is a upland-field crop. An immersion of kenaf seed in water for a long time caused a decrease vitality and life-shortening of seed, resulting in a reduction of germination percentage. So, it is important to identify a induction period of water-uptake preventing water absorption due to a balance of water potential. Before priming treatment, seed moisture was about 10%. With an immersion times increasing, seed moisture gently increased up to 1.93 times at 24 hours compared to 0 hour. At the early times, priming treated seeds, particularly by 3 hours, had rapid water uptake compared to 0 hour. Beyond 12 hours, its content was statistically same. We thought that a critical point for soaking water in kenaf seeds was 12 hours. From 12 hours, a part of seeds broke seedcoat by coming out radicle (Fig. 3). Similar result was observed by Shekari *et al.* (2015).

Effect of various KNO_3 and immersion times on ion leachate

In this part of study, kenaf seeds only exposed to 0% KNO_3 was expressed as HP (hydro-priming) (Table 2). As time

Table 2. Ion leakage difference of priming solutions containing seeds following priming with various KNO_3 concentrations from 0 hour to 24 hours.

KNO_3 (mM)	EC value (mS/cm)					
	0 hrs	1 hr	3 hrs	6 hrs	12 hrs	24 hrs
[J]0	0.7g	2.5g	4.9g	5.5g	6.4g	8.7g
50	34.5f	36.4f	36.2f	37.8f	35.9f	36.8f
100	63.2e	65.6e	65.1e	67.5e	66.2e	67.6e
150	90.9d	91.6d	93.1d	92.2d	94.0d	93.4d
200	117.6c	118.4c	119.2c	119.5c	120.7c	120.1c
250	142.5b	143.2b	144.7b	145.7b	145.4b	144.3b
500	2,586a	2,536a	2,590a	2,620a	2,600a	2,606a

* [J] : Seeds grown in solution without KNO_3 were denoted as HP (hydro-priming).
* Different letter in each column indicate significant difference ($p < 0.05$) as determined by Duncan's multiple range test.

Table 3. Responses of germination percentage (%) of kenaf seed under various KNO_3 concentration based on time.

Solutions	Hours	KNO_3 (mM)							
		[J]Control	[†]0	50	100	150	200	250	500
H_20	12	0d	77.5a	77.5a	85.0a	36.3b	40.0b	32.5c	0d
	24	78.8ab	85.0a	85.0a	91.3a	68.8bc	61.3c	38.8d	5.0e
	48	97.5a	85.0b	85.0b	91.3ab	68.8c	61.3c	43.8d	7.5e
	72	90.0a	65.0b	58.8b	62.5b	40.0c	38.8c	31.3c	1.3d
0.3% NaCl	12	0d	73.8a	62.5ab	58.8b	32.5c	22.5c	18.8c	0d
	24	58.8c	82.5a	72.5ab	65.0bc	53.8c	40.0d	37.5d	2.5f
	48	86.3a	83.8a	72.5ab	65.0bc	55.0c	41.3d	38.8d	2.5f
	72	82.5a	73.8a	50.0b	47.5b	42.5bc	30.0cd	25.0d	1.3e

* [J] : Seeds untreated with water and solution of KNO_3 are denoted as the Control.
* [†] : Seeds grown in solution without KNO_3 are denoted as HP (hydro-priming).
* Different letter in each row indicate significant difference ($p<0.05$) as determined by Duncan's multiple range test.

passed, electrical conductivity (EC) value of HP consistently increased. In the presence of KNO_3 (from 50 to 500 mM) in solution, the ion leakage concentration sharply increased (Table 2). However, there was no difference in EC of these solutions even if times passed (Table 2).

By adding KNO_3, the EC value increased step-by-step by 250 mM. Its value incredibly soared at 500 mM. It is clear that an alteration of EC value was affected by KNO_3 rather than water. The EC increase demonstrated the degree of the loss of solutes from the seeds, which reflects the extent of membrane deterioration resulting from seed aging (Roberts, 1986).

Effect of KNO_3 on surging the germination percentage

In this part, non-treated seeds without being exposed to water and KNO_3 was expressed as Control. The seeds only exposed to water was used as hydro-priming (HP) (Table 3). Germination of kenaf seed was generally poor in the control condition based on the kenaf seed maturity. So, it is necessary for kenaf seed to apply priming technique of KNO_3 so as to surge germination uniformity and capability. The efficacy of KNO_3 priming on the germination of kenaf seed was investigated at 25°C. Table 3 illustrate the final germination against time respectively. KNO_3 concentration in this study was the same range as that of other previous studies (Bae *et al*., 2014; Ismail *et al*., 2005; Kang *et al*., 2003).

As shown in Table 3, there was a significant difference between germination percentage of the early time under 24 hours and germination percentage of the late time over 48

hours when compared Control and priming-treatment. The seeds primed with both hydro-priming and osmopriming (KNO_3) showed a fast germination percentage at the early time under 24 hours compared to Control. It means that a priming treatment would promote germination rate of kenaf seeds. Priming at 100 mM KNO_3 concentration for 12 hours increased the germination upto 85% under H_20 solution and at HP upto 73.8% under 0.3% NaCl solution compared to Control (0%). The highest germination percentage was recorded at a range of 24-48 hours under H_20 and 0.3% NaCl solutions. The sample treated with 100 mM KNO_3 at 24 hours showed the highest germination percentage with 91.3% under H_20 solutions. Under 0.3% NaCl solution, it was turned out as 0 mM KNO_3 (82.5%) termed as HP (hydro-priming). Compared to Control, the germination percentage was increased up to 100 mM KNO_3, it kept decreasing under both solutions more than this concentration. As we can see from Table 2, a high EC value posed by loss of solutes from the seeds was related with the reduction of germination percentage. It is concluded that a critical concentration of KNO_3 to surge germination was identified as 100 mM. We thought that this method considerably reduced the time required to initiate the germination of seeds. Kim *et al*. (2014) reported that the gardenia germination was increased up to 100 mM KNO_3, whereafter decreased. These results may be attributed to chemical effects of KNO_3 due to cell wall looseness increasing water uptake of cell. The most probable mechanism for KNO_3 enhancement of germination is the intensification of mass transfer and easier access of the water to the interior of the cell wall structure.

On the contrary of the result of 24 hours, the highest germination percentage with 97.5% at 48 hours was shown in Control in H_20 solution and turned up 86.3% in Control at previous same hours under 0.3% NaCl solution in comparison to KNO_3 priming treatments. The priming treatments (HP and KNO_3) were only germinated by 24 hours, but Control was germinated up to 48 hours. As to a findings of 72 hours, it was represented with a survival seedlings. Significant difference in survival rate of seedlings also occurred among treatments under both solutions. The survival rate of Control was significantly greater than the other priming treatments. A part of seedlings treated with KNO_3 was earlier dead than that of Control. Previous study reported that studies with cotton and kenaf have shown a strong association between rapid germination and seedcoat susceptibility to mold growth, as well as seed rot and diseased seedling roots (Bird, 1982; Cook *et al*., 1992). Germination synchronization, shoot length, and leaf unfolding of primed seed was greater than those of Control. Also, main root or hair root appeared faster in the priming treated seeds and grew abundantly compared to the Control (Fig. 4).

Effect of KNO_3 on surging both T50 and MDG

The findings of T50 (times to reach 50% of the final germination percentage) and MDG (mean number of days to germination) after KNO_3 treatment are as follows. The T50 of Control under both H_20 and 0.3% NaCl solutions was 18 and 22 times, respectively. However, when treated KNO_3 priming (0 to 100 mM) in H_20 and 0.3% NaCl solution, 9 times was sufficient to reach T50. It is 2 times

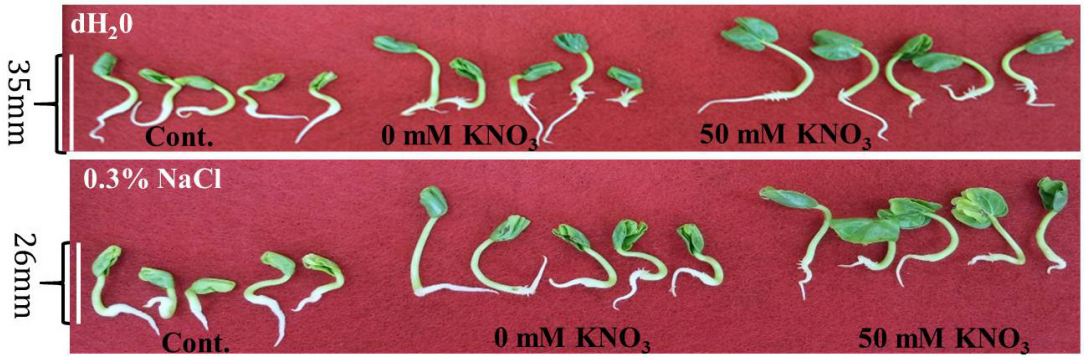

Fig. 4. Comparison of seedlings at various KNO_3 concentrations at 3 days after germination under distilled water and 0.3% NaCl conditions. Cont. : Control, 0 mM KNO_3 : Seeds grown in solution without KNO_3 are denoted as HP (hydro-priming).

as high as that of Control (Table 4). MDG decreased from 1.13 days being maximum for Control to 0.6 days being minimum for 50 mM KNO_3 in H_20 solution and from 1.31 days being maximum for Control to 0.62 days being minimum for 100 mM KNO_3 in 0.3% NaCl solution (Table 4). The analysis of the data indicates that the T50 and MDG were significantly affected by the priming during the germination test. Previous study demonstrated that a increase of germination percentage and MDG in primed barley seeds may be due to initiating metabolic events in primed seeds (Yaldagard *et al*., 2008). Kang *et al.* (2003) reported that the seed primed with KNO_3 showed a fast uptake of K^+ ion to use as substrate for germination as well as a highly metabolic activation of cell. Nitrate-containing salts like KNO_3 were more efficient than nitrate-free salts in promoting low-temperature germination (Nerson & Govers, 1986).

Table 4. Difference in T50 and MGD of kenaf seeds under various KNO_3 concentration after 48 hours.

Solutions	KNO_3 (mM)	GP	T50 (Times)	MDG
H_20	[J]Control	97.5a	18	1.13
	[†]0	85.0b	9	0.62
	50	83.8b	9	0.60
	100	91.3ab	9	0.61
	150	68.8c	17	0.78
	200	61.3c	18	0.73
	250	43.8d	48	0.80
	500	7.5e	-	1.33
0.3% NaCl	[J]Control	90.0a	22	1.31
	[†]0	65.0ab	9	0.65
	50	56.7bc	9	0.64
	100	62.5cd	9	0.62
	150	40.0d	18	0.77
	200	38.8e	50	0.80
	250	31.3e	-	0.83
	500	1.3f	-	1.00

* GP : Germination percentage at 48 hours.
* T50 : Times to reach 50% of the final germination percentage.
* MDG : Mean number of days to germination.
* [J] : Seeds untreated with water and solution of KNO_3 are denoted as the Control.
* [†] : Seeds grown in solution without KNO_3 are denoted as HP (hydro-priming).
* Different letter in each column indicate significant difference ($p<0.05$) as determined by Duncan's multiple range test.

Effect of KNO_3 on the dry weight

Results indicated a effect of priming on dry weight in 3 days after initiating germination (Fig. 5). We did not measure the dry weight of plants treated in more than 150 mM KNO_3 due to death of seedling. Under H20 solution, the dry weight of plant showed a increasing tendency after the priming treatment of HP and KNO_3. However, there was no statistical difference among all treatments. No significant difference of dry weight under abiotic stress (0.3% NaCl) was observed between Control and primed seeds. But, a dry weight following priming was more decreased than that of Control. According to Singh *et al*. (2014), a dry weight of cowpea after KNO_3 treatment was soared sharply at 3 weeks after sowing. It is opposite to our findings. We thought that this cause is brought about by the difference of priming duration (10 hours at cowpea and 24 hours at kenaf) and of investigation period to estimate dry weight (in 3 weeks after sowing in field to cowpea and 3 days after sowing in growth chamber to kenaf). It is possible for cowpea to photosynthesize because of growth for 3 weeks in field, resulting in increasing dry weight. But, kenaf was grown for just 3 days, causing exhaustion of energy sources from cotyledon of kenaf for this period and having little production of organic matter by photosynthesis.

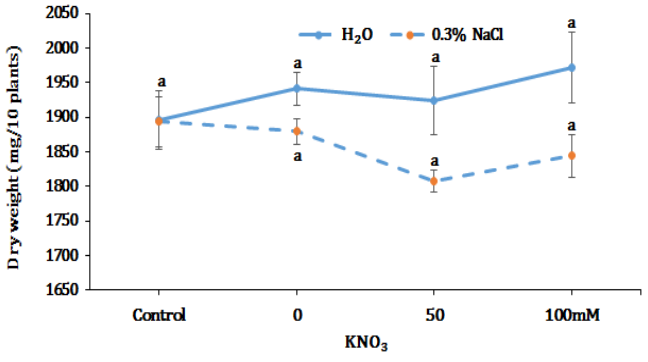

Fig. 5. Dry weight of seedlings at various KNO_3 concentrations at 3 days after germination under H_2O and 0.3% NaCl condition. Control : Seeds untreated with water and solution of KNO_3 are denoted as the Control. 0 mM KNO_3 : Seeds grown in solution without KNO_3 are denoted as HP (hydro-priming). The same letter for each treatment indicates no significant difference as determined by Duncan's multiple range test.

In conclusion, the study performed on kenaf suggested that its seeds can be primed with both hydro-priming (water) and osmopriming (KNO_3) for soared germination. Based on the MDG, osmopriming with 50-100 mM KNO_3 for 24 hours could be greater than non-priming (Control) and hydropriming regardless of abiotic stress. Finally, we will make a point of using this technique for producing a higher economic yield of kenaf.

We primed kenaf seeds for 24 hours depending on the best result of Daniel *et al*., 2004. However, further research is needed to reduce durations of priming treatment for decreasing operation costs.

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