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Pre-Sowing Seed Treatment: Effect of Deep Sea Water Priming on Germination of Wild Vegetables; Gondalbi (*Cirsium setidens*), Jandae (*Adenophora triphylla* var. *Joponica* Hara) and Deoduck (*Codonopsis lanceolata* Trautv.)

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Abstract - This experiment was conducted on wild vegetables; Gondalbi (*Cirsium setidens*), Deoduck (*Codonopsis lanceolata* Trautv.), and Jandae (*Adenophora triphylla* var. *Joponica* Hara) seed to study whether priming with deep sea water results in enhancement of seed germination and identify the optimum concentration of the priming solution, and duration of priming. Seeds were primed with 5 various concentrations (5%, 10%, 15%, 20% and 30%) of deep sea water (DSW) in 12 hours, 24 hours, and 48 hours at 24℃. Since Jandae had seed dormancy, it was kept for four weeks in refrigerator at 2℃ after priming treatment. In Deoduck, 5 percentage DSW priming significantly improved the early germination percentage, radicle length, and plumule emergence percentage. Among the priming period of treatments, 24 hours priming showed better performance in this treatment whereas, in Jandae, 12 hours priming with 10 percentages DSW significantly improved the germination percentage and germination rate. This treatment had increased the final germination percentage by 54%, 15% and 40% compared with control, plain water and KNO_3 priming respectively. But in Gondalbi, priming did not improve the germination of seed. However, among the priming treatments, 12 hours priming with 3% KNO_3 and 20% DSW gave better performance. In both the wild vegetables; Deoduck and Jandae, priming in deep sea water had improved the germination percentage and germination rate as compare to plain water, KNO_3, and without priming treatment. Hence the best seed priming treatment on Deoduck and Jandae are 24 hours with 5% DSW and 12 hours with 10% DSW respectively.

Key words - Deep sea water, Gondalbi, Deoduck, Jandae, Seed priming, Germination percentage and rate

Introduction

Seed priming is a controlled seed hydration treatment (Hudson *et al.*, 2002) that is sufficient to permit pre-germinative metabolic events but insufficient to allow radicle protrusion through the seed coat (Heydecker *et al.*,1975; Hudson *et al.*, 2002). Primed seeds will usually show higher seed vigor compared with raw seed. Priming can provide faster, more uniform seedlings emergence (Hudson *et al.*, 2002; Korkmaz, 2005; Damier *et al.*, 1999; Kang *et al.*, 1996; Ashraf and Foolad, 2005; Soon *et al.* 2000; Giri and schillinger, 2003) and vigor (Shazad *et al.*, 2005; Fly and Heydecker, 1981; Brocklehurst *et al.*, 1987). Hence, rapid seedling establishment might minimize crop risk due to environmental conditions or insect and disease problems during field emergence; which is another advantage of primed seeds especially under adverse condition. Rapid stand establishment may result in a shorter cycle (Passam *et al.*,1989). Priming resulted in improved root proliferation that resulted in improved nitrogen uptake, and enhanced amylase activity that increased starch hydrolysis, which resulted in increased contents of total and reducing sugars (Shazad *et al.*, 2005). It improved the water use efficiency of drought stressed plants (Ajori *et al.*, 2004) and grain yield (Shazad *et al.*, 2005; Ajori *et al.*, 2004).

Seed priming is used in Zimbabwe and India to improve establishment of some summer grain crops like grain sorghum. On-farm seed priming is a 'key' technology – low cost with low risk to produce an immediate benefit unlocking the farming system and giving the farmer reasonable access to further benefits (Harris *et al.*, 2001).

The most frequently used seed priming techniques involve polyethylene glycol (PEG), sodium chloride (NaCl) or potassium nitrate (KNO_3). One method of increasing crop resistance to NaCl levels in soil is salt priming of the seeds before planting (Milligan *et al.*, 2003; Sirvritepe *et al.*, 2004).There is a possibility to increase plant tolerance for these abiotic stress through effective priming of preexisting defense pathways without resorting to genetic alterations (Metraux and Mani, 2005).

Deep sea water from a depth of more than 200m has cold temperature, abundant nutrients, and good water quality that is pathogen-free and stable. Currently, the utilization of deep sea water is receiving

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much attention due to high productivity, large quantity, and potential for recycling energy (Nakasone and Sadamitsu, 1997). Various foods and beverage are being produced using desalinized or concentrated deep sea water (Histake, 1997). Application of deep sea water for agriculture is performed at NELHA in Hawai, U.S.A., where they have succeeded in producing various cold season vegetables and crops in the tropics (Daniel, 1994).

Several kinds of wild edible plants have been already grown as vegetable crops (Kim, 1986). It is probable that edible plants growing wild in fields and mountain have been used as food resources in Korea is from the prehistoric age (Lee, 1978). Wild edible plants are great value as food resources (Kim, 1986) and were frequently used as foods for the relief of the sufferers from famine at that time of war, disaster, the spring food shortage or crop failures (Lee, 1978). In addition, quite a few of those wild plants are regarded as delicacies of the season because of peculiar flavor. Wild vegetables have promising value as food resources in respect that their edible portions are variable. Fresh and tender young leaves or sprouts can be continuously gathered throughout considerable picking season. Recently the utilization of wild vegetables is very popular in respect that agriculture chemical was not used for them. Domestication of wild edible plants as vegetable crops seems to be an urgent need for establishment of food resources in Korea. A hindrance in domestication of wild vegetables is low germination rate. Accordingly the study on the germination physiology of wild vegetables would be the greatest problem to be solved for cultivation of wild vegetables (Kim, 1986). Germinating and growing plants from seed is an inexpensive way of obtaining local native species for use in revegetation (QNRM, 2001).

The objective of this experiment was to study whether priming with deep sea water results in enhancement of seed germination and identify the suitable concentration and period of priming. The optimization of seed priming techniques becomes very important to seed priming.

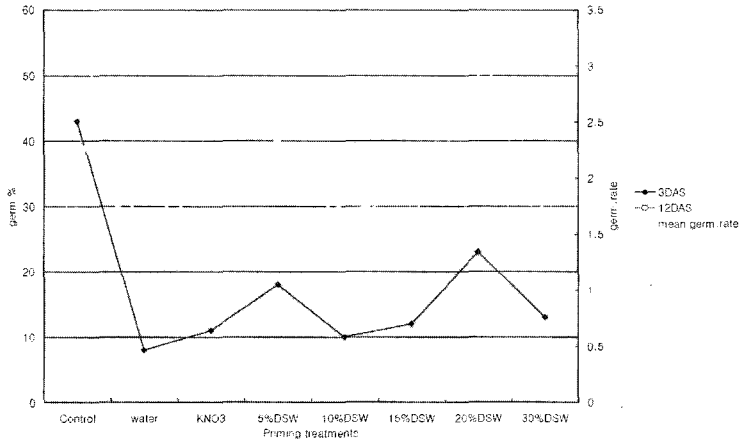

Fig. 1. Effect of Deep Sea Water priming on germination (%) and mean germination rate of Gondalbi seed. Even though, among the priming treatments, 20% DSW improves the germination percentage and rate, priming is not required.

Materials and Methods

The experiment was conducted in Plant molecular and physiology lab of Kangwon National University in 2006. Gondalbi seeds (*Cirsium setidens*), Deoduck (*Codonopsis lanceolata* Trautv.) and Jandae (*Adenophora triphylla* var. *Joponica* Hara) was used in the experiment. Following pre-sowing seed treatments were included.

Factor A	**Factor B**
12 hours priming	Control (without priming)
24 hours priming	Control (priming with distilled water)
48 hours priming	3% KNO_3
	5% Deep sea water
	10% Deep sea water
	15% Deep sea water
	20% Deep sea water
	30% Deep sea water

30 seeds were used in each treatment. In addition to plain water and 3% KNO_3, required concentrations of the Deep Sea Water (5%, 10%, 15%, 20% and 30%) were prepared and seeds were fully immersed in priming media at a temperature of 25℃ for duration of 12, 24 and 48 hours after the first batch, respectively, so that all the seed was removed from media at the same time. After the priming, seeds were rinsed with distilled water. But for seeds of Jandae, for breaking the dormancy of seed, after seeds were kept in refrigerator at 2℃ for 4 weeks after treatment. Ten seeds per petridish were sown and the experiment was replicated thrice. This experiment was laid out in factorial Completely Randomized Design (CRD). Germination of seeds was evaluated on 55 millimeter diameter Whatman no. 2 moist filter paper in petridishes. The petridishes were covered with lips and placed in an germinator at 25℃. The data of final germination percentage (%), mean germination time (MGT) (days) and radicle length (cm) were recorded until no further germination occurred.

The data collected was analyzed using the Fisher's analysis of variance technique under completely randomized design (CRD) with MSTAT software program and the treatment means were compared by Least Significant Difference (LSD) test at 0.05 probability level (Steel and Torri, 1984).

Results and Discussion

Gondalbi

Germination of seed was recorded in three days interval; three, six, nine and twelve days after sowing. Even though there was not interaction between period of priming and concentration of priming, effect of them were significant. But, no any treatment was superior to control. Hence, priming is not required to enhance the germination of this seed. The trend of declining its germination with increasing the period of priming showed that even the 12 hours soaking is long period for this. Hence, further observation is required under less than 12 hours priming. However, within the priming treatments, 12 hours priming with 20% DSW improved the germination followed by 3% KNO_3 (Table 1). Hence, as compare to water priming, deep sea water is preferred. Even though, among the priming treatments, 20% DSW improves the germination percentage and rate, priming is not required for Gondalbi seeds (Fig. 1).

Table 1. Effect of priming period and concentration on germination of Gondalbi seed

Treatments	Germination (%)				Mean germ rate
	3DAS	6DAS	9DAS	12DAS	
48hours					
Control	50 a	63 a	67 a	67 a	2.8 ab
Water	13 b	23 c	30 b	30 c	1.6 b
KNO_3	10 b	57 ab	63 a	63 a	3.3 a
5%DSW	27 b	37 abc	40 b	47 b	2.5 ab
10%DSW	20 b	30 bc	40 b	40 bc	2.0 ab
15%DSW	20 b	37 abc	37 b	37 bc	1.6 b
20%DSW	30 ab	50 abc	63 a	63 a	3.1 a
30%DSW	20 b	30 bc	37 b	37 bc	1.8 b
Mean	**24 a**	**41 a**	**47 a**	**48 a**	**2.3 a**
24hours					
Control	47 a	50 a	57 a	57 a	2.6 ab
Water	10 b	20 b	27 c	27 c	1.4 bc
KNO_3	13 b	37 ab	47 a	47 a	2.5 abc
5%DSW	17 b	37 ab	43 ab	43 ab	2.2 abc
10%DSW	7 b	17 b	17 c	20 c	1.3 c
15%DSW	10 b	30 ab	30 bc	30 bc	1.4 bc
20%DSW	23 b	37 ab	53 a	53 a	3.1 a
30%DSW	10 b	23 ab	27 c	30 bc	1.5 bc
Mean	**17 ab**	**31 ab**	**36 b**	**38 b**	**2.0 ab**
12hrs					
Control	30 a	47 a	47 a	47 a	1.9 ab
Water	0 b	1 c	13 c	17 c	0.8 bc
KNO_3	10 b	13 bc	13 c	17 c	0.6 c
5%DSW	10 b	20 abc	27 bc	30 bc	1.8 abc
10%DSW	3 b	23 abc	27 bc	27 bc	1.4 abc
15%DSW	7 b	23 abc	30 b	30 bc	1.8 abc
20%DSW	17 ab	40 ab	40 ab	47 a	2.4 a
30%DSW	10 b	23 abc	33 ab	33 ab	1.7 abc
Mean	**11 b**	**25 b**	**29 c**	**31 c**	**1.5 b**
CV%	76	52	41	40	40.8
A	**(.774)	**(.977)	**(.566)	**(.485)	** (.463)
B	**(2.19)	**(2.73)	**(1.60)	**(1.37)	** (1.309)
AxB	Ns	Ns	Ns	Ns	Ns

A: Duration of priming B: Different concentration DFP: Days from priming

Deoduck

Effect of duration of priming was significant on plumule emergence but not on germination of seed. However, among the three duration of priming, 12 hours priming had enhanced the germination percentage followed by 24 hours and 48 hours priming. 24 hours priming improved the plumule emergence percentage significantly. There was also significant interaction effect between period of priming and priming treatments on germination percentage (5DAS and 9DAS) and plumule emergence percentage (Table 2).

The effect of priming treatment was highly significant on germination percentage, radicle length and germination rate. The highest percentage of germination, radicle length and plumule emergence percentage was noticed in 5% DSW treatment followed by 10% DSW (Fig. 2). The 5% DSW gave significantly highest percentage of germination, radicle length (24mM) and plumule percentage (57%) in 24 hours priming. 53% of germination was counted in early days

Table 2. Effect of duration and concentration of DSW priming on Deoduck seed

Treatments	Germination (%)				Radicle length (cm)	Plumule emerg(%)	
	5DAS	7DAS	9DAS	11DAS	10 DAS	23 DAS	Mean germ rate
12hours							
Control	20 bc	47 ab	63 ab	67 ab	10 c	47 b	4.9 a
Water	30 ab	57 ab	63 ab	70 a	11 bc	53 a	5.1 a
KNO_3	10 c	37 b	47 b	47 b	13 bc	30 b	3.9 a
5%DSW	27 abc	53 ab	57 ab	63 ab	17 abc	40 ab	4.1 a
10%DSW	37 ab	67 a	77 a	80 a	22 a	50 ab	5.5 a
15%DSW	27 abc	50 ab	53 ab	67 ab	12 bc	30 b	4.7 a
20%DSW	40 a	57 ab	60 ab	63 ab	24 a	50 ab	4.2 a
30%DSW	30 ab	63 a	77 a	77 a	19 ab	33 ab	4.7 a
Mean	**28**	**54**	**62**	**67**	**16**	**42**	**4.6**
24hours							
Control	30 bc	47 bcd	63 ab	67 ab	14 c	40 abc	4.3 bc
Water	33 bc	60 abc	67 ab	77 ab	15 bc	43 abc	5.3 ab
KNO_3	7 d	23 d	30 c	43 c	10 c	7 d	3.4 c
5%DSW	53 a	77 a	83 a	87 a	24 a	57 a	5.2 ab
10%DSW	30 bc	57 abc	60 ab	67 ab	23 ab	30 bc	4.2 bc
15%DSW	23 cd	47 bcd	60 ab	63 bc	15 bc	23 cd	4.2 bc
20%DSW	20 cd	40 cd	53 bc	57 bc	16 abc	23 cd	3.7 bc
30%DSW	43 ab	67 ab	73 ab	77 ab	15 bc	50 ab	6.0 a
Mean	**30**	**52**	**61**	**67**	**16**	**34**	**4.5**
48 hrs							
Control	30 bc	60 abc	67 ab	70 ab	18 ab	47 ab	4.4 a
Water	27 bc	37 cd	50 b	57 b	11 b	30 bc	3.8 a
KNO_3	13 c	17 d	17 c	20 c	0 c	0 d	1.2 b
5%DSW	50 a	63 ab	80 a	83 a	22 a	53 a	5.1 a
10%DSW	40 ab	60 abc	73 ab	73 ab	18 ab	47 ab	4.3 a
15%DSW	43 ab	67 a	70 ab	77 ab	17 ab	43 ab	4.6 a
20%DSW	33 ab	63 ab	77 a	80 a	17 ab	27 bc	5.2 a
30%DSW	13 c	40 bcd	57 ab	63 ab	15 ab	17 cd	4.9 a
Mean	**31**	**51**	**61**	**65**	**15**	**32**	**4.2**
CV%	40.3	29.5	23.3	20.	33	33	22.7
A	Ns	Ns	Ns	Ns	Ns	**	Ns
B	**(1.964)	** (2.53)	**(2.36)	** (2.18)	** (8.07)	** (2.01)	**(1.65)
AxB	*	Ns	*	Ns	Ns	**	Ns

A: duration of priming B: different concentration DFP: Days from priming

(5DAS) where as it was only 7%, 33% and 30% in KNO_3, plain water priming and without priming respectively. As far as the final germina tion percentage is concerned, highest germination (87%) was obtained in this treatment whereas it was 43%, 77% and 67% in KNO_3, plain water priming and without priming respectively. Similarly, plumule emergence percentage was also highest (57%) in this treatment. Beside this, in 48 hours and 12 hours priming too, this treatment gave superior result in these parameter (Table 2). If priming is done for 12 hours, 10% DSW is preferred. Hence, as priming period with DSW was increased from 12 hours to 24 hours, concentration of DSW should be decreased from 10% to 5% to get the superior result. This positive result of deep sea water supports the finding of Demir, (1999) who had found salt priming as a useful tool for improving germination, seedling growth and uniformity of water melon seeds in early spring sowing. But it modified the findings of Korkmaz, (2005) who had found better result of pepper seeds primed in KNO_3 solution. This experiment showed the better response of DSW as compare to KNO_3.

Table 3. Effect of priming period and concentration on germination of Jandae

Treatments	Germination (%)				Mean germ rate
	5DAS	7DAS	9DAS	11DAS	
12hours					
Control	10 c	23 b	33 b	33 b	2.2 c
Water	47 b	73 a	73 a	73 a	3.8 ab
KNO_3	13 c	40 b	47 b	47 b	2.8 bc
5%DSW	47 b	70 a	70 a	70 a	3.5 ab
10%DSW	70 a	87 a	87 a	87 a	4.2 a
15%DSW	23 c	37 b	47 b	47 b	2.7 bc
20%DSW	50 ab	73 a	73 a	73 a	3.7 ab
30%DSW	57 ab	70 a	70 a	70 a	3.4 abc
Mean	**40**	**59**	**62 a**	**62 a**	**3.3 a**
24hours					
Control	13 c	23 c	30 d	33 d	1.9 c
Water	40 b	53 b	57 abc	57 abc	3..0 abc
KNO_3	20 bc	27 c	37 cd	37 cd	2.2 c
5%DSW	33 bc	43 bc	47 bcd	47 bcd	2.4 bc
10%DSW	67 a	77 a	77 a	77 a	3.6 a
15%DSW	40 b	50 b	53 bc	57 abc	2.7 abc
20%DSW	20 bc	23 c	37 cd	37 cd	2.2 c
30%DSW	37 b	53 b	63 ab	67 ab	3.6 bc
Mean	**34**	**44 b**	**50 b**	**51 b**	**2.7 b**
24hrs					
Control	13 c	23 d	30 d	30 e	1.9 cd
Water	47 ab	53 bc	67 ab	67 ab	3.6 ab
KNO_3	23 c	27 d	30 d	30 e	1.7 d
5%DSW	50 a	60 ab	60 bc	63 bc	2.9 bcd
10%DSW	27 bc	40 bcd	43 cd	43 cde	2.3 cd
15%DSW	67 a	80 a	87 a	87 a	4.4 a
20%DSW	50 a	53 bc	60 bc	60 bcd	3.0 bc
30%DSW	23 c	33 cd	37 d	40 de	2.0 cd
Mean	**37**	**46 b**	**52 b**	**52 b**	**2.7 b**
CV%	37.8	27	23.7	23.8	26.2
A	Ns	**(0.78)	** (0.75)	**(.764)	*(.442)
B	** (2.297)	** (2.21)	**(2.13)	**(2.172)	**(1.249)
AxB	**	**	**	**	*

A: Duration of priming B: Different concentration DFP: Days from priming

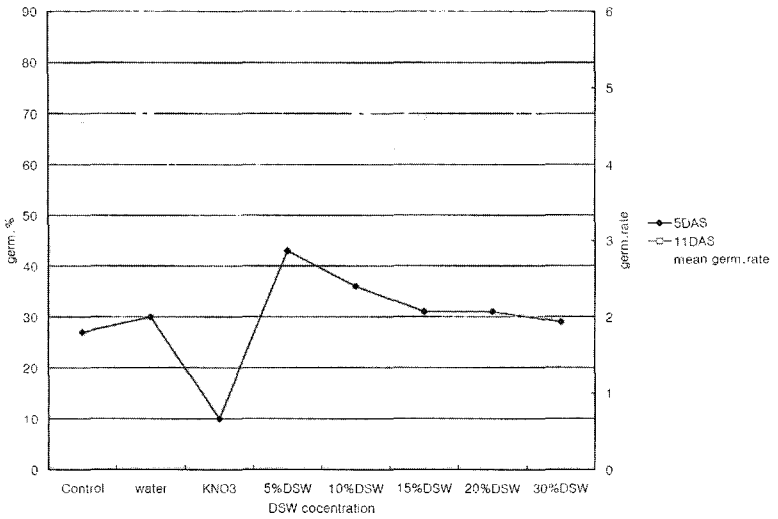

Fig. 2. Effect of Deep sea water priming on Deoduck germination (%) and germination rate. Deep sea water priming improves the germination percentage and rate.

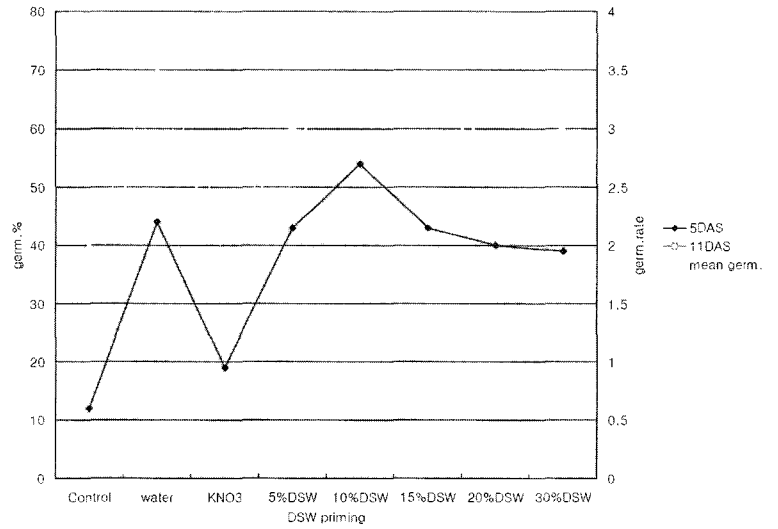

Fig. 3. Effect of Deep sea water priming on germination (%) and germination rate of Jandae seed. Deep sea water priming improves the germination percentage and rate.

Jandae

Effect of duration of priming was highly significant on early to final germination percentage except on 5DAS. Among the three duration of priming, 12 hours of priming had significantly enhanced the germination percentage and germination rate. Highest germination percentage; 48%, 59%, 62% and 62% was recorded on 5DAS, 7DAS, 9DAS and 11DAS, respectively whereas it was only 34%, 44%, 50% and 51% in 24 hours priming, and 37%, 46%, 52%, and 52% in 48 hours priming on above mentioned days respectively. Similarly, the significantly highest germination rate (3.3) was obtained in 12 hours priming as compare to 24 hours (2.7) and 48 hours (2.7) (Table 3).

The effect of priming treatment was highly significant on germination percentage and germination rate. The highest percentage of germination on all the recorded days was noticed in 15% DSW treatment followed by 3% KNO_3. Hence, deep sea water priming improved the germination percentage and rate (Fig. 3).

There was also significant interaction effect on germination percentage during all these recorded days and on germination rate. Among the treatments in 12 hours priming, significantly highest percentage of germination and germination rate were obtained in 10% DSW treatment. It was 70% germination on early days (5DAS) whereas control, plain water and KNO_3 treatment had only 10%, 47% and 13%, respectively. Similarly, significantly highest germination rate (4.2) was recorded in this treatment (Table 3). As far as the final germination percentage is concerned, this 10% DSW treatment also gave the highest germination (87%) whereas KNO_3, plain water and without priming had 47%, 73% and 33% respectively. This result supports the finding of Fly and Heydecker (1981) who had found that priming improved the germination of Parsley seed.

Acknowledgements

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