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The Effect of Deep Sea Water on Seed Priming of Sweet Pepper (*Capsicum annum* L.), Rice (*Oryza sativa* L.) and Ginseng (*Panax ginseng* C.A.Meyer)

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Abstract - This experiment was conducted to study whether priming with deep sea water results in enhancement of seed germination and to identify the optimum concentration of the priming solution, and duration of priming using sweet pepper (Cv. California wonder), rice (Cv. Ilpum) and ginseng seed. Sweet pepper and rice seeds were primed with 5 various concentrations (5%, 10%, 15%, 20% and 30%) for deep sea water for 48 hours, 24 hours and 12 hours at 25℃ and ginseng seeds in 5%, 10%, 15%, 20%, 25% and 30%, and 2,4,6, and 8 electrical conductivity (EC) which were made by desalinating deep sea water. Priming in deep sea water (DSW) improved the early and final germination percentage, mean germination rate, emergence percentage and root and shoot length, compared with plain water, KNO_3 and without priming treatments. In sweet pepper, 24 hours priming with 5 percentage DSW significantly improved the early germination percentage and radical length. It has also improved the mean germination and emergence days and early emergence percentage, compared with KNO_3 and control. Whereas, in rice, 48 hours priming with 10 percent DSW significantly improved the early germination percentage, plumule emergence percentage, root length and shoot height. Hence the best seed priming treatment on sweet pepper and Rice are 24 hours with 5 percentage DSW and 48 hours with 10 percentage DSW, respectively, whereas in ginseng, priming with EC4, EC8 and 25% DSW had shown better germination.

Key words - Germination, Root, Emergence, Radicle, Imbibition

Introduction

Seed priming is a pre-sowing treatment that involves exposure of seeds to low external water potential that limits hydration (Heydecker *et al.*, 1975) that can reduce the time it takes for seedlings to emerge (Giri and Schillinger, 2003; Fly & Heydecker, 1981). It used basic principles of water potential to hold seeds in an imbibed condition, but prevent germination (radical emergence). The three early phases of germination are: (1) imbibition, (2) lag phase, and (3) protrusion of radicle through the testa (Simon, 1984). Primed seeds will usually show higher seed vigor compared with raw seed (Shazad *et al.*, 2003). Priming can provide faster, more uniform seedlings emergence (Hudson *et al.*, 2002; Soon *et al.*, 2000; Demir, 1999; Korkmaz, 2005; Kang *et al.*, 1996; Brocklehurst *et al.*, 1987). Rapid seedling establishment might minimize crop risk due to environmental conditions or insect and disease problems during field emergence, which is an advantage of primed seeds especially under adverse condition (Passam *et al.*, 1989). Priming 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 (Ashraf and Foolad, 2005).

One method of increasing crop resistance to NaCl levels in soil is salt priming of the seeds before planting (Milligan *et al.*, 2003).The use of NaCl pretreatments could be a useful strategy to increase the salt tolerance of melon plants in long term and also to permit the establishment of melon crop by direct sowing in a saline medium (Sivritepe *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). In this way, on-farm seed priming is a 'key' technology that is a 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) and potassium nitrate (KNO_3).

Deep sea water from a depth of more than 200 m has cold temperature, abundant mineral nutrients, and good water quality that is pathogen-free and stable. Basic research on the utilization of this water for fisheries in Japan began in 1976. Currently, the utilization of deep sea water (DSW) is receiving much attention due to high productivity, large quantity, and potential for recycling energy. Future investigations should focus on future explorations of deep sea water

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attributes a cascade system for using deep sea water, reduction of cost, and potential environmental impacts (Nakasone and Sadamitsu, 1997). Various foods and beverages are being produced using desalinized or concentrated deep sea water (Hisatake, 1997). The application of deep sea water for agriculture are 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).

For more than half of humanity, rice is the staple diet and is one of the most important cereals in the world. In Korea it occupies 980,000 hectare with the average yield of 4900 kg per hectare. Rice transplanting requires a large amount of labors, usually at a critical time which often results in shortage and increasing labor costs. Alternate methods of establishing crops, especially rice that require less labor and water without sacrificing productivity are needed. Considering water availability and opportunity cost of labor, dry seeding of rice is an appropriate alternative for transplanting method. Sub-optimum plant population and uneven crop stand resulting from poor nursery seedlings is one of the most important yield limiting factors in the rice production system. Similarly, in Sweet pepper which is a fast emerging vegetable and cash crop in Korea has also the problem of low germination and slow growth. Beside this, seeds are quite expensive as compare to other vegetable crops.

Ginseng is an herbaceous perennial that is known widely for the medicinal properties of its root. It has played an integral part of Chinese medicine for centuries (Chang, 1995). Fruit known as berries, set soon after flowering begins. Each berry contains at least 2 seeds, although variation does occur. Seed is also an economically important portion of the plant (Proctor and Bailey, 1987). The seed is the most widely used means of propagation and should, therefore, be the main focus for crop improvement. A long after-ripening period, (18-22 months) is required for the germination of seed. The seeds are traditionally stratified in the imbibed state, at temperatures which give the seed alternating periods of warm and cool. Two cool periods (winters) are required to break dormancy (Stoltz and Snyder, 1985). Hence germination of seed is a main problem in ginseng.

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

Materials and Methods

The experiment was conducted in plant molecular and physiology lab of Kangwon National University in 2006. Rice seeds of cv. Ilpum, sweet pepper of cv. California wonder and Ginseng were used in the experiment. Following pre-sowing seed treatments were included in rice and sweet pepper experiment.

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

A counted number of seeds (50 in rice and 30 in sweet pepper) were used in each treatment. Required concentrations of the Deep sea water were prepared and seeds were primed for above mentioned duration and concentration at 25℃. After the priming, seeds were rinsed with distilled water. Experiment was laid out in factorial Completely Randomized Design (CRD). The treated seeds were compared with control ones for germination, shoot and root growth, mean germination and emergence days.

Vigor evaluation

Sweet pepper

Germination test: Germination of seeds was evaluated on moist filter paper in petridishes. Ten seeds per petridish were sown and the experiment was replicated thrice. The petridishes were covered with lips and placed in an germinator at 16℃. The data on germination percentage (%), mean germination days (MGD), and radicle length (cm) were recorded up to 15 days after sowing.

Emergence test: The experiment was carried out in plastic plug trays filled with readymade horticultural soil (Best Company). Six seeds per treatment at the rate of one seed per plug were sown at the depth of one cm and the replication was replicated five times. The trays were placed in green house where temperature was ranged generally 20℃ to 25℃. The data regarding the final emergence percentage (%), and mean emergence days (MED) were recorded up to the 10 days of sowing.

Rice

Germination test: Germination of seed was evaluated on test tubes filled with distilled water and covered with nylon mesh over the mouth Five seeds per test tubes were sown and the experiment was replicated ten times. These test tubes were kept in a germinator. The data of germination percentage (%), mean germination days (MGD), shoot length (cm), and root length (cm) were recorded up to 29 days after sowing.

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

Ginseng

Ginseng seeds were primed in deep sea water with 5%, 10%, 15%, 20%, 25% and 30% concentration. In other set of experiment, concentrations were made 2, 4, 6, and 8 electrical conductivities (EC) from deep sea water, and seeds were primed and germination percentages were recorded.

Results and Discussion

Sweet pepper

Effect on germination and radicle length

Effect of duration of priming was highly significant on early to final germination percentage, radicle length, and mean germination days. Among the three duration of priming, 24 hours priming had significantly enhanced the germination percentage, radicle length and mean germination days. There was also significant interaction effect on germination percentage (6DFP and 8DFP) and radicle length. Highest germination; 32% and 84.6% was recorded on 6 DFP and 8 DFP respectively where as it was only 8.3% and 52.5% in 48 hours priming, and 9.2% and 77.1% in 12 hours priming respectively. Similarly, the highest mean radicle length (0.96 cm) was measured in 24 hours priming (Table 1). Among the treatments in 24 hours priming, significantly highest percentage of germination and radicle length was recorded on 5% DSW treatment. It was 53% germination on 6 DFP where as control, water and KNO_3 treatment had only 6%, 30% and 40% respectively. Similarly, significantly highest radicle length (1.09 cm) was measured in 5% DSW treatment where as it was only 0.62, 0.87 and 0.87 cm in control, water and KNO_3 treatment respectively (Table 1). This result supports the finding of Demir (1999), who had found salt priming as a useful 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. Over all, the germination rate for the primed seeds was much higher than the nonprime seeds. Therefore, mean germination days are significantly reduced in primed seeds.

Among the treatments in 24 hours priming, 5% DSW and plain water priming gave 100% final germination. No any difference was obtained between 10% DSW, 15% DSW, and 20% DSW treatments on the germination percentage. However, all the deep sea water priming had improved the germination of seed compared to without priming (Fig 1).

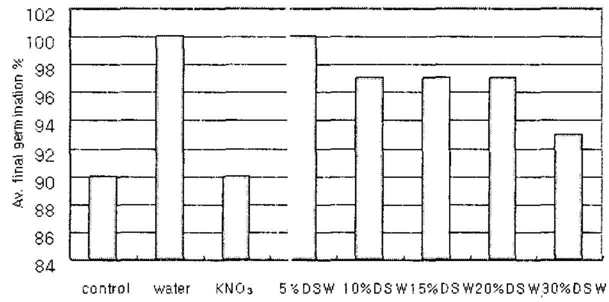

Fig 1. Effect on average final germination percentage of sweet pepper. Priming improves the final germination percentage.

Effect on emergence

Even though there was no any significant effect, the treatment 5% DSW treatment with 24 hours priming had given 100% emergence (Table 1). It had taken 5.1 mean germination days which is at par with KNO_3 but had decreased the mean germination days as compare to control (5.3days) and plain water (5.4 days) treatment (Fig 2).

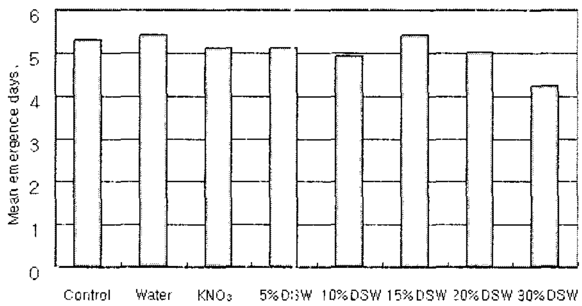

Fig 2. Effect on mean emergence days of sweet pepper. Generally priming with DSW reduces the mean germination days.

Rice

Effect on germination and emergence

Effect of duration of priming was highly significant on early to final germination percentage, plumule emergence percentage, root length, and shoot height. Among the three duration of priming, 48

Table 1. Effect of priming period and concentration on germination and emergence of sweet pepper(Cv. California wonder)

Treatments	Germination (%)			Radicle (cm)		Mean germ days	Emergence		
	6DFP	8DFP	10DFP	13DFP	15DFP		(%) 6DAP	(%) 9DAP	rate
48hours									
Control	3 bc	20 e	80 d	0.54 e	0.82 c	9.4 a	83	100	5.2
Water	3 bc	67 bc	87 bcd	0.57 de	0.76 c	8.2 b	88	100	4.7
KNO_3	3 bc	57 cd	90 abc	0.74 ab	0.93 ab	8.2 b	83	100	5.0
5%DSW	3 bc	50 d	90 abc	0.65 bcd	0.82 c	7.7 bc	95	100	5.0
10%DSW	30 a	80 a	97 a	0.79 a	0.98 a	7.4 c	78	95	4.7
15%DSW	13 b	30 e	83 cd	0.58 cde	0.79 c	7.8 bc	62	100	5.8
20%DSW	0 c	70 ab	87 bcd	0.67 bc	0.97 a	8.0 bc	72	95	4.6
30%DSW	10 bc	47 d	93 ab	0.61 cde	0.86 bc	7.8 bc	55	100	4.9
Mean	**8.3 b**	**52.5 c**	**88.3 b**	**0.65 b**	**0.87 b**	**8.0 a**	**77**	**99**	**5.1**
24hours									
Control	6 e	40 c	87 b	0.62 e	0.89 d	7.7 a	72	95	5.3
Water	30 cd	93 a	100 a	0.87 d	1.10 c	7.0 b	67	100	5.4
KNO_3	40 bc	80 b	87 b	0.87 d	1.10 c	6.8 bc	62	100	5.1
5%DSW	53 a	93 a	97 a	1.09 ab	1.26 a	6.8 bc	83	100	5.1
10%DSW	47 ab	93 a	97 a	0.93 cd	1.12 bc	6.9 bc	88	100	4.9
15%DSW	23 d	87 ab	97 a	1.01 bc	1.15 bc	6.9 bc	72	100	5.4
20%DSW	27 d	93 a	97 a	1.15 a	1.21 ab	6.7 bc	78	100	5.0
30%DSW	30 cd	93 a	93 ab	1.12 a	1.17 abc	6.4 c	83	100	4.2
Mean	**32.1 a**	**84.6 a**	**94.2 a**	**0.96**	**1.12 a**	**6.9 b**	**75**	**99**	**5.1**
12hours									
Control	3 bc	60 b	93 ab	0.56 c	0.85 bc	8.6 a	67	95	5.4
Water	10 abc	83 d	100 a	0.80 b	0.94 ab	7.5 cd	83	100	5.0
KNO_3	20 a	97 a	100 a	0.93 a	1.00 a	7.3 d	55	95	5.2
5%DSW	13 ab	63 d	97 ab	0.61 de	0.74 d	8.3 ab	83	100	5.2
10%DSW	0 c	80 bc	90 b	0.67 cd	0.82 cd	7.3 d	62	100	5.7
15%DSW	0 c	80 bc	97 ab	0.68 cd	0.90 abc	7.9 bc	78	100	5.4
20%DSW	10 abc	70 cd	100 a	0.67 cd	0.81 cd	7.9 bc	83	100	5.1
30%DSW	17 a	83 b	100 a	0.76 bc	0.99 a	7.5 cd	95	100	5.1
Mean	**9.2 b**	**77.1 b**	**97.1 a**	**0.71 b**	**0.88 b**	**7.8 a**	**75**	**99**	**5.3**
CV%	66	18	9.3	15.3	12.7	7.6	26	4.4	13.5
A	** (0.64)	**(0.75)	**(0.50)	** (0.069)	**	** (0.55)	Ns	Ns	Ns
B	** (1.04)	** (1.06)	Ns	** (0.097)	Ns	** (.0.37)	Ns	Ns	Ns
A×B	**	**	Ns	*	*	Ns	Ns	Ns	Ns

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

Table 2. Effect of duration and concentration of seed priming on rice seed(cv. Ilpum) germination

Treatments	Germination (%)			Plumule emerg (%)		Root (cm)/sedlg		Shoot (cm)/sedlg	
	12DFP	14DFP	16DFP	14DFP	16DFP	23 DFP	29 DFP	23 DFP	29 DFP
48hours									
Control	54 d	74 b	92	22 c	80	0.8	7.7	0.2c	32
Water	64 bcd	76 ab	86	26 c	70	1.1	4.9	0.3 c	23
KNO_3	60 cd	78 ab	94	22 c	78	1.1	5.3	0.3 c	33
5%DSW	72 ab	80 ab	98	46 ab	82	2.1	6.2	0.5 b	27
10%DSW	82 a	82 ab	96	56 a	90	3.0	4.5	0.7 a	20
15%DSW	66 bc	78 ab	92	34 bc	76	1.8	6.8	0.6 ab	27
20%DSW	82 a	84 a	96	54 a	84	3.6	7.2	0.7 a	31
30%DSW	72 ab	74 b	96	48 ab	80	2.0	5.9	0.5 b	29
Mean	**70 a**	**78 a**	**94 a**	**38 a**	**80 a**	**1.9 a**	**6.1 b**	**0.5 a**	**28**
24hours									
Control	36 b	46 c	90	2 b	78	0.5	7.1	0.2	30
Water	52 ab	80 a	94	14 ab	78	1.0	6.8	0.3	29
KNO_3	46 ab	62 bc	92	4 ab	74	0.5	6.3	0.3	32
5%DSW	52 ab	70 ab	94	6 ab	72	0.6	6.2	0.3	28
10%DSW	62 a	72 ab	92	18 a	76	0.8	5.6	0.3	26
15%DSW	52 ab	64 ab	90	10 ab	66	0.8	7.0	0.3	37
20%DSW	58 a	70 ab	86	4 ab	72	0.4	6.4	0.3	30
30%DSW	50 ab	74 ab	94	4 ab	76	0.6	6.2	0.3	23
Mean	**52 b**	**68 b**	**92 a**	**8 b**	**74 b**	**0.7 c**	**6.4 b**	**0.3 c**	**29**
12 hours									
Control	34 b	54 ab	80	0	58	1.8	9.1	0.3	30
Water	44 ab	58 ab	86	0	74	1.7	9.0	0.3	27
KNO_3	54 a	68 a	86	4	74	1.8	11.5	0.4	35
5%DSW	42 ab	58 ab	82	0	60	1.3	9.1	0.4	29
10%DSW	38 ab	54 ab	82	0	62	0.9	10.8	0.4	36
15%DSW	36 ab	50 b	82	4	66	0.7	8.9	0.3	22
20%DSW	36 ab	58 ab	90	0	72	0.9	10.9	0.3	27
30%DSW	38 ab	64 ab	90	2	74	0.9	8.2	0.4	20
Mean	**40 c**	**58 c**	**84 b**	**2 c**	**68 c**	**1.3 b**	**9.7 a**	**0.4 b**	**28**
CV%	38	28	15	111	25	63	50	42	49
A	** (9.1)	** (3.0)	** (2.1)	** (0.27)	** (2.97)	** (0.25)	** (1.15)	** (0.049)	Ns
B	* (0.53)	* (0.85)	Ns	** (0.77)	Ns	* (11.6)	Ns	** (1.39)	Ns
AxB	Ns	Ns	Ns	**	Ns	**	Ns	**	Ns

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

hours priming had significantly enhanced the germination percentage, plumule emergence, root and shoot length. Highest germination; 70%, 78% and 94% was recorded on 12 DFP, 14DFP and 16 DFP, respectively, where as it was only 52%, 68% and 92% in 24 hours priming, and 9.2% and 40%, 58% and 84% in 12 hours priming on 12DFP, 14 DFP and 16 DFP, respectively (Table 2). Similarly, the significantly highest plumule emergence percentages (38 and 80) were observed on 14 DFP and 16 DFP respectively, as compare to 24 hours and 12 hours priming (Table 2). Among the treatments in 48 hours priming, significantly highest percentage of germination and plumule emergence were obtained in 10% DSW treatment. It was 82% germination on 12 DFP where as control, plain water, and KNO_3 treatment had only 54%, 64% and 60%, respectively. Similarly, significantly highest plumule emergence percentage (56% and 90%) was recorded on 14 DFP and 16DFP, respectively (Table 2).

Effect on root and shoot growth

Effect of duration of priming was highly significant on root length and shoot height. Among the three duration of priming, 48 hours priming had significantly improved the root length (23 DFP) and shoot height (23 DFP). There was also significant interaction effect on root growth and shoot growth on early days (23 DFP). Highest root length (1.9 cm) was measured on 23 DFP in 48 hours priming where as it was only 0.7 cm and 1.3 cm in 24 hours and 12 hours priming, respectively. Similarly, the highest mean shoot height (0.5 cm) was measured in 48 hours priming (Table 2). Among the treatments in 48 hours priming, 10% DSW and 20% DSW produced significantly higher root length; 3.0 and 3.6 cm respectively on early days (23DFP) as compare to the rest of the treatments. Similarly average shoot height; 7.0cm was also higher on these days as compare to control, plain water and KNO_3 treatments. This result supports Shazad *et al.*, (2003) findings', they had found root proliferation in salt primed wheat seeds.

Ginseng

Among the five treatments of electric conductivities, highest final germination percentage (65%) was recorded in EC4 followed by EC8 (60%) priming. But the germination percentage, which was recorded three days before of the final observation was highest in EC8 (55%). It showed that, electrical conductivity 4 and 8 prepared by DSW enhanced the germination of ginseng seeds as compare to control and the rest of the treatments (Fig 3).

In the second experiment of ginseng seed, among the six levels of DSW priming, highest germination percentage was obtained by 25% DSW priming on both the observation period. It was 75% and 85% in first and second observation, respectively. Result showed that, DSW priming had enhanced the germination percentage of ginseng seeds as compare to without DSW priming (Fig 4).

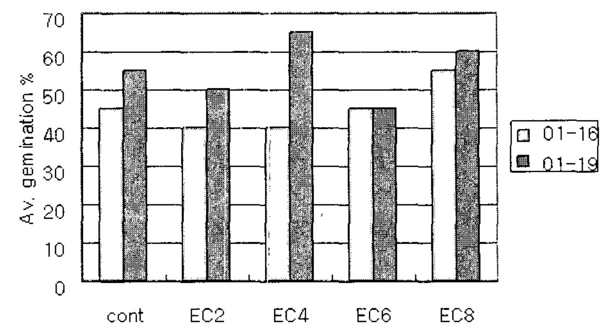

Fig 3. Effect on average germination percentage of ginseng.

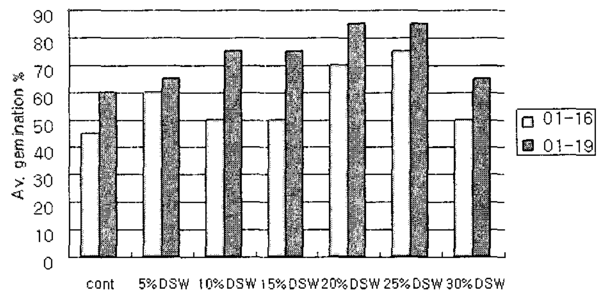

Fig 4. Effect on average germination percentage of ginseng. Over all positive effect of deep sea water priming was found on all the treatment except 30% DSW.

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