International Journal of Plant & Soil Science

33(21): 1-8, 2021; Article no.IJPSS.74817
ISSN: 2320-7035

Impact of Various Sources of Seed Priming on Growth, Yield and Quality of Spinach Local (*Spinacia oleracea*) *in-vitro* and *in-vivo* Condition

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2021/v33i2130651
Editor(s):
(1) Dr. Hon H. Ho, State University of New York, USA.
Reviewers:
(1) Itagi Ravi Kumar, SVYASA Deemed-to-be University, India.
(2) Rajendra Prasad Nagar, Indian Grassland & Fodder Research Institute, India.
(3) Kamlesh Kumar, ICAR-Central Institute for Arid Horticulture, India.
Complete Peer review History: https://www.sdiarticle4.com/review-history/74817

Original Research Article

Received 17 July 2021
Accepted 27 September 2021
Published 11 October 2021

ABSTRACT

Seed germination, following seedling growth are basic but crucial steps in a plant life cycle which includes proper seed germination which is a basic pre-requisite for a better crop growth and yield. The experiment was conducted in Vegetable Science Department under the Defence Institute of Bio-Energy and Research (DIBER), (DRDO), Pithoragarh, Uttarakhand in the year 2020-2021 to standardize the best treatment of seed priming specific to Spinach (*Spinacia oleracea)*. An investigation was conducted both in a laboratory and on the field with the same treatment that was: (T1) Distilled water, (T2) 1%NaCl, (T3) 1%H_2O_2, (T4) 1%Na_2CO_3, (T5) Tap water, (T6) 1%DAP, (T7) Control. Seeds were soaked for 16 hrs and then dried to original moisture content for 2 days. It was found that all the priming treatments showed a significant difference with the control and the highest germination %(lab) with 71% was observed in seeds treated with DAP (T6) and the highest crop yield (field) were observed for 1% Na_2CO_3. Na_2CO_3 showed the best result in the field as well as in lab conditions based on different quality parameters followed by DAP. This study showed that seed priming treatments help in the enhancement of seed quality parameters, proved cost-effective

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and the most economical method. The experiment helps to improve the seed quality using different priming treatments which are cost-effective, economic, environment friendly and one of the quickest methods used for seed enhancement.

Keywords: Seed priming; spinach; germination%; seed enhancement.

1. INTRODUCTION

Spinach (*Spinacia oleracea*) commonly known as palak is one of the important cool season green leafy vegetables especially in tropical and sub-tropical region. It is an annual crop belongs to the family Amaranthaceae with chromosome no. 2n=24. Spinach is most probably a native of central and western Asia region.

Spinach is rich source of many nutrients and forms a major category of vegetable groups that have been designated as 'nature anti-ageing wonders and medicinal value [1]. Spinach seeds have laxative and cooling properties [2,3] and is also a good source of minerals like iron, copper, phosphorous, zinc, selenium), and vitamins like vit B complex (niacin and folic acid), ascorbic acid, carotenoids (ß-carotene, lutein, zeaxanthin), and Omega-3-fatty acids.

Proper seed germination is a basic pre-requisite for the good crop yield. An important problem that occurs in spinach cultivation is poor seed germination that results in late germination, reduces uniformity in total seed establishment which lastly results in poor quality and yield of spinach. Different techniques can be adopted to enhance the seed quality and germination either under adverse condition or may be sometimes for early germination under same favourable condition. Seed priming can be adopted to enhance seed germination and seed viability.

Heydecker proposed seed priming in 1973. Seed priming is a pre-sowing technique that results in increased germination rate, uniformity, and in improving crop growth. It is a simple and low-cost method used for seed enhancement. Seed priming reduces the time between seed sowing and emergence of seedling and synchronizes the process of emergence [4]. Priming involves treating of seeds with different organic and inorganic chemicals either in high or low temperature [5]. It is the technique of slow adsorption and post-dehydration [6]. Seed priming is the imbibition of seeds in water sufficient for pre¬ germinative metabolic activity to occur while preventing radical emergence [7]. An experiment was conducted in spinach (*Spinach oleracea*) seeds by soaking the seeds for different intervals @ 12, 16, 18, 24, 28 and 32 hours which showed that seeds soaked for 24 hours favoured improved seed quality parameters and germination increased by 94.25% [8]. Seed priming with 2-10 mM H_2O_2, is used for enhancing drought tolerance in rice which results in increased antioxidant activity and reduces oxidative stress and damages to the cellular components [9]. Seed priming with NaCl has been found to be better treatment as compared to non-primed seeds in case of hot pepper for improving the seedling vigour and seedling establishment under salt-stressed conditions [10]. Hydrogen peroxide @ 0.25% recorded maximum germination in all the varieties when compared with hydro-primed and unprimed seeds irrespective of the salt concentrations. This treatment improved the germination performance of the salt sensitive rice variety (ADT (R) 49) under higher NaCl concentrations [11]. An experiment to study the effect of effect of various sources and duration of priming on spinach seeds was conducted in which, four priming sources (Distilled water, DAP, SSP, SSP+Na_2CO_3) and soaking durations of 4-hour interval (4 hour to 24 hour) along with control were taken, resulted in overall SSP+Na_2CO_3 solution proved the best in most of the parameters while distilled water (control) showed comparatively poor performance [12]. Seeds treated with Na_2HPO_4, and $MgSO_4$ resulted in good plant growth, better quality and highest yield under both normal irrigation condition and stress condition as compared to seed priming with KCl which gave lower positive effects but showed better response than hydro-primed seeds [13]. At last, he concluded that okra plants could resist the applied water stresses treatments as a result of seed priming processes used which were found to cause accumulation of some osmolytes as proline. Fredj *et al.* [14], noticed that best germination percentage in coriander (*Coriandrum sativum)* was obtained by applying NaCl @ 4g/L for 12 h which resulted in increased germination percentage compared with non-primed seeds. Keeping all these points in view, the present investigation entitled **"Impact of various sources of seed priming on growth, yield and**

quality of Spinach local (*Spinacia oleracea*) *in-vitro* and *in-vivo* condition" is being planned with the following objective:

1.1 Objective

A. To study about the effect of different priming methods on the seed quality parameters of spinach under *in-vitro* condition (lab).
B. To study about the priming effect on the quality and yield of spinach under *in-vivo* condition (field).

2. METHODOLOGY

The present investigation on "Impact of various sources of seed priming on growth yield and quality of Spinach local (*Spinacia oleracea*) *in-vitro* and *in-vivo* condition" was carried out during 2020-2021 at Defence Institute of Bio-energy and Research (DIBER), DRDO Research station, Pithoragarh, Uttarakhand. The details of the materials used and methods adopted during the course of the investigation are presented here under. Seeds required for the experimental investigation were local spinach (DIBER-Sel.) developed through selection under vegetable department of DIBER, DRDO of Pithoragarh district in Uttarakhand. Spinach crop can be grown on a wide range of well fertilized and well drained soils. The experiment was laid out in both laboratory and field. It was conducted with 6 priming treatments with control. Completely Randomized Design were followed for lab with 4 replication and Randomized block design was followed for field experiment.

Treatments used for the experiment were same for both lab and field condition that were as: Distilled water (T1), 1% NaCl (T2), 1% of H_2O_2 (T3) ,1% of Na_2CO_3 (T4), Tap water (T5),1% of DAP T6, and T7 was control.

Seeds were soaked for 16 hrs and were dried upto original moisture content for 2 days at room temperature.

Chart 1. Experimental details
Lab condition

1.	Treatments	7(6+1)
2.	Replication	4
3.	Number of seeds/ replications	100
4.	Method adopted	Top of paper

Before placing seeds, petri-dishes were properly sterilised and then 100 seeds were placed in each petri-dish with 4 replications of each treatment. And then petri-dishes were placed in a germinator at 24℃ temperature for 14 days and then data was taken on the basis of different quality parameters.

2.1 Chemical Characteristics of the Experimental Field

Soil sample from the experimental site were taken prior to sowing and the chemical properties of soil were: EC 0.047dsm^{-1}, pH 7.50, Organic matter 2.09 % along with P_2O_5 27.23kg/hac and K_2O 439.04kg/hac

Prior to seed sowing, the field was thoroughly ploughed, harrowed and levelled to have efficient distribution of irrigation water. Farm yard manure (FYM) was added to the soil. Three main plots divided into 7 sub-plots (3m×1.8m). Field was irrigated just after sowing and then frequent irrigation were given after every 3 days were given.

Chart 2. Field condition

1.	Test crop and Variety	Spinach local (DIBER sel)
2.	Design	RBD
3.	Replication	3
4.	Treatment combination	7(6+1)
5.	Total no. of Plots	21
6.	Gross plot size	22.5 m×7.3 m
7.	Net plot size	3m×1.8 m
8.	Spacing (row *plant)	30cm×10cm
9.	Planting season	November 2020-March 2021
10	Recommended dose of Fertilizer	Cow dung manure @200q/ha

3. RESULTS AND DISCUSSION

3.1 Effect of Different Priming Treatments on the Basis of Different Quality Parameters under *in- vitro* Condition

3.1.1 Root length (cm)

It is evident from the Table 1 that among various priming treatments, root length showed significant difference. However, seeds primed with DAP at 1% (T6) registered significantly highest root length (5.96cm) compared to all the other treatments and was at par with seeds primed with Na_2CO_3at 1% (T4) which recorded 5.46cm of root length. While, lowest root length was recorded in control (T7) (3.56 cm).

3.1.2 Shoot length (cm)

Shoot length as displayed in Table 1 was significantly affected by priming treatments, Spinach seeds primed with Na_2CO_3(T4) registered significantly highest shoot length (3.50cm) followed by the seeds treated with DAP at1% (T6) which recorded (3.40cm), respectively and significantly lowest shoot length was recorded in control (T7) (2.32cm).

3.1.3 Seedling length (cm)

As showed in mean value Table 1 significant difference was observed in seedling length due to different priming treatments, Spinach seeds treated with DAP (T6) registered significantly highest seedling length (9.36cm) followed by the seeds primed with Na_2CO_3(T4) at1% which recorded 8.97cm seedling length, respectively and significantly lowest seedling length was recorded in control (T7) (5.89cm).

3.1.4 Seedling fresh weight (g)

Significant difference for seedling fresh weight was observed due to priming treatments which is presented in Table 1 Spinach seeds treated with Na_2CO_3 (T4) registered highest fresh weight (0.14g) followed by seeds treated with DAP (T6) (0.120g) and the lowest seedling dry weight was recorded seed soaked in Tap water (T5) (0.06g).

3.1.5 Seedling dry weight (g)

The mean value Table 1 showed a significant difference in seedling dry weight, seeds primed with Na_2CO_3, at 1 % (T4) registered significantly highest seedling dry weight (0.02g) and was on par with seeds treated with DAP at 1% (T6), which recorded (0.018g) of seedling dry weight and the lowest seedling dry weight was recorded in control (T7) (0.01g).

3.1.6 Seedling moisture percentage

Spinach seeds treated with Na_2CO_3(T4) registered highest moisture content (90.75%), and the lowest moisture percentage was recorded in seed soaked in tap water (81.08%).

3.1.6.1 Seedling vigour index 1

Significant difference for seedling vigour index 1 was observed due to priming treatments and data is given in Table 1 Spinach seeds treated with DAP @ 1% (T6) registered significantly highest seedling vigour index 1 (659.90) and followed by the seeds primed with Na_2CO_3at 1% (T4) which recorded (607.06) and the lowest seedling vigour index 1 was recorded in control (T7) (295.55).

3.1.6.1 Seedling vigour index 2

The mean value Table 1 showed a significant difference seed vigour Index 2 seeds soaked in Na_2CO_3 at1% (T4) registered significantly highest seedling vigour index 2 (1.35) and followed by the seeds primed with DAP(T6) which recorded (1.21) and the lowest seedling vigour index 2 was recorded in control (T7) (0.50).

3.1.7 Germination percentage

It is evident from the Table 1 that among various priming treatments, spinach seeds primed with DAP at 1% (T6) registered significantly highest seed germination (71%) and was at par with seeds primed with Na_2CO_3at 1%(T4) (68%) and significantly lowest germination percentages was recorded in control (T7) (50%).

3.2 Observation 2: Effect of Different Priming Treatments on the Basis of Different Quality Parameters Under *In- Vivo* Condition.

3.2.1 Root length(cm)

The results on root length in field varied significantly due to various priming treatments and is printed in Table 3 Among various priming treatments, root length showed significant difference. However, seeds primed with DAP at 1% (T6) showed significantly highest root length (8.77cm) followed by seeds hydro-primed with water(T3) which recorded (8.16cm) of root length. While, lowest root length was recorded in control (T7) (7.50cm).

Table 1. Mean value Table for the effect of different quality parameters of spinach seeds under laboratory condition

Treatment	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Fresh weight (g)	Dry weight (g)	Moisture%	Seedling vigour index 1	Seedling vigour index 2	Germination %
T1	4.95	3.50	8.45	0.10	0.01	86.45	524.63	0.81	62.0
T2	5.28	3.18	8.46	0.11	0.01	84.42	416.87	0.85	52.0
T3	3.94	2.86	6.80	0.10	0.01	81.08	345.46	0.68	51.0
T4	5.46	3.50	8.97	0.14	0.02	90.75	607.06	1.35	68.0
T5	3.84	2.42	6.25	0.06	0.01	82.83	349.62	0.56	56.0
T6	5.96	3.40	9.36	0.12	0.01	84.18	659.99	1.21	71.0
T7	3.56	2.32	5.89	0.08	0.01	87.91	295.55	0.50	50.0
MEAN	**4.71**	**3.02**	**7.74**	**0.10**	**0.01**	**85.37**	**457.02**	**0.85**	**58.6**
C.V.	**0.19**	**0.16**	**0.18**	**0.26**	**0.27**	**0.03**	**0.30**	**0.37**	**0.1**
F. ratio	**3.59**	**2.58**	**6.54**	**11.15**	**4.57**	**2.75**	**11.36**	**7.13**	**12.1**
***P.* ratio**	**0.013***	**0.04***	**0***	**0***	**0.004***	**0.03***	**0***	**0***	**0***
S.E.	**0.35**	**0.19**	**0.52**	**0.01**	**0.001**	**1.23**	**53.44**	**0.12**	**3.22**

Table 2. ANOVA Table for the effect of different quality parameters of spinach seeds under laboratory condition

Si. No.	Parameters	Source of variation	Mean sum of square	F-ratio	*P*-value
1	**Root Length(cm)**	**Treatment (df=6)**	3.45	3.59	0.01*
		Error (df=21)	0.96		
2	**Shoot Length(cm)**	**Treatment (df=6)**	1.01	3.58	0.04*
		Error (df=21)	0.39		
3	**Seedling Length(cm)**	**Treatment (df=6)**	7.79	6.64	0.005*
		Error (df=21)	1.19		
4	**Fresh weight (g)**	**Treatment (df=6)**	0.0029	11.15	0*
		Error (df=21)	0.00026		
5	**Dry weight (g)**	**Treatment (df=6)**	0.000059	4.57	0.004*
		Error (df=21)	0.0000129		
6	**Moisture %**	**Treatment (df=6)**	42.62	2.75	0.03*
		Error (df=21)	15.47		
7	**Seedling Vigour Index 1**	**Treatment (df=6)**	80273.18	11.36	0*
		Error (df=21)	7062.28		
8	**Seedling Vigour Index 2**	**Treatment (df=6)**	0.41	7.13	0*
		Error (df=21)	0.05		
9	**Germination%**	**Treatment (df=6)**	281.55	12.05	0*
		Error (df=21)	23.36		

**Significance level at 5%*

3.2.2 Shoot length(cm)

As shown in Table. shoot length showed a significant difference among various priming treatments, However, seeds primed with Na_2CO_3at 1% (T4) registered significantly highest shoot length (9.72cm) compared to all the other treatments and was at par with seeds primed with DAP at 1% (T6) which recorded (9.60cm) of shoot length. While, lowest shoot length was recorded in control (T7) (7.50cm).

3.2.3 Number of leaves /plants

The data as per Table 3 showed significant differences due to various priming treatments on number of leaves/plants, maximum number of leaves were recorded (10.13) in seeds treated with 1% Na_2CO_3 (T4) which was followed DAP(T6) which recorded (9.77) while the lowest number of leaves was recorded in control (T7) which recorded as (8.66)

3.2.4 Plant height (cm)

The data on plant height showed significant differences due to various priming treatments and is given in Table 3 Significant differences were observed among the priming treatments, seeds treated with 1% Na_2CO_3 (T4) shows the highest plant height (18.31 cm) compared to all the other treatment and was on par with 1% DAP treated seeds (T6) which recorded (18.24cm) while the lowest plant height was recorded in seeds hydro-primed with tap water (T5) which was recorded as (13.39 cm).

3.2.5 Crop yield /treatment (kg)

It is evident from the Table 3 that among various priming treatments, crop yield/treatment showed significant difference, maximum yield was recorded (2.96kg) when seeds were treated with Na_2CO_3 at 1% (T4) followed by seeds primed with DAP at 1% (T6) which was recorded (2.60kg) while the lowest yield was of control (T7) (1.96 kg).

3.2.6 No. of seeds/plant

Significant differences were observed in number of seeds/plants as shown in Table 3, highest number of seeds were recorded in seeds primed with Na_2CO_3(T4) (66.21) followed by seeds treated with DAP (T6) which was recorded (65.98) while the lowest number of seeds was recorded in control (T7) with (55.89).

3.2.7 Seed weight/plant (g)

Significant differences were observed as shown in mean value Table 3 in seed weight/plant, the highest weight of seeds was recorded when seeds primed with Na_2CO_3 (T4) (0.78g) which was followed by DAP (T6) recorded as (0.72g) while the lowest weight of seeds was recorded in control (T7) with (0.53g).

Table 3. Mean value Table for the effect of different quality parameters of spinach seeds under field condition

Treatments	Shoot length (cm)	Root length (cm)	Plant height (cm)	No. Leaves/ plant	Crop yield (greens) (kg)	Number of seeds/plant (avg. 5)	Seed weight /plant (g) (avg. 5)
T1	7.42	7.65	15.13	8.73	2.43	57.32	0.61
T2	9.28	7.77	17.39	9.33	2.33	61.76	0.63
T3	7.22	8.16	15.48	8.72	2.43	58.06	0.63
T4	9.72	8.12	18.31	10.13	2.96	66.21	0.78
T5	7.94	7.45	13.39	8.86	2.30	58.71	0.62
T6	9.60	8.77	18.24	9.73	2.60	65.98	0.72
T7	7.70	7.50	15.20	8.66	1.96	55.89	0.53
MEAN	**8.41**	**7.92**	**16.16**	**9.16**	**2.44**	**60.56**	**0.65**
C.V.	**3.75**	**1.14**	**2.81**	**2.17**	**2.61**	**0.94**	**0.81**
S.E.	**0.73**	**0.27**	**1.06**	**0.46**	**0.14**	**1.33**	**0.01**
C.D. @ 5%	**2.31**	**0.87**	**3.32**	**2.17**	**0.46**	**4.17**	**0.03**
LOWEST RANGE	**7.22**	**7.50**	**13.39**	**8.66**	**1.96**	**55.89**	**0.53**
HIGHEST RANGE	**9.72**	**8.77**	**18.31**	**10.13**	**2.96**	**66.21**	**0.78**

Table.4. ANOVA Table for the effect of different quality parameters of spinach seeds under field condition

Si. No.	Parameters	Mean sum of square			F-value	
		Replication (df=2)	Treatment (df=6)	Error (df=12)	Replication	Treatment
1	Shoot Length (cm)	3.71	34.87	4.89	0.758	7.04
2	Root Length (cm)	0.226	28.92	0.70	0.32	41.20
3	Plant Height (cm)	10.86	124.64	10.13	1.07	12.30
4	No. of leaves/plant	8.53	37.79	1.94	4.39	19.46
5	Crop Yield(kg)	1.89	2.88	0.20	9.48	14.39
6	No. of seeds/plant	62.11	1657.09	15.09	3.89	103.81
7	Weight of seeds/plant(g)	0.01	0.20	0.001	7.77	147.37

** 5% level of significance*

4. CONCLUSION

Based on current findings, it can be concluded that seed primed with 1% DAP solution produced best results under laboratory experiment, followed by Na_2CO_3 and gave more germination% as compared to un-primed seed. While 1% Na_2CO_3 treated seeds gave best response under field condition which was at par with DAP. Based on current experiments, and considering both laboratory and field experiment seed priming with Na_2CO_3 is recommended for priming treatment in spinach while DAP can also be suggested as per the response. The study proved the positive effect of priming on spinach. However, there is a dire need for further investigations to explore the effect of Na_2CO_3 and DAP under different concentrations, to envisage the secretes of priming effects at molecular and biological levels.

ACKNOWLEDGEMETS

Authors are thankful to all the members of the DIBER, DRDO, Fld st. Pithoragarh, Uttarakhand and Department of Genetics and Plant Breeding, Sam Higginbottom University of Agriculture, Technology and Sciences, Naini, Prayagraj, UP, for their encouragement and support.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/74817