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Effect of mechanical scarification and biofertilizer treatments on seed quality enhancement in Senna (*Senna alexandrina* Mill.)

Kalyanrao, Dhimant Desai, Sudeshna Chakraborty, Anil Rawat and Ujjval Solanki

Department of Seed Science and Technology, B. A. College of Agriculture, Anand Agricultural University, Anand 388 110, Gujarat, India

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ABSTRACT

Senna (*Senna alexandrina* Mill.) is an annual medicinal plant commonly known as "senna" or "Alexandrian senna" primarily known for its agricultural, industrial and ethno-medicinal purposes which belong to family *leguminoceae.* Senna seeds usually exhibit seed coat-imposed dormancy which may be due to impermeability of testa to water and gases. Seed scarification treatment is one of the methods adopted for seed dormancy breaking and better avenues for their establishment, growth and development of seedlings. In the present investigation seeds were treated with different mechanical scarification and biofertilizer treatments and their combined effect on germination and other seed quality attributes were studied. It was found that the seeds scarified for two **(S_3)** to three minutes **(S_4)** with mechanical scarification followed by seed priming treatment with Bio NPK (5ml/kg seeds) showed the highest seed germination percentage, highest seedling vigour index I and highest seedling vigour index II compared to control and other treatments.

Key words: *Senna, Mechanical scarification, Germination, Seedling vigour*

Introduction

Senna is predominantly self-pollinated crop but out crossing could be high (20%) through buzz-crossing. It is primarily grown in Egypt, India, and Sudan and is widely in the western countries for making 'herbal tea' and as a mild laxative. Senna species are also used for constipation, fungal skin infectious and hemorrhoids (Agarwal and Bajpai, 2010). Senna is mainly grown during the *rabi* and summer season under residual moisture after the *kharif* harvest of paddy and cotton crop. The plant is in high demand in the international market because of its potential use in a variety of medications based on Sennosides A and B which are purgative, antibacterial, anticancer, and antioxidant compounds. Senna is currently commercially produced in India and there is potential for large-scale production and profitable export.

Seed dormancy is an important evolutionary factor in plants, ensuring their survival in unfavorable conditions as Senna seeds exhibits seed coat-imposed dormancy, which means that the seed coat prevents water and gases from entering the seed and inhibits germination. Seed treatment is one of the methods adopted for quality seed production as it not only reduces the deleterious effects of damage to seed viability and vigour but also provides better means for the establishment, growth and development of seedlings. To overcome seed dormancy and promote germination, seed scarification and seed priming treatment can be used.

A scarification method is the most appropriate way to break downseed dormancy without damaging embryo during the germination process. This involves physically breaking or weakening of the seed coat allowing water and gases to penetrate and trigger the germination process. There are several methods of seed scarification, including mechanical scarification, chemical scarification, and hot water treatment. Mechanical scarification involves nicking or scratching the seed coat with a sharp blade or sandpaper, while seed priming involves treating the seed with a chemical solution that dissolves or softens the coat. Seed priming is a technique of controlled hydration in which seed is soaked in water or low osmotic potential solution to a point where germination related metabolic activities begin in the seed but radical emergence remains on hold. Some pre-sowing treatments are reported to be effective in optimizing germination in seeds of various medicinal plant species, therefore application of pre-sowing treatments (both physical and chemical) also need to be investigated in senna. Moreover, possibility of application of biofertilizers for producing quality seedlings in medicinal plants needs to be explored.This study therefore, intends to identify the best pre-treatment methods to break seed dormancy, promote germination and other seed quality parameter of senna seed.

Materials and Methods

The present investigation was carried out to elicit the effect of mechanical scarification and biofertilizer treatments on seed quality enhancement in senna. The experimental materials used for the present investigation includes variety GAS 1 (Gujrat Anand Senna 1) evaluated under Factorial Completely Randomized Design under two factors comprising ten treatments with three repetitions over three years 2020-22. The experiment was conducted at Department of Seed Science and Technology Laboratory, B. A. College of Agriculture, AAU, Anand during 2020-22.

The two-factors used in FCRD testing were mechanical scarification and seed priming with biofertilizer. Mechanical scarification was done by taking five grams of seeds at random from the seed lot and scarified in electrical seed scarifier for one minute **(S_1)**, two minutes **(S_2)** and three minutes **(S_3)** separately. Seeds were treated with biofertilizer cultures as per the recommended dose for seed treatment @5 ml/kg seeds diluted with required quantity of water and were soaked in liquid biofertilizers culture for 2-3 hrs and thereafter, germination test was conducted. The different treatment under biofertilizer priming treatments were **B_1**: Control; **B_2**: *Azospirillium*(*Azospirilliumlipoferum*); **B_3**: *Azotobacter* (*Azotobactrerchroococcum*); **B_4**: Beejamrut; **B_5**: GA_3 90% WP @100 ppm (3 hours soaking).

Table 1. Details of treatments for seed enhancement

Factor I: Mechanical Scarification
S_1: Control
S_2: Mechanical Scarification (1 minute)
S_3: Mechanical Scarification (2 minute)
S_4: Mechanical Scarification (3 minute)
Factor II: Seed priming treatments
B_1: Control
B_2: *Azospirillum lipoferum* (5ml/kg seeds)
B_3: *Azotobacter chroococcum* (5ml/kg seeds)
B_4: Beejamrut
B_5: Bio NPK (5ml/kg seeds)
B_6: GA_3 90% WP @100 ppm (3 hours soaking)

As given in Table 1. The untreated seeds were considered as control and it was taken from fresh seed lot. Test for seed quality were conducted for nine parameters *viz.*, Germination (%), Seedling length (cm), Seedling dry weight (g), Seedling vigour index-I and Seedling vigour index-II.The seedling vigour indices were calculated by using the procedure suggested by Abdul- Baki and Anderson (1973) and expressed in whole number.

Results and Discussion

Effect of mechanical scarification and priming treatment on seed quality enhancement of senna variety GAS 1 was studied for three consecutive years based on different treatment of time framed mechanical scarification and fertilizer priming treatments *viz.*, priming with *Azotobacter*, *Azospirilium*, Beejamrut, Bio NPK and GA_3along with control. The critical difference (CD) and standard error (SE) were worked out for each case and the interaction and pooled data for three years were worked on basis of Factorial Completely Randomized Design based statistical analysis.

Germination %

Among the scarification treatment, **S_4** (3 Minutes) treatment recorded highest germinationpercentage of 91.94 % during year 2020 in compared to the control treatment S_1 with germination % of (67.94 %). On the basis of pooled year data seeds scarified with treatment S_3 (2 Minutes) showed higher germination % (87.14 %) in compared to control treatment S_1 value of 65.94 %. While, priming treatment B_5 (Bio NPK @ 5ml/kg) recorded significantly highest germination percentage of 90.16 % in the year 2020in contrast to the 67.94 % of germination in control treatment B_1. Pooled year data for priming treatment showed significant interaction on pooled basis were treatment B_5 (Bio NPK @ 5ml/kg) had highest germination percentage of 87.00 %, while the control treatment B_1 on pooled basis had germination of 73.22 % (Table 2).

Mechanical scarification pre-treatment in senna improves germination which indicates the significance of scratching seed coat surface to allow, water and oxygen readily to the embryo resulted into higher germination. These results are in conformity with the findings of Patel *et al.* (2020) in Senna and Sapra *et al*. (2020) in Ashwagandha through 2 minutes of mechanical scarification. Teketay (1996) analysed five senna species for pre sowing treatments and observed that the mechanical scarification resulted in 100% germination among different pretreatments in the species viz., *S. bicapsularis*, *S. didymobotrya*, *S. multiglandulosa* and *S. occidentalis*. Additionally, Ardiarini *et al*. (2021) in long bean (*Vigna sinesis*); Mohan *et al.* (2012) in various medicinal crops *viz*., Ashwagandha (*Withanias omnifera* L.), Senna (*Cassia angustifolia* L.), Tulasi (*Ocimum sanctum* L.) and Adavivulavalu (*Cassia absus*) observed improvement in germination percent in case of different physical/mechanical scarification methods. However, observed better germination % with Bio NPK might be due to the activation of enzyme in embryo and favourable metabolic processes and releasing growth favorable substances, enabling mobilization of food reserves to the embryo. Additionally, bio-primed seed did not have an inhibitory effect on germination and promoted the early seedling growth of most plant species (Melissa *et al*., 2020). The advantageous effects of chemical/biofertilizer seed priming treatments on favorable germination % and vigour were reported by earlier scientist (Mohan *et al*., 2012); (Tiwari *et al*., 2018) in various medicinal plants.

Seedling length (cm)

The scarification treatment effect of treatment S_3 (2 Minutes) recorded significantly highest seedling length 17.31cm followed by treatment S_4 (3 Minutes) having seedling length of 17.04 cm during the year 2020 in compared to the control treatment S_1 (13.98 cm). While, the scarification treatment S_3 (2 Minutes) recorded higher seedling length of 16.33 cm over the three years period on pooled basis in compared to control treatment S_1 (13.35 cm). Among the priming treatments, seeds primed with B_5 (Bio NPK @ 5ml/kg) recorded highest Seedling length of 17.50cm during 2020 while, the control treatment S_1 had seeding length of 15.15 cm. Furthermore, pooled

Table 2. Effect of mechanical scarification and seed priming treatments on germination (%), seedling length (cm) and seedling dry weight (g)

Source	Germination (%)				Seedling length (cm)				Seedling dry weight (g)			
	2020	2021	2022	Pooled	2020	2021	2022	Pooled	2020	2021	2022	Pooled
S1	67.94	65.11	64.77	65.94	13.98	13.61	12.47	13.35	0.08	0.08	0.08	0.08
S2	89.44	88.38	78.00	85.27	16.98	14.55	12.83	14.79	0.10	0.08	0.08	0.09
S3	90.05	90.61	80.77	87.14	17.31	15.35	16.35	16.33	0.10	0.09	0.08	0.09
S4	91.94	90.33	77.44	86.55	17.04	15.29	15.07	15.80	0.10	0.09	0.06	0.08
S. Em ±	0.60	0.60	0.31	1.75	0.25	0.18	0.13	0.48	0.002	0.001	0.002	0.005
CD (P=0.05)	1.73	1.73	0.88	6.06	0.72	0.53	0.38	1.66	0.005	0.004	0.005	NS
B1	70.08	78.16	71.41	73.22	15.15	13.28	13.09	13.84	0.08	0.08	0.07	0.08
B2	87.66	82.08	69.33	79.69	16.17	14.67	13.05	14.63	0.10	0.08	0.08	0.08
B3	89.00	84.66	76.66	83.44	16.05	13.69	12.91	14.21	0.10	0.08	0.07	0.08
B4	85.08	83.16	77.50	81.91	16.02	13.83	14.61	14.82	0.09	0.08	0.08	0.08
B5	90.16	89.16	81.66	87.00	17.50	15.54	16.21	16.42	0.10	0.08	0.08	0.09
B6	87.08	84.41	74.91	82.13	17.06	17.17	15.22	16.48	0.10	0.10	0.07	0.09
S. Em ±	0.74	0.74	0.38	2.01	0.31	0.22	0.16	0.39	0.002	0.002	0.002	0.003
CD (P=0.05)	2.10	2.11	1.00	6.30	0.84	0.64	0.47	1.24	0.006	0.004	0.003	NS
CV %	3.05	3.08	1.75	2.76	6.57	5.36	4.03	5.55	6.86	6.02	9.79	7.56

year data suggest seeds primed with treatment B_6 (GA_3 90% WP @100 ppm)(16.48 cm) and B_5 (Bio NPK @ 5ml/kg)(16.42 cm) showed higher Seedling length. A perusal of data indicated that interaction between scarification and seed priming treatment have been found to be significant over the years. These results are in conformity with the findings of Patel *et al.* (2020) in Senna through mechanical scarification for 2 minutes as well as Sapra *et al*. (2020) in Ashwagandha. Further, biofertilizer improve plant performance by releasing plant growth hormones, mobilizing nutrients and suppressing pests and diseases. Thereby, priming with microbes will improve root and shoot length of plant (Sarkar *et al.,* 2020) (Table 2).

Seedling dry weight (g)

The scarification treatments $\mathbf{S_2}$ (1 Minute),$\mathbf{S_3}$ (2 Minute) and $\mathbf{S_4}$ (2 Minute) recorded significantly highest seedling dry weight 0.104 g during 2020. As well as the same treatments $\mathbf{S_3}$ (2 Minute) and $\mathbf{S_2}$ (1 Minute) recorded high seedling dry weight of 0.092 g and 0.091 g respectively, on pooled year basis. Among the priming treatments, seeds primed with B_2, B_3, B_5 and B_6 treatments recorded highest Seedling dry weight of 0.10 g during year 2020. Furthermore, the treatments B_5 (Bio NPK @5ml/kg) and B_6 (GA_3 90% WP @100 ppm) have similar high seedling dry weight of 0.094 g on the basis of pooled year analysis. Higher seedling growth (Seedling length, fresh and dry weight) in seeds treated with biofertilizers might be due to the increased cytokinin production which triggers the cell division, thus seedling or plant growth maximized (Marriapan *et al*., 2014) (Table 2).

Seedling vigour index-I

The result pertaining to scarification treatments suggests that the treatment S_4 (3 Minute) has significantly highest seedling vigour index-I of 1617 during 2020 as compared to SV-I value of 951 for treatment S_1 (Control). While, the treatment S_3 (2 Minute) had superior value of 1444 for seedling vigour index-I on pooled year basis in contrast to 883 value of SV-I for treatment S_1 (Control). Among the priming treatments, seeds primed with B_5 (Beejamrut) recorded highest seedling vigour index-I of 1593 during year 2020 and the same treatment B_5 (Beejamrut) primed seed showed higher Seedling vigour index-I (1441) on pooled year basis (Table 3).

Seedling vigour index-II

Based on mechanical scarification, treatment S_4 (3 Minute) recorded significantly high seedling vigour index-II of 9.78 in year 2020 in compared to treatment S_1 (Control) with SV-II value of 5.50. While, based on the pooled year data treatment S_3 (2 Minute) had high Seedling vigour index-II of 7.04 in compared to SV- II value of 4.51 for treatment S_1 (Control). Optimum time of scarification impose positive impacts on seed germination and vigour. Similar outcome for higher seedling vigour index-I

Table 3. Effect of mechanical scarification and seed priming treatments on Seedling vigour index I and Seedling vigour index II

Source	Seedling vigour index I				Seedling vigour index II			
	2020	2021	2022	Pooled	2020	2021	2022	Pooled
S1	951	887	812	883	5.50	5.20	2.83	4.51
S2	1568	1288	1005	1287	9.66	7.65	2.85	6.72
S3	1612	1392	1326	1444	9.73	8.25	3.14	7.04
S4	1617	1384	1167	1389	9.78	8.15	2.34	6.76
S. Em ±	23.35	17.37	11.39	55.10	0.15	0.11	0.10	0.64
CD (P=0.05)	66.64	49.57	32.50	190.68	0.45	0.31	0.28	NS
B1	1281	1042	946	1090	7.36	6.26	2.19	5.27
B2	1443	1210	906	1183	8.97	7.12	2.60	6.23
B3	1440	1166	999	1202	9.07	6.96	2.72	6.25
B4	1377	1149	1135	1220	8.10	6.87	2.97	5.98
B5	1593	1394	1336	1441	9.44	8.10	3.95	7.16
B6	1498	1465	1142	1369	9.06	8.57	2.30	6.64
S. Em ±	28.60	21.27	13.95	44.64	0.19	0.13	0.12	0.29
CD (P=0.05)	81.62	60.72	39.81	140.66	0.55	0.39	0.35	0.93
CV %	6.89	5.95	4.48	6.12	7.78	6.47	15.32	8.56

and II were also reported by Patel *et al.*, (2020) in Senna and Sapra *et al.*, (2020) in Aswagandha (2020). The priming treatment B_5 (Bio NPK @5ml/kg) has recorded highest seedling vigour index-II of 9.40 during year 2020, which also has shown superior performance on pooled year basis with high value of SVI-II (7.16). A perusal of interaction result indicated that interaction between scarification and seed priming treatment have been found to be significant over the years for seedling vigour index-II. The effectiveness of mechanical scarification may vary depending on the genus and species. Effectiveness of the methods varies depending on the duration of imposed treatments (Table 3).

Interaction effect results

A perusal of treatment, treatment effect and their combination/interactions based on pooled FCRD analysis indicates that interaction between scarification and seed priming treatment have been found to be significant over the years for all the character studied. So, further interaction study has been carried out to find out the best treatment combination of mechanical scarification followed by seed priming suitable for seed quality enhancement.

Interaction effect between mechanical scarification and seed priming treatment have shown significant relationship. The result suggests that treatment combination, $\mathbf{S_4B_5}$ (Mechanical scarification for 3 Minutes followed by seed priming with Bio NPK @ 5 ml/kg) and $\mathbf{S_2B_5}$ (Mechanical scarification for 1 Minutes followed by seed priming with Bio NPK @ 5ml/kg) recorded highest germination percentage of 98.00 % during year 2020. While, the control treatment $\mathbf{S_1B_1}$ recorded 61.00 % germination for year 2020. On the basis of pooled year data analysis, treatment combination, $\mathbf{S_4B_5}$ (Mechanical scarification for 3 Minutes followed by seed priming with Bio NPK @ 5 ml/kg) showed highest germination percentage (92.88 %) in relation to which control treatment $\mathbf{S_1B_1}$ recorded germination percentage of 60.33 %. However, senna seeds that did not germinate well (S1B1) because of the treatment process which made the seeds absorbed little amount of

Table 4. Interaction effects of different scarification and seed priming treatments on seed germination (%) and seedling length (cm)

Treatments	Germination %				Treatments	Seedling length (cm)			
	2020	2021	2022	Pooled		2020	2021	2022	Pooled
S1B1	61.00	58.66	61.33	60.33	S1B1	12.56	12.56	10.17	11.76
S1B2	70.66	61.33	62.33	64.77	S1B2	13.30	14.16	11.21	12.89
S1B3	70.66	66.66	63.00	66.77	S1B3	14.06	12.34	11.21	12.54
S1B4	67.00	66.00	66.33	66.44	S1B4	13.72	13.84	13.85	13.80
S1B5	70.66	72.66	69.66	71.00	S1B5	15.27	13.95	14.35	14.52
S1B6	67.66	65.33	66.00	66.33	S1B6	14.96	14.84	14.01	14.60
S2B1	70.00	83.33	73.00	75.44	S2B1	15.79	13.81	11.26	13.62
S2B2	91.33	86.66	74.33	84.11	S2B2	15.84	12.69	11.00	13.18
S2B3	96.00	89.33	79.33	88.22	S2B3	16.60	13.63	11.23	13.82
S2B4	89.33	87.33	80.33	85.66	S2B4	16.53	14.04	11.80	14.79
S2B5	98.00	94.66	81.33	91.33	S2B5	19.19	15.33	15.12	16.55
S2B6	92.00	89.00	79.66	86.88	S2B6	17.92	17.80	14.58	16.76
S3B1	73.66	84.66	76.66	78.33	S3B1	16.02	13.19	15.62	14.94
S3B2	95.33	91.00	76.33	87.55	S3B2	18.43	15.83	14.91	16.39
S3B3	94.00	93.33	81.66	89.66	S3B3	16.80	14.39	15.06	15.41
S3B4	90.00	89.33	84.00	87.77	S3B4	16.82	13.72	15.46	15.33
S3B5	94.00	94.00	90.33	92.77	S3B5	17.72	16.59	19.54	17.95
S3B6	93.33	91.33	75.66	86.77	S3B6	18.08	18.30	17.54	17.97
S4B1	75.66	86.00	74.66	78.77	S4B1	16.21	13.57	15.31	15.03
S4B2	93.33	89.33	64.33	82.33	S4B2	17.12	16.00	15.07	16.06
S4B3	95.33	89.33	82.66	89.11	S4B3	16.74	14.40	14.14	15.09
S4B4	94.00	90.00	79.33	87.77	S4B4	17.02	13.70	15.34	15.35
S4B5	98.00	95.33	85.33	92.88	S4B5	17.84	16.29	15.83	16.65
S4B6	95.33	92.00	78.33	88.55	S4B6	17.30	17.76	14.75	16.60
S. Em±	1.29				S. Em±	0.48			
CD (P=0.05)	3.59				CD (P=0.05)	1.25			

water or due to embryo damage. In case of biofertilizer treatments, these results may be due to the role of *Azotobacter* and *Azospirillum* in providing growth promoting substances such as indole acetic acid and gibberellins. Germination of *Cassia fistula* L. was improved when seeds were mechanically scarified (84%) or treated with concentrated sulphuric acid for 90 minutes (84%) (Babeley and Kandya 1988) (Table 4).

A perusal of results indicate significant interaction between scarification and seed priming treatment for seedling length over the years. The treatment combination, S_3B_5 (Mechanical scarification for 2 Minutes followed by seed priming with Bio NPK @ 5ml/kg) recorded highest seedling length 19.54 cm during the year 2022. Moreover, the control interaction treatment S_1B_1 in year 2022 had seedling length of10.17cm. While, the treatment combinations, S_3B_6 (Mechanical scarification for 2 Minutes followed by seed priming with GA_3 90% WP @100 ppm) recorded higher seedling length of 17.97 cm followed by S_3B_5 (Mechanical scarification for 2 Minutes followed by seed priming with Bio NPK @ 5ml/kg) with seedling length of 17.95 cm over the pooled years seedling length values. The corresponding control treatment S_1B_1 recorded seedling length of 11.76 cm on pooled year basis.

Mechanical scarification and seed priming treatment showed significant interaction on pooled year basis for seedling dry weight over the years. The treatment combination, S_3B_2 (Mechanical scarification for 2 Minutes followed by seed priming with *Azospirillumlipoferum* @5ml/kg) and S_3B_6 (Mechanical scarification for 2 Minutes followed by seed priming with GA_3 90% WP @100 ppm) recorded highest seedling dry weight 0.117 g during 2020. While, the control treatment S_1B_1 had a dry weight of 0.0078 in year 2020. Furthermore, treatment combination S_3B_6 (Mechanical scarification for 2 Minutes followed by seed priming with GA_3 90% WP @100 ppm) recorded high seedling dry weight of 0.101 g on pooled year basis. The corresponding control treatment combination S_1B_1 (0.073 g) recorded low seedling dry weight on pooled year basis.

Table 5. Interaction effect of different scarification and seed priming treatments on seedling vigour index-I and seedling vigour index-II

Treatments	Seedling dry weight (g)				Treatments	Seedling vigour index-I			
	2020	2021	2022	Pooled		2020	2021	2022	Pooled
S1B1	0.078	0.078	0.063	0.073	S1B1	766	736	623	708
S1B2	0.082	0.085	0.085	0.084	S1B2	939	869	699	836
S1B3	0.080	0.073	0.081	0.078	S1B3	993	822	706	840
S1B4	0.078	0.083	0.092	0.084	S1B4	919	912	918	917
S1B5	0.084	0.72	0.092	0.082	S1B5	1079	1014	999	1031
S1B6	0.081	0.087	0.090	0.086	S1B6	1011	971	925	969
S2B1	0.093	0.082	0.082	0.086	S2B1	1369	1151	822	1114
S2B2	0.110	0.077	0.078	0.088	S2B2	1443	1100	817	1120
S2B3	0.107	0.081	0.079	0.089	S2B3	1591	1217	891	1233
S2B4	0.096	0.084	0.082	0.087	S2B4	1476	1227	1108	1270
S2B5	0.110	0.087	0.091	0.096	S2B5	1880	1452	1230	1521
S2B6	0.109	0.105	0.080	0.098	S2B6	1646	1585	1161	1464
S3B1	0.082	0.079	0.072	0.078	S3B1	1475	1117	1197	1263
S3B2	0.117	0.089	0.077	0.094	S3B2	1757	1441	1138	1445
S3B3	0.106	0.084	0.080	0.090	S3B3	1579	1342	1229	1383
S3B4	0.100	0.082	0.107	0.096	S3B4	1512	1226	1298	1345
S3B5	0.102	0.102	0.078	0.094	S3B5	1664	1559	1765	1663
S3B6	0.117	0.108	0.078	0.101	S3B6	1687	1670	1327	1561
S4B1	0.096	0.080	0.072	0.083	S4B1	1512	1167	1142	1274
S4B2	0.093	0.093	0.083	0.090	S4B2	1597	1431	961	1332
S4B3	0.109	0.087	0.068	0.088	S4B3	1597	1285	1169	1350
S4B4	0.100	0.081	0.069	0.083	S4B4	1600	1233	1217	1350
S4B5	0.116	0.097	0.060	0.091	S4B5	1749	1553	1351	1551
S4B6	0.102	0.101	0.061	0.088	S4B6	1651	1635	1156	1480
S. Em±	0.004		44.20						
CD (P=0.05)	0.011		122.52						

Significant differences for interaction effect among treatment combination of mechanical scarification and seed priming treatment over the year suggests that the treatment combination S_2B_5 (Mechanical scarification for 1 Minutes followed by seed priming with Bio NPK @5ml/kg) has highest seedling vigour index-I of 1880 during 2020. While, the control treatment S_1B_1 had seedling vigour index-I of 766 during year 2020. In pooled year data, interactive treatment S_3B_5 (Mechanical scarification for 2 Minutes followed by seed priming with Bio NPK @5ml/kg) recorded high value of 1664.00 for seedling vigour index-I in comparison to the controlled treatment combination S_1B_1 which had value of 708 for seedling vigour index-I (Table 5).

Table 6. Interaction effect of different scarification and seed priming treatments on seedling vigour index-II

Treatments	2020	2021	2022	Pooled
S1B1	4.79	4.60	2.06	3.82
S1B2	5.84	5.26	3.15	4.75
S1B3	5.65	4.91	2.54	4.37
S1B4	5.28	5.48	3.46	4.74
S1B5	5.96	5.23	3.66	4.95
S1B6	5.49	5.73	2.09	4.44
S2B1	8.06	6.87	2.43	5.79
S2B2	10.11	6.75	2.54	6.47
S2B3	10.27	7.26	2.71	6.75
S2B4	8.65	7.34	3.15	6.38
S2B5	10.82	8.31	3.71	7.61
S2B6	10.03	9.37	2.54	7.31
S3B1	7.61	6.69	2.45	5.58
S3B2	11.22	8.11	2.51	7.28
S3B3	9.96	7.89	2.91	6.92
S3B4	9.04	7.32	2.79	6.38
S3B5	9.61	9.61	5.53	8.25
S3B6	10.96	9.87	2.62	7.82
S4B1	9.00	6.87	1.84	5.90
S4B2	8.73	8.35	2.18	6.42
S4B3	10.38	7.80	2.70	6.96
S4B4	9.43	7.34	2.45	6.41
S4B5	11.38	9.25	2.90	7.84
S4B6	9.78	9.30	1.95	7.01
S. Em±	0.31			
CD (P=0.05)	0.087			

A perusal of interaction result indicates significant interaction effect between scarification and seed priming treatment seedling vigour index-II. Treatment combination, $\mathbf{S_4B_5}$ (Mechanical scarification for 3 Minutes followed by seed priming with Bio NPK @5 ml/kg) recorded highest seedling vigour index-II of 11.38 in year 2020. While, control treatment S_1B_1 had seedling vigour index-I of 4.79 during first year 2020. Based on the pooled year data treatment combination, S_3B_5 (Mechanical scarification for 2 Minutes followed by seed priming with Bio NPK @5ml/kg) had highest Seedling vigour index-II of 8.25. While, the control treatment S_1B_1 recorded value of 3.82 seedling vigour index-II on pooled year basis.

The role of biofertilizers *Azospirillum* and phosphobacteria in enhancing the availability of nitrogen and phosphorus and other nutrients with consequent enhancement in the metabolic activity resulted in higher germination and seed quality traits in Ashwagandha (Sapra *et al.*, 2020). The enzymatic and hormonal mechanism stimulates metabolic process such as sugar mobilization, protein hydrolysis, oxidation etc., which leads to increase in seedling fresh weight and seedling dry weight (Mohan *et al.*, 2012).

Conclusion

Study of three consecutive years, the pooled data revealed that the seeds scarified for two (S_3) to three minutes (S_4) with mechanical scarification followed by seed priming treatment with Bio NPK (5ml/kg seeds) showed the highest seed germination percentage of 92.78 % and 92.89 % respectively, as compared to absolute Control ($S_1 B_1$) which recorded the lowest germination of 66.22 %. The highest seedling vigour index I was observed in the seeds treated for two to three minutes of mechanical scarification followed by seed priming treatment with Bio NPK (5ml/kg seeds) *i.e.,* 1663.33 & 1551.33, respectively. However, the lowest value was observed in absolute Control ($S_1 B_1$). The highest seedling vigour index II was observed in the seeds treated for two to three minutes of mechanical scarification followed by seed priming treatment with Bio NPK (5ml/kg seeds) *i.e.,* 8.25 & 7.85, respectively. However, the lowest value was observed in seed priming treatment with B_3-*Azotobacter chroococcum* (5 ml/kg seeds) ($S_1 B_3$).

Conflict of interest: None

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