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Research Article

Phenotypic evaluation and seed dormancy studies in selected *Stylosanthes seabrana* genotypes

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Abstract

A study of seven *Stylosanthes seabrana* genotypes was conducted during the 2022-23 *kharif* and *rabi* season at ICAR-Indian Grassland and Fodder Research Institute, Dharwad. Additionally, the effect of dormancy-breaking treatments on seed quality was assessed at the Seed Testing Lab of NSP/BSP Unit, University of Agricultural Sciences, Dharwad. Prominent variability was observed in plant height, growth habit, green fodder yield/plant, dry matter yield/per plant and 1000 seed weight. Notably, *S. seabrana*-11595 (G_4) excelled in morphology, germination rate (33.28%), minimal hard seed percentage (62.72%), and seedling attributes, while *S. seabrana*-2534 (G_2) had the highest hard seed percentage (77.53) and least favorable seedling characteristics. For dormancy breaking, several treatments, including FeNPs and concentrated H_2SO_4, were applied to freshly harvested seeds to improve germination and other seed parameters. *S. seabrana* -11595 (G_4) showed the highest germination rate (67.1%) and lowest hard seed percentage (27.59%), benefiting significantly from FeNPs treatment (80 ppm for 12 hours), which effectively reduced hard seed percentages and enhanced seedling length and vigor index. Biochemical analyses indicated that FeNPs treatment increased total dehydrogenase and α-amylase activities in *S. seabrana*-11595 (G_4) and *S. seabrana* -104710 (G_3). The findings highlight the substantial genotypic variability, with G_4 demonstrating superior potential across various traits and benefiting from effective dormancy-breaking treatments.

Keywords: Dormancy, Enzyme activity, Forage legume, Germination, Hard-seededness, Iron nanoparticles.

Introduction

Enhancing food security and alleviating poverty in developing nations depend significantly on the preservation and expansion of genetic resources in food and forage crops. Unfortunately, there is a notable lack of efforts to boost productivity and broaden the genetic diversity of forage crops in tropical, subtropical, mountain, and dryland regions. Preservation of germplasm for key forage genera is inadequately addressed, with only a minimal proportion securely stored in long-term facilities. Identifying, collecting, and evaluating the extensive range of germplasm is crucial. It requires collaboration with traditional guardians, pastoralists, and farmers who have actively preserved and enhanced their forage resources through judicious use and management. In India, livestock farming plays a pivotal role, contributing 25% to agricultural income. Despite a vast livestock population exceeding 535.78 million (Anon, 2019a) and a thriving poultry sector, India paradoxically ranks low globally in milk and livestock product production. This production gap is attributed to issues such as malnutrition and under-nutrition among livestock, which hinder their genetic potential and overall productivity (Anon, 2021). In these challenging environments, the availability of nutritious fodder crops is crucial for livestock sustenance. Among these, legumes are an exceptional source of protein, energy, and essential minerals for cattle.

Stylosanthes, which is closely related to the peanut genus *Arachis*, boasts a rich diversity of over 40 species. In the 1970s, five primary *Stylosanthes* species (*S. scabra, S. hamata, S. viscosa, S. humilis,* and *S. guianensis*) from northern Australia were evaluated across India. More recent developments have led to the exploration of a new

Table 1a. Morphological observations of *S. seabrana* genotypes (qualitative traits)

Genotype		Growth habit	Stem pigmentation	Flower colour	Seed shape	Seed colour
Code	Name (G)					
G1	*S. seabrana* - 2523	Erect	Medium	Pale yellow	Kidney shape with hook	Dark brown
G2	*S. seabrana* - 2534	Erect	Strong	Yellow		Dark brown
G3	*S. seabrana* -104710	Erect	Absent	Pale yellow		Light brown
G4	*S. seabrana* - 11595	Semi erect	Medium	Yellow		Light brown
G5	*S. seabrana* - MPKV Rahuri-1	Semi Erect	Absent	Dark yellow		Light brown
G6	*S. seabrana* - MPKV Rahuri-2	Erect	Absent	Yellow		Light brown
G7	*S. seabrana* - Phule Kranti	Semi erect	Strong	Dark yellow		Light brown

species, *S. seabrana*, which was introduced to India in 1988 under an ACIAR project (Chandra *et al.,* 2006). This perennial legume from Bahia, Brazil, is valued for its ability to fix nitrogen, improve soil fertility, and tolerate drought, making it a promising crop for agriculture. *S. seabrana* has shown promising potential, surpassing *S. scabra* in frost tolerance, essential amino acids, and seedling vigor (Bhatt *et al.,* 2008). However, it faces challenges such as physical dormancy due to a hard seed coat, which affects germination rates (Mott and McKeon, 1982). Since these are minor crops and information on germplasms, morphological characters, variability, etc., is very limited, which hinders the crop improvement programmes. Characterization of germplasm provides vital baseline information on morphological traits, growth behavior, fodder yield, dormancy patterns, and seed quality (Kumar *et al*., 2024). Such information is crucial for the genetic improvement of forage legumes like *Stylosanthes seabrana* and for guiding future breeding programmes (Pereira *et al*., 2011). An important constraint in *Stylosanthes* species is the prevalence of large-scale seed dormancy. Being rangeland legumes, these species commonly exhibit hard seed coat–induced dormancy, which significantly influences germination and establishment. Dormancy, an adaptive trait, allows seeds to remain dormant until favorable conditions arise, promoting species survival in harsh climates (Chibani *et al*., 2006). Overcoming hard seed dormancy in *Fabaceae*, including *S. seabrana*, often involves techniques such as sulfuric acid treatment, hot water, sandpaper, or mechanical methods. Despite these approaches, detailed information on seed dormancy for *S. seabrana* remains limited, highlighting the need for further research to improve seed germination and optimize its use in forage breeding programs. Accordingly, the present study was undertaken.

Materials and Methods

Study site and plant materials: Experiment-I: An experiment was conducted to characterize the seven *S. seabrana* genotypes on the basis of morphology, fodder yield, seed yield and seed quality during kharif and rabi seasons of 2022 and 2023 at ICAR-Indian Grassland and Fodder Research Institute, SRRS Dharwad. The mean weekly maximum and minimum temperatures ranged from 29.6°C to 15.6°C, with a mean relative humidity of 78.4%, and total weekly rainfall of 124.67 mm during the experimental period as per the meteorological observatory at College of Agriculture, Dharwad, during 2022-23. The experimental material was composed of seven Stylosanthes seabrana genotypes (Table 1a). Morphological data were analyzed using the mean values of five randomly selected plants in three replications. A total of 15 traits were recorded, comprising qualitative (descriptive) and quantitative (measurable) parameters. Qualitative traits included growth habit, stem pigmentation, flower colour, seed shape, and seed colour (Table 1a), while quantitative traits comprised plant height (cm), number of primary branches per plant, central leaf length (cm), days to 50% flowering, green fodder yield per plant (g), dry matter yield per plant (g), number of seeds per cluster (a cluster refers to a group of flowers/pods borne together at a single node on the inflorescence axis), test weight (g), number of seeds per plant and seed yield per plant (g) (Table 1b). Observations on morphological traits were recorded at 50% flowering stage, while yield parameters were recorded at harvest maturity. Seed quality parameters were assessed immediately after harvest and after dormancy-breaking treatments. The experiment was conducted by using a randomized block design (RBD) with three replications.

Experiment-II: After the harvest of the fresh seeds of seven *S. seabrana* genotypes were subjected to analysis of seed quality parameters. However, all the genotypes have exhibited severe dormancy and resulted in very poor germination (Table 2). In order

Table 1b. Morphological observations of *S. seabrana* genotypes (quantitative traits)

Genotypes (G)	Plant height (cm)	No. of primary branches/ plant	Central leaf length (cm)	Days to 50% flowering	Green fodder yield/ plant (g)	Dry matter yield/plant (g)	Number of seeds/ cluster	Test weight (g)	Number of seeds/ plant	Seed yield/ ha (kg)
G1	58.00	6	2.37	58	52.30	22.74	8	2.46	3825	268.2
G2	49.50	4	1.67	49	54.65	26.2	9	2.51	3984	285.0
G3	63.00	10	3.10	64	91.70	59.85	10	2.93	5188	433.2
G4	67.80	11	3.15	69	94.80	62.77	10	3.04	5315	460.5
G5	53.00	8	2.17	55	66.70	34.67	9	2.69	4717	361.7
G6	54.00	8	2.27	59	64.75	31.95	9	2.73	4502	350.3
G7	51.50	6	2.20	53	68.80	36.78	9	2.71	4856	375.1
SEM	1.68	0.41	0.08	2.19	2.87	3.07	0.27	0.11	137.67	10.98
CD<0.05	5.17	1.26	0.25	6.76	8.84	6.70	0.83	0.34	424.19	33.82

to break the dormancy, a laboratory experiment was conducted during 2022-23 and 2023-24 assessed at the Seed testing lab of NSP/BSP Unit, University of Agricultural Sciences, Dharwad. Then the freshly harvested seeds of all seven *S. seabrana* genotypes were subjected to different dormancy breaking seed treatments viz. (Table 1c), T_1: Mechanical scarification by using sand paper for 5 min, T_2: Soaking the seeds in hot water treatment at 80°C for 5 minutes, T_3: Soaking of seeds in Cow urine for 24 hours, T_4: Immersion in (98%) Conc. H_2SO_4 for 2 minutes and subsequent washing in water, T_5: Incubation at 80°C for 10 min (hot air oven), T_6: Soaking of seeds in (0.2%) KNO_3 for 24 hours, T_7: Seed soaking in FeNPs (iron nanoparticles) at 80 ppm for 12 hours and T_8: Control (untreated). The 80 ppm FeNPs were synthesized from *Moringa oleifera* in the Green Nanotechnology Laboratory, University of Agricultural Sciences, Dharwad. Seeds were soaked

Table 1c. Treatment details of experiment - II

Treatments (T)	Treatment details
T1	Mechanical scarification for 5 min
T2	Soaking in hot water at 80°C for 5 min
T3	Soaking in cow urine for 24 hours
T4	Conc. H2SO4 (98%) for 2 min.
T5	Incubation at 80°C for 10 min
T6	Soaking in KNO3 (0.2%) for 24 hours
T7	Seed soaking in FeNPs (80 ppm) for 12 hours
T8	Control (Untreated)

Table 2. Initial seed quality parameters of freshly harvested seeds of *S. seabrana* genotypes

Genotypes (G)	Germination (%)	Hard seeds (%)	Dead seeds (%)	Abnormal seedlings (%)	Root length (cm)	Shoot length (cm)	Seedling dry weight (mg)	Seedling vigor index - I	Electrical conductivity (dSm-1)	Field emergence (%)
G1	21.00 (27.27)	73.25 (58.85)	1.75 {1.32}	4 {2.00}	1.9	2.25	18.88	87	0.184	17.6 (24.80)
G2	17.00 (24.35)	77.53 (61.71)	2.67 {1.63}	2.8 {1.67}	2.1	2.11	19.10	72	0.134	12.7 (20.87)
G3	29.00 (32.58)	67.33 (55.14)	2 {1.41}	1.67 {1.29}	2.7	3.10	24.60	168	0.131	26.5 (30.97)
G4	33.28 (35.23)	62.72 (52.37)	1.3 {1.14}	2.7 {1.64}	2.9	3.20	26.14	203	0.115	30.1 (33.27)
G5	15.91 (23.51)	74.59 (59.73)	2.5 {1.58}	7 {2.65}	2.2	2.75	22.66	79	0.146	14.0 (21.97)
G6	25.00 (30.00)	68.40 (55.81)	2.1 {1.45}	4.5 {2.12}	2.18	2.71	23.42	122	0.142	21.3 (27.48)
G7	19.00 (25.84)	74.25 (59.51)	1.75 {1.32}	5 {2.24}	2.4	2.56	21.83	94	0.134	17.2 (24.50)
SEM	0.65	1.10	0.03	0.04	0.05	0.07	0.37	1.33	0.002	0.32
CD<0.01	2.72	4.63	0.12	0.16	0.20	0.30	1.54	5.60	0.007	1.35

Table 3. Effect of dormancy breaking treatments on germination (%) of *S. seabrana* genotypes

Interaction (T×G)	Genotypes (G)							Mean factor II (Treatments)
Treatments (T)	**G_1**	**G_2**	**G_3**	**G_4**	**G_5**	**G_6**	**G_7**	
T1	41.0 (39.8)	39.0 (38.6)	59.4 (50.4)	62.0 (51.9)	51.0 (45.6)	49.0 (44.4)	54.0 (47.3)	50.8 (45.4)
T2	54.2 (47.4)	49.5 (44.7)	68.0 (55.5)	74.7 (59.8)	58.0 (49.6)	44.0 (41.5)	60.6 (51.1)	58.4 (49.8)
T3	37.0 (37.5)	43.9 (41.5)	60.0 (50.8)	63.0 (52.5)	40.0 (39.2)	46.0 (42.7)	51.0 (45.6)	48.7 (44.3)
T4	66.7 (54.7)	71.9 (58.0)	76.7 (61.1)	77.9 (61.9)	70.2 (56.9)	67.6 (55.3)	62.2 (52.4)	69.3 (56.3)
T5	41.0 (39.8)	37.0 (37.5)	72.0 (58.0)	67.2 (55.0)	40.0 (39.2)	33.0 (35.1)	44.0 (41.5)	44.7 (43.7)
T6	47.7 (43.7)	32.0 (34.4)	69.2 (56.3)	72.0 (58.0)	29.0 (32.6)	64.0 (53.1)	45.0 (42.1)	51.3 (45.7)
T7	62.1 (52.0)	66.0 (54.3)	81.0 (64.2)	86.6 (68.5)	70.0 (56.8)	71.0 (57.4)	65.0 (53.8)	70.4 (56.8)
T8	21.0 (27.3)	15.9 (23.5)	29.0 (32.6)	33.3 (35.2)	25.0 (30.0)	17.0 (24.3)	19.0 (25.8)	22.9 (28.6)
Mean Factor I (Genotypes)	46.3 (42.9)	44.4 (41.8)	64.4 (53.4)	67.1 (55.0)	47.9 (43.8)	49.0 (44.4)	48.9 (44.3)	-
	SEM		CD<0.01		CV (%)			
Genotype (G)	0.338		1.25					
Treatment (T)	0.361		1.34		3.56			
Interaction(G×T)	0.956		3.54					

Table 4. Effect of dormancy breaking treatments on seedling length of *S. seabrana* genotypes

Interactions (T×G)	Genotypes (G)							Mean factor II (Treatments)
Treatments (T)	**G1**	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**	
T1	4.65	3.76	5.9	6.14	3.56	4.46	4.26	4.68
T2	5.44	4.61	5.3	6.24	3.96	5.25	5.39	5.17
T3	4.66	3.59	5.9	6.07	4.04	4.97	4.46	4.81
T4	5.52	4.9	3.88	4.41	4.53	4.71	4.48	4.63
T5	4.26	3.86	5.84	6.12	5.72	5.25	4.75	5.11
T6	4.98	4.17	4.34	4.2	3.93	4.95	4.75	4.47
T7	5.11	4.04	6.6	6.95	4.53	5.32	5.22	5.40
T8	5.08	4.16	4.42	4.7	3.42	4.86	4.66	4.37
Mean Factor I (Genotypes)	4.96	4.14	5.27	5.60	4.21	4.97	4.75	-
	SEM		CD<0.01		CV (%)			
Genotype (G)	0.040		0.149					
Treatment (T)	0.044		0.160		4.10			
Interaction (G×T)	0.114		0.423					

in the 80 ppm iron nanoparticles for 12 hours, then the solution was drained out and the seeds are shade dried and used for further studies. Observations on seed quality parameters *viz*., germination (%), hard seeds (%), seedling length, seedling vigor index (Abdul-Baki and Anderson, 1973), enzyme activity of dehydrogenase (Kittock and Law, 1968) and alpha amylase activity (Bernfeld,1955) were taken after exposing to dormancy-breaking treatments to analyze their effect on seed quality. Germination tests were conducted using four replications of 100 seeds per under controlled conditions at 25 ± 1°C with 95± 1°% relative humidity, following ISTA guidelines (Anon, 2019b). Final germination count was recorded on the 10th day. The experiment was conducted using a completely randomized design with three replications. Data was statistically analyzed using SPSS software 25, and values were transformed using the arcsine function and the square root function for seed quality percentages. The significance of differences between pairs of means was evaluated with significance levels set at 5 and 1%, respectively as per Gomez and Gomez (1984).

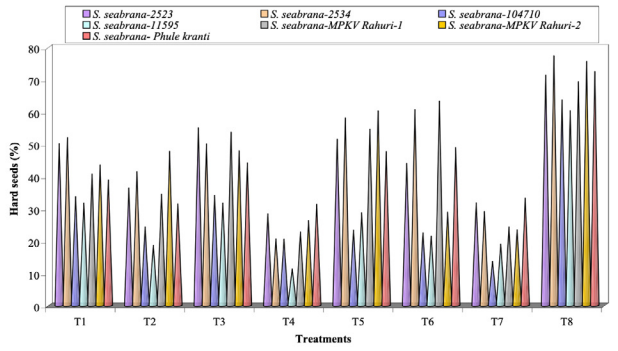

Fig 1. Effect of dormancy breaking treatments on hard seeds (%) of *S. seabrana* genotypes

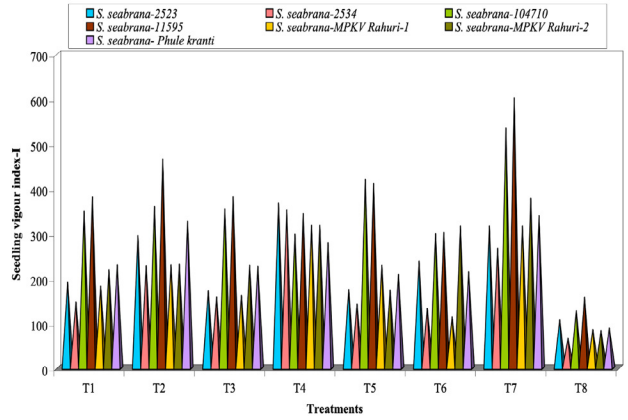

Fig 2. Effect of dormancy breaking treatments on seedling vigor index-I of *S. seabrana* genotypes

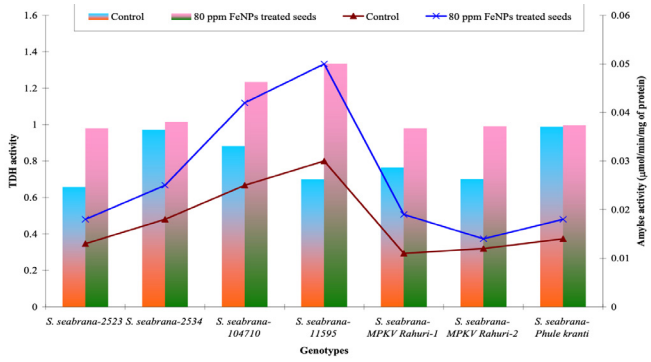

Fig 3. Effect of dormancy breaking treatments on total dehydrogenase activity and amylase activity of *S. seabrana* genotypes

Results and Discussion

The assessed seedling traits were examined in accordance with the UPOV (International Union for the Protection of New Varieties of Plants) guidelines. Since UPOV does not provide specific guidelines for *S. seabrana*, the observations were recorded based on general UPOV descriptors for forage legumes, along with standard descriptors used in *Stylosanthes* characterization studies.

The findings of the study revealed that although certain genotypes displayed similarities in specific traits, they also showcased notable variations in other attributes. Morphological characters were recorded as per Table 1a and 1b. In terms of leaf length, the spectrum spanned from short in *S. seabrana*-2534 to long in *S. seabrana*-MPKV Rahuri-2. Stem pigmentation patterns also exhibited diversity, with strong pigmentation evident in *S. seabrana*-2534 and *S. seabrana* - Phule Kranti. The genotypes were further distinguished by their plant heights, ranging from dwarf varieties to taller ones, while their growth habits were categorized as either erect or semi-erect. The number of primary branches/plant showcased a wide range, with fewer branches in *S. seabrana*-2523 and *S. seabrana*-2534, but a higher count in *S. seabrana*-104710 and *S. seabrana*-11595 (G_4). In terms of flowering duration, the genotypes were classified as early, mid, and late flowering. The color of their flowers ranged from pale yellow to dark yellow, adding to the overall variability (Kumar *et al*., 2024).

Fodder yield exhibited significant diversity, with *S. seabrana*-104710 (G_4) and *S. seabrana*-11595 showing particularly high yields, while others like *S. seabrana*-2534 and *S. seabrana*-2523 displayed lower yields. Dry matter yield/plant also demonstrated variations, with high values recorded in multiple genotypes. Seed traits encompassed a range of color from light brown to dark brown, consistent in *S. seabrana*-2534 (G_2) and *S. seabrana*-2523 (G_1), respectively. All seven genotypes shared a kidney-shaped seed with a hook-like feature. Moreover, the 1000 seed weight differed across the genotypes, with *S. seabrana*-2534 (G_2) and *S. seabrana* - 2523 (G_1) categorized as light-weight, *S. seabrana*-MPKV Rahuri-1 (G_5), *S. seabrana* - Phule Kranti (G_7), and *S. seabrana*-MPKV Rahuri-2 (G_6) as medium-weight, and *S. seabrana*-11595 (G_4) and *S. seabrana*-104710 (G_3) as high-weight (Table 1b). The findings related to various morphological characteristics were in confirmation with Khade (2007) who conducted an investigation on morphological and biological differentiation of *Stylosanthes* cultivars and reported that the most significant differences were noted in terms of plant height, growth habit, green fodder yield/plant, dry matter yield/plant and 1000 seed weight. This phenomenon could potentially be attributed to the positive interplay between the specific genotype of the plants and the prevailing environmental conditions. Since seed yield depends on many factors, *i.e.*, leaf length, branching habit, amount of photosynthesis, synchronization of flowering *etc*.

The initial seed quality parameters exhibited notable variations among the seven genotypes. Significantly highest germination (33.28%), lowest hard seeds (62.72%), minimum number of abnormal seedlings (1.3%), highest root length (2.9 cm), shoot length (3.20 cm), seedling dry weight (26.14 mg), seedling vigor index-I (203),

lower electrical conductivity (0.115 dSm-1) and field emergence (30.1%) was recorded in *S. seabrana*-11595, whereas, significantly lowest germination (15.91%) and highest abnormal seedlings (7%) was observed in (*S. seabrana*-MPKV Rahuri-1). Whereas, *S. seabrana*-2534 recorded the highest hard seeds of 77.53%, the highest dead seeds (2.67%), the lowest root length (2.11 cm), the lowest seedling vigor index-I (72), and the lowest field emergence percentage (12.7%). Likewise, *S.seabrana*-2523 was reported to have the lowest root length and seedling dry weight (Table 3).

The initial seed germination analysis resulted in very poor germination among the genotypes. The genotype *S. seabrana*-11595 recorded the highest (33.28%) germination, followed by *S. seabrana* - MPKV Rahuri-2 (25%) and the lowest (15.91%) germination was recorded in *S. seabrana* MPKV Rahuri- 1.

Influence of seed treatments on dormancy: The results obtained from the impact of different dormancy treatments on seed quality parameters of *S. seabrana* genotypes are presented in Tables 4 & 5 and Figures 1 & 2. The dormancy-breaking treatments have significantly improved the seed germination and improvement was significantly higher (67.1%) in *S. seabrana*-11595; it was on par with *S. seabrana*-104710, which recorded 64.4%, whereas the lowest improvement (44.4%) in germination was observed in *S. seabrana* - 2534 (Table 4). Regarding the treatments, a notably elevated germination (%) of 70.38% over the control was documented for T_7 (seeds soaked in FeNPs at a concentration of 80 ppm for a duration of 12 hours). Following closely behind was T_4 (seeds treated with conc. H_2SO_4 for 2 min), resulting in a germination percentage of 69.30% over its control. Conversely, the lowest germination percentage of 22.88% was conspicuously noted among untreated seeds, denoted as T_8 and depicted in Table 4. This observed phenomenon could be attributed to the potential presence of impermeable seed coats, often linked with the existence of one or more layers of impermeable palisade cells. Notably, in Fabaceae, seed coats that are impermeable tend to possess a single palisade layer. In the context of germination, iron nanoparticles (FeNPs) adhere to the external surface layer of seeds, thus facilitating their penetration into Stylosanthes seeds. This particular interaction ushers in advantageous outcomes for seed germination, a phenomenon underscored by the work of Oskoiee *et al*. (2021). Similar successes have been documented in the context of breaking dormancy caused by hard seed coats, in Stylosanthes species by Bhatt *et al*. (2008). This could be attributed to the gradual and limited release of Fe^{2+} ions, which could be a driving factor for the significant impact of Fe_3O_4 on the germination process, as elucidated by Jayaramudu *et al*. (2017) in mungbean; similar findings were also reported by Tovar *et al*. (2020).

Due to seed dormancy breaking treatments, the hard seeds (%) of the *S. seabrana* genotypes varied significantly (Fig 1). Out of the seven genotypes evaluated, the genotype G_4 (*S. seabrana*-11595) displayed the notably lowest proportion of hard seeds (27.59%). Following closely were G_3 (*S. seabrana*-104710) and G_2 (*S. seabrana*-2534), both recording a hard seed percentage of 29.42 percent. Conversely, the highest percentage of hard seeds (46.02%), was significantly noted in G_1 (*S. seabrana*-2523). Distinct levels of hard-seededness emerge as seeds progress towards maturity and shed moisture to achieve a balance in alignment with the prevalent atmospheric humidity conditions. A high content of hard seeds with variation between lines and accessions has been reported in many species (Tovar *et al*., 2020). Within the scope of treatments evaluated, a notably reduced proportion of hard seeds, amounting 23.90%, was observed in T_4, where seeds were subjected to a treatment involving concentrated H_2SO_4 for a duration of 2 minutes. On a similar note, T_7 which encompassed the immersion of seeds in FeNPs at a concentration of 80 ppm for 12 hours, demonstrated a comparable result with a hard seed percentage of 24.23%. Conversely, the highest proportion of hard seeds, reaching 69.98%, was significantly documented in the case of untreated seeds (T_8).

Out of seven genotypes, the significantly highest seedling length (5.60 cm) was observed in *S. seabrana*-11595, followed by *S. seabrana*-104710, which recorded (5.27 cm) (Table 4). Significantly lower seedling length (4.14 cm) was seen in *S. seabrana*-2534. Among several treatments, significantly maximum seedling length of 5.40 cm was recorded in T_7 (seeds soaked in FeNPs at 80 ppm for 12 hours), significantly minimum seedling length of 4.37 cm was recorded in T_8 (untreated seeds) (Table 4). The increase in germination percentage was accompanied by a corresponding improvement in root and shoot length, indicating enhanced seedling vigor. These results are in agreement with the findings of Joshi and Pant (2010). A similar trend was evident in *Stylosanthes* species, as indicated by the findings of Jesse *et al*. (2011). Regarding the various genotypes, a notably elevated seedling vigor index-I (381) was distinctly noted in *S. seabrana*-11595, followed by *S. seabrana*-104710, which recorded vigor index-I (343). In contrast, the most significantly diminished seedling vigor index-I (187) was observed in *S. seabrana* - 2534 (Table 2).

Interactions of seed treatments and genotypes indicated the highest germination in G_4T_7 (86.6%), followed by G_3T_7 (81%). However, G_6T_8 (17%) recorded the lowest germination. In terms of seedling length highest was in G_4T_7 (6.95), followed by G_3T_7 (6.6), whereas G_2T_1 (3.76) recorded the lowest seedling length (Table or figure?). The correlation of all these factors could influence seed

yield as reported by Boelt *et al.* (2015) in legumes. He opined that seed yield is a genetically complex trait and in the perennial, insect-pollinated forage legumes, it is further highly influenced by environmental conditions and crop management factors. The seed yield is directly affected by branching pattern, growth pattern, and pod photosynthesis. Significantly lower mean vigor index-I (102) was recorded in untreated seeds (T_8). Significantly higher seedling vigor index-I of 602 was observed in G_4T_7 (*S. seabrana*-11595 seeds soaked in FeNPs at 80 ppm for 12 hours), while significantly the lowest seedling vigor index-I of 66 was recorded in G_2T_8 (untreated seeds of *S. seabrana*- 2534) (Fig. 2). Since the FeNPs treatment has shown the best results in terms of seed quality parameters, the iron-nanoparticle treated seeds of all seven genotypes are taken for biochemical analysis along with the control.

The FeNPs treatment has shown a significant influence on both total dehydrogenase activity and α-amylase activity of *S. seabrana* genotypes. G_4 (*S. seabrana*-11595 seeds soaked in FeNPs at 80 ppm for 12 hours) and G_3 (*S. seabrana*-104710 seeds soaked in FeNPs at 80 ppm for 12 hours) showed higher total dehydrogenase activity of 1.334 and 1.233, compared to their respective controls, which have recorded enzyme activity of 0.699 and 0.881. Similarly, G_4 (*S. seabrana*-11595 seeds soaked in FeNPs at 80 ppm for 12 hours) and G_3 (*S. seabrana*-104710 seeds soaked in FeNPs at 80 ppm for 12 hours) recorded significantly higher levels of α-amylase activity. Specifically, G_4 exhibited α-amylase activity of 0.050 μ mol/min/mg of protein, while G_3 displayed α-amylase activity of 0.042 μ mol/min/mg of protein (Fig 3). As a crucial micronutrient, iron (Fe) serves as a pivotal cofactor for a majority of dehydrogenase enzyme complexes engaged in seed germination and nutrient mobilization (Sridhar, 2012). The heightened availability of these micronutrients at the nanoscale, coupled with heightened chemical reactivity, leads to an escalation in the synthesis and activity of dehydrogenase enzymes and α-amylase enzyme (Vijayalaxmi *et al*., 2013; Korishettar *et al*., 2016).

Conclusion

The study revealed significant diversity among *S. seabrana* genotypes in morphological, yield, and seed traits, including variation in growth, flowering, seed color, and 1000-seed weight, with a consistent kidney-shaped seed form. *S. seabrana*-11595 (G4) stood out for its superior morphological characteristics and seed quality parameters. The research underscores the effectiveness of pre-treating seeds with 80 ppm iron nanoparticles (FeNPs) to enhance seed quality, leading to improved germination, root and shoot lengths, and other seed traits. This improvement is attributed to increased α-amylase and total dehydrogenase activities and enhanced protein synthesis, making FeNPs a promising treatment for overcoming dormancy in forage legumes with hard seed coats.

In the future, this approach can be expanded through multi-location trials and validation across a wider range of forage species to assess consistency under diverse agro-climatic conditions. Further studies focusing on optimization of nanoparticle concentration, long-term soil and environmental safety, and integration with seed treatment technologies could facilitate large-scale adoption and contribute to improved forage productivity and sustainable livestock systems.

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