



RESEARCH ARTICLE

Comparing dormancy-breaking treatments for enhancing seed germination in *Tephrosia purpurea* (L.) Pers. under laboratory conditions

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Abstract

Tephrosia purpurea (L.) Pers. (wild indigo) is a leguminous plant valued for its medicinal and soil-enriching properties. However, its propagation is limited by hard seed coat induced dormancy, which restricts water absorption and delays germination. To overcome this constraint, various physical, mechanical and chemical dormancy-breaking treatments are used. The present study was conducted to assess the efficiency of different dormancy-breaking treatments on germination of *T. purpurea*. The study was conducted during 2021 at Dr. M.S. Swaminathan Agricultural College and Research Institute, Tamil Nadu Agricultural University, Thanjavur, Tamil Nadu, India. *Tephrosia purpurea* seeds were exposed to various dormancy-breaking treatments, namely heat, mechanical, chemical and growth regulator applications. The data were subjected to statistical analysis. The study revealed that dormancy in *T. purpurea* is mainly physical due to a hard, impermeable seed coat. Among all treatments, hot water treatment for 6 min was the most effective, resulting in 65 % germination compared with 4 % in the untreated control. Acid scarification (H₂SO₄ for 5 min; 31 % germination) and mechanical scarification (sandpaper for 15 min; 26 % germination) also improved germination but were less effective. Excessive heat or acid exposure damaged embryos, ultimately inhibiting germination. Potassium nitrate (KNO₃) and gibberellic acid (GA₃) treatments showed minor effects on germination, indicating that dormancy in *T. purpurea* is predominantly physical rather than physiological. In conclusion, hot water treatment for 6 min is recommended as a simple, safe and eco-friendly dormancy-breaking method for *T. purpurea* under laboratory conditions.

Keywords: dormancy-breaking treatments; germination; green manure; physical dormancy; *Tephrosia*

Introduction

Current Indian agriculture faces major challenges of land degradation and depleting soil fertility due to continuous crop cultivation and overuse of inorganic fertilisers. This impaired soil health needs to be reclaimed through organic farming which depends on compost and green manures (1). Among various green manure crops, the legume based green manures are more advantageous as they fix atmospheric nitrogen and convert it into an available form to the succeeding crop (2). Within the category of legume-based green manures, the genus *Tephrosia* (3) comprises perennial crops encompassing about 400 species (4), with a growth habit ranging from herbs to shrubs, distributed widely across Asia, Sri Lanka and Africa as a common wasteland weed (5). It holds primary importance as a green manure crop in single cropped wetland conditions of Delta regions. Besides serving as a green manure crop, it also exhibits diverse insecticidal and pharmacological properties (6).

Tephrosia purpurea (L.) Pers. is propagated mainly through seeds. Many plants in the family Fabaceae, including *Tephrosia* species, have hard seed coats that inhibit germination (7, 8). The impermeable seed coat acts as a protective barrier, which prevents water and gases from entering the seed, both of which are essential for germination. In *T. purpurea*, as in many members of the Fabaceae, physical dormancy is primarily associated with a thick, highly lignified and water-impermeable seed coat composed of macrosclereid cells covered by a waxy cuticle. This specialised seed coat restricts water uptake and gaseous exchange, thereby preventing embryo hydration and delaying germination until the seed coat is naturally disrupted by environmental factors such as temperature fluctuations, microbial activity or mechanical abrasion. Consequently, dormancy-breaking treatments such as hot water, acid and mechanical rubbing are intended to alter the integrity of the seed coat, increase its permeability and facilitate water imbibition,

leading to successful germination. This dormancy mechanism is a challenge for growers because it reduces the germination rate and plant population when seeds are sown directly in the field. The natural impermeability of the hard seed coat can result in suboptimal germination rates when these seeds are sown in the field. Nevertheless, this impermeability gradually diminishes over time (9) and only a certain percentage of the seeds will eventually germinate. As a consequence, this particular crop has the potential to persist in the field for many years once initially sown. Farmers are therefore not required to sow seeds annually in the same field, a practice commonly associated with other green manure crops.

Therefore, the presence of a hard seed coat causing dormancy in *Tephrosia* serves as a mechanism for perpetuation. While seed dormancy is generally considered advantageous, it poses challenges for farmers in the modern era of intensive agriculture due to poor germination. Because it is not grazed by cattle, *Tephrosia* is highly sought after as a green manure crop by farmers, especially in arid and semi-arid regions. To ensure uniform germination and a healthy plant population in the field, it becomes necessary to break the dormancy present in the crop. Scientists have employed various seed dormancy-breaking treatments to break the physical dormancy induced by the hard seed coat in different crops including *Albizia schimperiana* Oliv. (10), *Andrographis paniculata* (Burm.f.) Wall. ex Nees (11), *Ziziphus nummularia* (Burm.f.) Wight & Arn. (12), *Acacia nilotica* (L.) Willd. ex Delile (13), *Crotalaria juncea* L. (14), *Elaeocarpus prunifolius* (Müll.Berol.) Wall. ex Mast. (15), *Indigofera tinctoria* L. (16), *Stylosanthes humilis* Kunth (17), *Senna obtusifolia* (L.) H.S.Irwin & Barneby (18), *Sesbania sesban* (L.) Merr. (19), *Vigna sinensis* (L.) Savi ex Hassk. (20), *Medicago* spp (21, 22) and *Tephrosia* spp (23–25)

Although dormancy-breaking treatments such as hot water soaking, acid scarification and mechanical scarification have been reported in several leguminous species, including some members of the genus *Tephrosia*, information on the optimum treatment protocol for *T. purpurea* remains limited. Earlier studies on *Tephrosia* species reported variable germination responses depending on species, seed source and treatment duration highlighting the need for species specific standardisation of dormancy-breaking treatments due to differences in seed coat characteristics (23–26). Consequently, there is no widely recommended dormancy-breaking treatment for *T. purpurea*. Therefore, the present study was undertaken to evaluate chemical, hormonal and physical treatments for breaking seed dormancy in *T. purpurea* under laboratory conditions and to identify the most effective method for improving its seed germination. The findings are expected to provide a simple and reliable protocol for large-scale propagation of this important green manure and medicinal legume.

Materials and Methods

The current study was conducted at the Crop Improvement Laboratory, Dr. M.S. Swaminathan Agricultural College and Research Institute, Tamil Nadu Agricultural University, Thanjavur, Tamil Nadu, India, during 2021.

Seed collection and storage

Fully mature, dry pods of *T. purpurea* were collected from 65 healthy plants to ensure representative seed sampling in March, 2021 from naturally growing plants at Pulianthoppu village, Thanjavur taluk, Thanjavur district, Tamil Nadu, India. The pods were air-dried, manually threshed and the seeds were separated. Damaged and shriveled seeds were removed by visual inspection. The selected seeds were stored in cotton bags under ambient laboratory conditions for one month prior to the experiment to facilitate natural after-ripening before dormancy-breaking treatments were imposed.

Seed sterilisation

Seeds were surface sterilised with 70 % ethanol for 1 min followed by 2.5 % sodium hypochlorite for 3 min and rinsed thoroughly with distilled water (27).

Dormancy-breaking treatments

Prior to the germination test, seeds were subjected to 28 dormancy-breaking treatments (including an untreated control) using standard procedures described previously (28). The treatments employed in the present study are summarised in Table 1. Following the treatments, the seeds were rinsed thoroughly with distilled water, where applicable and immediately subjected to germination testing.

Germination assay

Germination tests were conducted following the International Seed Testing Association guidelines (29). The experiment was laid out in a completely randomised design (CRD) with 3 replications of 25 seeds each (75 seeds per treatment) which commonly used in laboratory seed germination studies to provide reliable estimates of treatment effects while maintaining experimental precision. Seeds were germinated by the roll towel method in a seed germinator maintained at $25 \pm 2^\circ\text{C}$ and 80–85 % relative humidity.

Data collection

Germination was recorded daily for four weeks. A seed was considered germinated when the radicle reached 2–5 mm in length (30). Root length, shoot length, seedling length, seedling dry weight, germination percentage, germination rate index (GRI), mean germination time (MGT), seedling vigour index-I and seedling vigour index-II were recorded using standard procedures (31–33). The corresponding equations are presented in Eq. 1–6.

Table 1. Dormancy-breaking treatments imposed on *Tephrosia purpurea* seeds

Treatment	Description	Treatment	Description
T1	Control (untreated seeds)	T15	25 % H_2SO_4 for 10 min
T2	Dry heat at 50°C for 12 hr	T16	25 % H_2SO_4 for 15 min
T3	Dry heat at 50°C for 24 hr	T17	50 % H_2SO_4 for 5 min
T4	Dry heat at 60°C for 12 hr	T18	50 % H_2SO_4 for 10 min
T5	Dry heat at 60°C for 24 hr	T19	50 % H_2SO_4 for 15 min
T6	Hot water (80°C) for 1 min	T20	0.5 % KNO_3 for 12 hr
T7	Hot water (80°C) for 2 min	T21	0.5 % KNO_3 for 18 hr
T8	Hot water (80°C) for 4 min	T22	0.5 % KNO_3 for 24 hr
T9	Hot water (80°C) for 6 min	T23	1 % KNO_3 for 12 hr
T10	Hot water (80°C) for 8 min	T24	1 % KNO_3 for 18 hr
T11	Sand paper rubbing for 15 min	T25	1 % KNO_3 for 24 hr
T12	Sand paper rubbing for 30 min	T26	GA_3 100 ppm for 24 hr
T13	Concentrated H_2SO_4 (98 %) for 5 min	T27	GA_3 300 ppm for 24 hr
T14	25 % H_2SO_4 for 5 min	T28	GA_3 500 ppm for 24 hr

Seedling length (cm) = Shoot length (cm) + Root length (cm)

Eq. (1)

Germination percentage =

$$\frac{\text{Total number of germinated seeds}}{\text{Total number of Seeds tested}} \times 100 \quad \text{Eq. (2)}$$

$$\text{Germination rate index (GRI)} = \frac{G_1}{1} + \frac{G_2}{2} + \dots + \frac{G_n}{n} \quad \text{Eq. (3)}$$

$$\text{Mean germination time (MGT)} = \frac{\sum(Dn)}{\sum n} \quad \text{Eq. (4)}$$

Seedling vigour index I =

$$\text{Germination (\%)} \times \text{Seedling length (cm)} \quad \text{Eq. (5)}$$

Seedling vigour index II =

$$\text{Germination (\%)} \times \text{Drymatter production (mg)} \quad \text{Eq. (6)}$$

Statistical analysis

Germination percentage data were subjected to arcsine square-root transformation before statistical analysis. Data were analysed using one-way analysis of variance (ANOVA) under a CRD. Treatment means were compared using Duncan's multiple range test (DMRT) at the 5 % probability level (34). Hierarchical cluster analysis was performed using SPSS Statistics based on standardised (Z-score) mean values. Similarity among treatments was determined using Squared Euclidean distance and clusters were generated using Ward's linkage method.

Results

The dormancy-breaking treatments significantly influenced seed germination and seedling vigour in *T. purpurea* (Table 2). Germination commenced on the 7th day after sowing and continued until the 13th day. Significant differences were observed among treatments for germination percentage, GRI, MGT and seed vigour indices (SVI).

Dry heat scarification

Dry heat scarification produced only a slight improvement in germination compared with the control (Table 2). Seeds exposed to 50 °C for 12 and 24 hr recorded germination percentages of 7 %, which were only marginally higher than the control (5 %). Exposure to 60 °C for 12 hr significantly improved germination to 12.00 %, with a GRI of 0.34 and seed vigour indices of 303.33 (SVI-I) and 39.64 (SVI-II). However, seeds treated at 60 °C for 24 hr failed to germinate.

Wet heat (hot water) scarification

Hot water treatment was the most effective dormancy-breaking method among all treatments tested. Germination percentage increased progressively with increasing treatment duration and reached a maximum of 65 % after 6 min of exposure. This treatment also recorded the highest GRI (2.14), SVI-I (1764.67) and SVI-II (161.76). The mean germination time was reduced to 7.69 days. A slight reduction in germination was observed when the treatment duration was extended to 8 min (Table 2).

Mechanical scarification

Mechanical scarification improved germination over the control (Table 2). Seeds scarified for 15 min recorded 29 % germination, a GRI of 0.82 and SVI-II of 173.98. However, prolonged rubbing for 30 min reduced germination to 9 %.

Chemical scarification

Responses to sulphuric acid (H₂SO₄) treatments varied with acid concentration and exposure duration. Concentrated H₂SO₄ for 5 min produced the highest germination (31 %) among chemical treatments, along with vigour indices of 615.87 (SVI-I) and 176.64 (SVI-II). Increasing acid concentration or extending exposure duration reduced germination substantially, with treatments involving 25 % and 50 % H₂SO₄ for more than 10 min resulting in germination below 6 %.

Chemical dormancy-breaking treatments

Potassium nitrate (KNO₃) treatments at 0.5 % and 1 % concentrations for 12 and 24 hr were ineffective, producing germination percentages ranging from 4 to 5 %, similar to the control (Table 2). The highest germination (9 %) was obtained with 100 ppm gibberellic acid (GA₃), while higher concentrations did not further improve germination (Table 2).

Discussion

The present study demonstrated that seed dormancy in *T. purpurea* is primarily associated with a hard and water-impermeable seed coat. The effectiveness of dormancy-breaking treatments varied considerably depending on their ability to weaken the seed coat without causing embryonic damage.

Dry heat scarification produced only a modest improvement in germination. The increase observed at 60 °C for 12 hr may be attributed to partial loosening of the seed coat, which enhanced permeability to water and gases. In contrast, no germination was observed following exposure to 60 °C for 24 hr. Although this may suggest that prolonged heat exposure adversely affected seed viability or embryo function (35), seed viability was not assessed using a tetrazolium or other viability test. Therefore, it cannot be conclusively established whether the seeds were non-viable or remained dormant after treatment. This response indicates that moderate dry heat was sufficient to increase seed coat permeability by partially disrupting the compact palisade layer without causing extensive structural damage, whereas prolonged exposure likely exceeded the thermal tolerance of the seed tissues. Similar responses have been reported in *Lupinus polyphyllus* Lindl. (36), where dry heat improved germination only within an optimum exposure range. These findings support earlier observations that controlled thermal treatments can alleviate physical dormancy in legumes by softening the seed coat (35, 37).

Among all treatments, hot water scarification proved most effective in enhancing germination and seedling vigour. The progressive increase in germination up to 6 min of exposure suggests that hot water effectively disrupted the physical barrier imposed by the seed coat. The reduced mean germination time further indicates improved water imbibition and rapid metabolic activation. Similar beneficial effects of hot water scarification have been reported in *Tephrosia* species (23–25), *A. schimperiana* (10) and *C. juncea* (14). However, although the general response to hot-water treatment was consistent across the genus, the optimum exposure

Table 2. Effect of seed dormancy-breaking treatments in *Tephrosia purpurea*

Sl. No.	Treatments	Germination percentage (%)	Germination rate index	Mean germination time (days)	Seed vigour index I	Seed vigour index II
1	Control	5fg	0.13efg	10.50a	58.67f	20.80def
2	Oven dry 50 °C for 12 hr	7efg	0.20efg	9.00abcdef	134.40ef	33.07def
3	Oven dry 50 °C for 24 hr	7efg	0.21efg	8.00ef	61.20f	37.32def
4	Oven dry 60 °C for 12 hr	12e	0.34e	8.75bcdef	303.33e	39.64def
5	Oven dry 60 °C for 24 hr	0h	0.00g	0.00g	0.00f	0.00f
6	Hot water for 1 min	28d	0.87d	8.21def	615.07d	77.51cdef
7	Hot water for 2 min	41c	1.32c	7.91ef	1029.73c	78.73cdef
8	Hot water for 4 min	52b	1.67b	7.82f	1370.53b	132.78abc
9	Hot water for 6 min	65a	2.14a	7.69f	1764.67a	161.76ab
10	Hot water for 8 min	53b	1.65b	8.16def	1196.80bc	105.01abcd
11	Sand paper rubbing for 15min	29d	0.82d	9.01abcdef	561.33d	173.98a
12	Sand paper rubbing for 30 min	9ef	0.27ef	8.72bcdef	174.00ef	83.81cdef
13	Conc. H ₂ SO ₄ for 5 min	31d	0.94d	8.23def	615.87d	176.64a
14	H ₂ SO ₄ 25 % for 5 min	8efg	0.23ef	8.61cdef	99.60ef	93.88bcde
15	H ₂ SO ₄ 25 % for 10 min	5fg	0.14efg	9.50abcde	78.40f	38.41def
16	H ₂ SO ₄ 25 % for 15min	5fg	0.14efg	9.50abcde	66.00f	29.93def
17	H ₂ SO ₄ 50 % for 5 min	4g	0.10fg	9.67abcd	91.33ef	35.83def
18	H ₂ SO ₄ 50 % for 10 min	5fg	0.16efg	8.50cdef	89.07f	17.03ef
19	H ₂ SO ₄ 50 % for 15 min	5fg	0.16efg	8.50cdef	124.53ef	180.21a
20	KNO ₃ 0.5 % for 12 hr	4g	0.11fg	9.00abcdef	42.93f	37.53def
21	KNO ₃ 0.5 % for 18 hr	4g	0.10fg	10.00abc	63.07f	25.15def
22	KNO ₃ 0.5 % for 24 hr	4g	0.10fg	10.33ab	54.00f	24.30def
23	KNO ₃ 1 % for 12 hr	5fg	0.16efg	8.33def	118.53ef	41.30def
24	KNO ₃ 1 % for 18 hr	5fg	0.14efg	9.50abcde	75.87f	52.61cdef
25	KNO ₃ 1 % for 24 hr	4g	0.12fg	8.67cdef	62.00f	35.60def
26	GA ₃ 100 ppm for 24 hr	9ef	0.27ef	8.94abcdef	133.20ef	80.42cdef
27	GA ₃ 300 ppm for 24 hr	4g	0.12fg	8.67cdef	23.47f	34.55def
28	GA ₃ 500 ppm for 24 hr	5fg	0.18efg	7.67f	101.60ef	64.03cdef
	SEd	3.00	0.09	0.68	90.70	34.60
	CD (P=0.05)	6.02	0.18	1.35	181.71	69.31

Means followed by the same letter(s) within each column are not significantly different at $p = 0.05$ according to Duncan's multiple range test (DMRT). Germination percentage data were arcsine-transformed [$\arcsin\sqrt{(p/100)}$] prior to analysis of variance; treatment means in the table are presented on the original percentage scale. DMRT grouping letters were derived from the arcsine-transformed ANOVA for germination percentage and from untransformed ANOVA for the remaining parameter.

duration differed among species, indicating species-specific differences in seed coat characteristics and dormancy intensity. The present study therefore identifies hot-water treatment for 6 min as an effective dormancy-breaking method specifically for *T. purpurea*. The enhanced germination is likely due to the formation of microcracks in the palisade layer of the testa, thereby increasing seed coat permeability and facilitating rapid water imbibition, gas exchange and subsequent metabolic activation of the embryo. The slight decline observed after 8 min of exposure suggests the onset of heat-induced embryo damage. Therefore, hot water treatment for 6 min appears to be the optimum method for overcoming dormancy in *T. purpurea*.

Mechanical scarification also improved germination, particularly at 15 min of sandpaper rubbing. The enhancement can be attributed to localised abrasion of the seed coat, which facilitates localised disruption of the impermeable seed coat, thereby increasing permeability to water and oxygen required for germination (28). However, prolonged scarification for 30 min reduced germination, indicating damage to the testa and embryo. Similar observations have been reported in lucerne (21, 22) and *Clitoria ternatea* L. (38), where moderate scarification enhanced germination while excessive abrasion reduced seed viability. Although effective, mechanical scarification may have limited

practical utility for large-scale seed treatment because of its labour-intensive nature and variability in abrasion depth.

Chemical scarification with concentrated H₂SO₄ for 5 min effectively improved germination, probably by etching the palisade layer of macrosclereids and increasing seed coat permeability by partially eroding the macrosclereid layer while maintaining embryo viability. Similar findings have been reported in *Medicago falcata* L. (21) and *C. ternatea* (38) where longer exposure periods and higher acid concentrations markedly reduced germination, indicating embryo toxicity and excessive thinning of the seed coat. However, concentrated H₂SO₄ failed to enhance germination in castor (*Ricinus communis* L.) seeds (39). In contrast, KNO₃ treatments failed to enhance germination. Although nitrate compounds are known to stimulate germination in physiologically dormant seeds through activation of nitrate-responsive metabolic pathways (40), their ineffectiveness in the present study suggests that dormancy in *T. purpurea* is predominantly physical rather than physiological. Similar observations were reported in *S. obtusifolia* (18).

The superior performance of hot-water scarification compared with H₂SO₄ treatment may be attributed to its more controlled modification of the seed coat. Exposure to hot water probably induced localised microfractures in the palisade layer, allowing gradual water uptake while preserving embryo viability. In

contrast, H_2SO_4 removes the protective seed coat through chemical erosion and even slight increases in concentration or exposure duration may result in excessive thinning of the testa and diffusion of acid into the embryo, thereby reducing seed viability. The rapid decline in germination observed with prolonged acid exposure in the present study supports the greater sensitivity of *T. purpurea* embryos to chemical injury than to short-duration thermal treatment. These findings indicate that controlled thermal scarification provides a wider margin of safety for overcoming physical dormancy while minimising embryonic damage.

The GA_3 treatments produced only marginal improvements in germination, with no additional benefit from increasing concentration. Comparable results were reported in *Sorbus domestica* L. and *Sorbus torminalis* (L.) Crantz, where exogenous GA_3 had minimal effects unless combined with prior physical scarification (41). The GA_3 primarily promotes embryo growth potential rather than permeability alteration (42). These findings concur with reports from other leguminous species where combined mechanical and hormonal treatments produced the highest germination (26). The limited response to GA_3 further supports the conclusion that dormancy in *T. purpurea* is mainly due to seed coat impermeability rather than hormonal or physiological constraints. Since GA_3 promotes embryo growth and enzyme activation primarily in physiologically dormant seeds, its low effectiveness in the present study indicates that seed coat restriction remains the principal barrier to germination (15).

The study confirmed that physical dormancy caused by a hard, impermeable seed coat is the main dormancy mechanism in *T. purpurea*. Among the treatments tested, hot-water scarification for 6 min was the most effective, producing the highest germination, faster germination and greater seedling vigour. The results provide a simple and effective dormancy-breaking protocol for the propagation and utilisation of *T. purpurea* as a green manure and medicinal plant. However, as the study was conducted under controlled laboratory conditions using a single seed lot, further validation across different seed lots and field environments is required. Ecologically, physical dormancy enables seeds to remain viable in the soil seed bank until favourable conditions promote germination, contributing to species persistence under variable environmental conditions.

Hierarchical clustering of seed dormancy-breaking treatments in *Tephrosia purpurea*

The cluster dendrogram (Fig. 1) groups the 27 seed dormancy-breaking treatments and the control based on their similarity across multiple traits viz., germination percentage, GRI, MGT and SVI. The left most lower branch forms a distinct cluster comprising the hot-water treatments for 6 min, 4 min and 8 min, which performed very similarly and emerged as the most effective dormancy-breaking methods in *T. purpurea*. A second cluster includes the hot water treatments for 1 and 2 min, sandpaper rubbing for 15 min and concentrated H_2SO_4 for 5 min. These treatments recorded moderate improvements in germination and vigour, performing better than the control but less effectively than the 6 min hot water treatment. They appear to partially break physical dormancy but with lower consistency. The third and largest cluster consists of all remaining treatments along with the control which exhibited very low and slow germination coupled with poor vigour indices. These treatments did not effectively break the hard seed coat and therefore failed to overcome physical dormancy.

Conclusion

The present investigation clearly demonstrates that dormancy in *Tephrosia purpurea* is primarily physical, imposed by a hard and water impermeable seed coat. Among the treatments evaluated, hot water treatment for 6 min proved the most effective, recording the highest germination, fastest germination rate and superior seed vigour indices. Concentrated sulphuric acid (H_2SO_4) for 5 min and sand paper rubbing for 15 min also showed significant improvement but were less efficient compared to hot water treatment. Prolonged or high intensity heat and acid exposures caused severe embryo damage and completely inhibited seed germination. Treatments with potassium nitrate (KNO_3) and gibberellic acid (GA_3) showed limited enhancement and were comparatively less effective in breaking dormancy. Therefore, hot water treatment for 6 min can be recommended as an effective laboratory-based dormancy-breaking protocol for *T. purpurea*. However, additional validation under nursery and field conditions using different seed lots, seasons and environments is required before this treatment can be recommended for large-scale agricultural propagation.

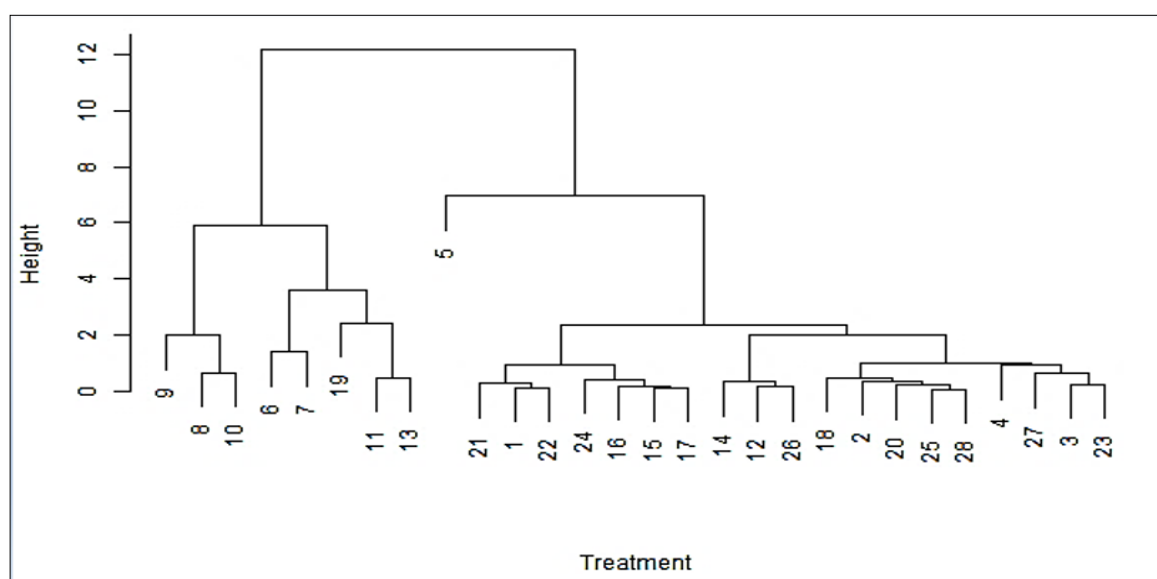


Fig. 1. Hierarchical clustering of seed dormancy-breaking treatments in *T. purpurea*.

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Authors' contributions

AS and TC carried out the experiments, collected and analysed the data and drafted the manuscript. KV and UM conceptualised the study, designed the experiments and performed the statistical analyses. SM¹, PD and SM² participated in the study design. All authors have read and approved the final manuscript [SM¹ stands for Selvamurugan M and SM² stands for Sabapathi M].

Compliance with ethical standards

Conflict of interest: Authors do not have any conflicts of interest to declare.

Ethical issues: None

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