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Enhancing seed germination and seedling vigour of Himalayan wild cherry (*Prunus cerasoides* D. Don) through chemical, hormonal and organic pre-sowing treatments

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Abstract
Poor seed germination caused by physical and physiological dormancy is a major constraint in the propagation and conservation of Himalayan wild cherry (*Prunus cerasoides* D. Don). The present study aimed to optimize different pre-sowing treatments for enhancing seed germination, seedling vigour, and early growth performance of this ecologically and economically important forest tree species. Seeds were collected from nine natural populations representing different altitudinal ranges of Himachal Pradesh, India, and subjected to chemical (sulfuric acid), hormonal (gibberellic acid, GA_3), and organic (cow dung slurry and Beejamrit) treatments during 2023 and 2024 under glasshouse conditions. Germination and seedling performance were evaluated for germination percentage, germination index, mean germination time, germination energy, seed vigour indices, and seedling growth traits. Among all treatments, sulfuric acid scarification at 15% for 20 min significantly improved germination percentage, seed vigour indices, and overall seedling performance, indicating effective removal of mechanical dormancy imposed by the hard endocarp. GA_3 at 500 ppm for 24 h also proved highly effective by enhancing germination rate and seedling growth, reflecting its role in overcoming physiological dormancy. Organic treatments, particularly cow dung slurry, showed promising results by improving germination energy and seedling vigour, offering a low-cost and eco-friendly alternative for nursery practices. The study demonstrates that a combination of chemical, hormonal, and organic pre-sowing treatments can substantially enhance the regeneration potential of *P. cerasoides*, providing practical implications for large-scale nursery production, restoration programs, and conservation of this threatened Himalayan species.

Keywords ·Seed dormancy · Pre-sowing treatments · Sulfuric acid scarification · Gibberellic

acid · Cow dung slurry · Beejamrit

Introduction-

Prunus cerasoides D. Don, commonly referred to as the Himalayan wild cherry, is a member of the Rosaceae family and holds a prominent position among all temperate fruits. It is a deciduous tree that can reach a height of ten meters. Its reddish-brown bark exfoliates in thin, circular stripes. Pinkish-white flowers are a good source of food for bees. Compared to traditionally consumed crops, these wild fruits may be a better source of nutrients [13]. In the temperate Himalayan region, which stretches from Kashmir to Bhutan, autumn-flowering wild cherries are abundantly found on all kinds of rocks and soil at elevations between 1100 and 2300 meters. The primary use of trees is as rootstock for cherry production. The herb is utilized in several ayurvedic compositions and has considerable medicinal value in traditional medical systems. Cherry brandy is made from the pulp of the edible fruit [21]. This species prefers areas with moderate light and shade. Bisexual flowers appear in rose-red fascicles that eventually turn almost white. Flowering from October to November, it has five sepals, five petals, and many stamens that can be found alone or in umbels of two to six, or occasionally more, racemes. The leaves are unlobed, lanceolate, simple, and alternating. At the base of the petiole are 2-4 glands. Fruiting takes place from December to February, and it ripens between March and April. Heartwood is reddish brown, tightly grained, and somewhat hard, whereas sapwood is white and shiny. It can withstand attacks by insects and fungi. Endemic to the Himalayan region, *Prunus cerasoides* is a rare and endangered deciduous tree [57]. Sometimes they can only be propagated by seed, and their continued survival will be threatened if they are hard to germinate. *Apis* species that visit *Prunus cerasoides* flowers in India in quest of nectar and pollen include *A. cerana, A. indica, A. dorsata, A. florae,* and *A. mellifera* [23]. Food, medicine, fuel, fodder, lumber, colors, and tannins can all be found in trees. Because its gum has antioxidant qualities, it is used in various religious events as well as the pharmaceutical business [29]. In addition to being utilized as a uterine tonic, it is used to treat skin conditions and improve complexion. Bark paste is used to treat forehead hemicranies, fractured bones, burns, indigestion, oral illnesses, wound healing, and more. It is antimicrobial, antibacterial, and anti-inflammatory. Kidney stones are treated with stem or bark, and joint pain is relieved by a bark infusion. Cynogenetic material is found in leaves, twigs, bark, and kernels. The oil found in kernels has a strong prussic acid flavor and resembles bitter almond oil. Ripe fruits taste astringent and can be eaten. Gum tragacanth can be replaced by chewing gum that exudes from the trunk and branches. On rare occasions, heartwood is utilized for construction and decorative furniture. Because their extract is used to treat urinary and prostate problems, leaves are well recognized for their diuretic qualities. Leaves can be used to make a green dye. Fruits improve digestion, and when consumed internally after being crushed and soaked in water, stems and branches prevent abortion. They are also used to treat scorpion stings. Branches and stems can aid with vomiting, leukoderma, and leprosy. Young twigs are utilized as toothbrushes and leaves as feed [42]. *Prunus cerasoides* is considered as an excellent tree species for the restoration of evergreen forest in seasonally dry tropical forest lands [33].

Poor seed germination remains a significant issue in many forest species. Germination inhibitors such benzoic acid, cinnamic acid, coumarin, naringenin, jasmonic acid, and abscisic acid (ABA) that are present in the seed coat are often the source of this. The use of these crops as rootstocks, which are necessary for budding and grafting procedures, is hampered by their poor germination rates, long germination times, and slow seedling growth rates. Pre-sowing treatments are being investigated to improve germination and subsequent seedling growth since dormancy or hard seed

coats are frequent causes of failed seed germination. Increasing seed coat permeability and improving germination rates are the goals of techniques like scarification or soaking in solutions like KNO3, urea, hot or cold water, thiourea, cow urine, cow dung slurry or GA3. These pre-sowing treatments weaken seed coverings, increase maximum germination percentages, shorten germination times, and improve embryo growth potential. Additionally, in addition to scarification, the use of plant growth regulators such as IBA and IAA in suitable concentrations can control growth behaviour and improve seed germination and seedling growth. Dormancy is a barrier to germination in some forest species that can be caused by either internal or external influences [43]. Tough and hard seed coverings can cause external dormancy, while internal seed inhibitors can prevent germination until they are eliminated. There are a number of ways to break dormancy, such as removing the seed coat, which encourages early germination by reducing mechanical limitations on embryo growth. According to [8], the extracted kernels germinated earlier than those that were sown with seedcoat because the kernels can directly absorb water, whereas the kernels with seedcoat took longer to germinate because the woody seed coat prevented the seed from absorbing water. Following the softening of the seed coat, germination began when water entered the seed coat. Additionally, the seed germinated earlier when soaked in GA3 at 100 ppm. Similarly, it was found that removing the seed coat reduced the amount of time needed for germination [31] as stony endocarp functions as a barrier to inhibitor escape [34].

Abbas et al. [1] found that unhusked seeds germinated later than husked seeds. Peach rootstock seed germination was increased by mechanically rupturing the seed coat [51]. Parvin et al. [36] noted better black walnut seed germination with cooling and GA3 treatment.

The percentage of seeds in a sample that truly sprout, regardless of time, is known as germination capacity. The percentage of seeds in a sample that have germinated up until the point at which the rate of germination reaches its maximum is known as the germination energy. The amount of time it takes for a seed to germinate is called the mean germination time [9].

Environment influences and genetic differences cause large variation in seed development and dimensions even within a single species. The seed size constitutes a significant factor in germination and early stage of plant growth [56]. Study of altitudinal variations is very important to find out variations in seed and effect of certain environmental conditions on germination. For most species significant altitudinal clines for growth were revealed. Despite the proximity of populations in their natural area, differences in altitude led to genetic differentiation in their phenology and growth [55]. Seed dormancy is the temporary inability of a viable seed to germinate under favourable environment conditions. There are a number of techniques to break seed dormancy, including stratification, chemical scarification (sulfuric acid), mechanical scarification (for impermeable seed coat), and treatment with growth regulators (gibberellin, cytokinin, ethylene, etc.). In general, these techniques are very helpful to increase seed germination [26].

Prunus cerasoides has poor regeneration in it's natural habitat due to it's hard seed coat and study on seed germination enhancement is lacking [50]. Presence of endocarp in form of hard and bony structure enclosing delicate seed which appears to be hermetically sealed and thus become interesting matter of observation and study. Prunus seeds often have exogeneous (mechanical) dormancy due to it's stony endocarp which restricts germination and they also have embryo dormancy which require period of after ripening in presence of moisture and oxygen to overcome it [16, 17, 54]. The goal of the current study was to provide a uniform pre-sowing treatment to enhance *P. cerasoides* reproduction, restoration, and conservation efforts in Himalayan regions.

Materials and methods

Study area

The study was carried out in the glasshouse of the Department of Tree Improvement and Genetic Resources at the Dr. Yashwant Singh Parmar University of Horticulture and Forestry in Nauni, Solan, Himachal Pradesh, India, between 2023 and 2024. The experimental location was situated in the mid-hill zone of Himachal Pradesh at 30°51′ N latitude and 77°09′ E longitude, with an elevation of 1388 meters above mean sea level. Mean annual rainfall of 1000–1300 mm, moderate temperatures and humidity, the area is ideal for controlled nursery experimentation. Nine wild populations from Himachal Pradesh's three altitudinal regions provided the seeds of *Prunus cerasoides* (Fig 1). To avoid selection of closely related individuals, three phenotypically superior and healthy trees were chosen from each site, with a minimum of 500 meters between trees. The geographic coordinates and altitude of each tree were recorded using a GPS-GPSMAP-67. The mature fruits were collected in April, and the seeds were de-pulped, cleaned, and shade-dried for ten to fifteen days. All damaged, undersized, and infected seeds were discarded before research.

Preparation and application of treatments

Ten treatments were examined in 2023, whereas thirteen treatments, including Beejamrit, were tested in 2024 (Table 1).

Water and fresh cow dung were combined to create a homogenous slurry. Depending on the treatment, seeds were placed in paper bags or muslin cloth and submerged in the slurry for two, four, or six days. Before being sown, seeds were carefully cleaned with clean water and allowed to dry in the shade. 5%, 10%, and 15% solutions were made by diluting concentrated sulfuric acid. The seeds were gently stirred while submerged in the acid solution for 20 minutes. Following treatment, seeds were air-dried in the shade after being repeatedly cleaned under running water to get rid of any remaining acid. Distilled water was used to create solutions of 500, 1000, and 1500 ppm of gibberellic acid (GA_3). Seeds were steeped in the appropriate solutions for 24 hours at room temperature and then surface-dried prior to sowing. Beejamrit was produced following traditional formulations by blending 5 kg fresh cow dung, 5 litres cow urine, 10 litres water, and 50 g lime. The mixture was stirred and left for fermentation upto one day. Before being sown, seeds were shade-dried after being soaked in Beejamrit for five, ten, or fifteen minutes. Pro-trays with a sterilized growing medium made up of soil, sand, and farmyard manure (FYM) in a 2:1:1 ratio were used to sow both treated and untreated seeds. The trays were set up in a controlled environment within the glasshouse. Depending on the humidity and temperature, regular irrigation was given once or twice a day. To reduce environmental diversity, all trays were subjected to the same cultural practices.

Observations

- Germination (%) $= \frac{\text{Number of seeds germinated}}{\text{Total seeds sown}} \times 100$
- Germination energy: Proportion of seeds germinated up to the day of peak germination.
- Germination index (GI) $= \frac{\text{Number of seeds germinated}}{\text{Day of first count}} + \frac{\text{Number of seeds germinated}}{\text{Day of final count}}$
- Mean germination time(MGT) $= \frac{\sum(n_i \times t_i)}{\sum n_i}$, where $n_i$is the number of seeds germinated on day t_i.

- Seed vigour index I (SVI 1) = Germination (%) × Seedling length (cm)
- Seed vigour index II (SVIII) = Germination (%) × Seedling dry weight (g)
- Root length and shoot length
- Seedling length= Root length + Shoot length
- Collar diameter, fresh and dry weights of seedling, root, and shoot, root–shoot ratio.

  Seedlings were oven-dried at 70 ± 2 °C until constant weight was achieved for dry weight estimation. Germinated seedlings were transplanted into nursery beds, and survival percentage after the first transplant was recorded to evaluate establishment success.

Statistical analysis and Experimental design

Experiment was set up using a completely randomized design (CRD). Each treatment was performed three times using seeds from each site. Standard statistical techniques were used to do analysis of variance (ANOVA) on all the data. Before being analysed, the germination percentage data were arcsine transformed. At the 5% level of significance, treatment averages were compared using the critical difference (CD). Where appropriate, interaction effects between seed sources and treatments were also examined.

Results

Morphological variation among selected trees

Significant variance was reported among seed sources for tree height and DBH. Tree height ranged from 5.88 to 8.00 m, with Churwadhar presenting the tallest trees and Thunag the shortest. DBH varied from 20 to 45 cm, maximum at Daroo and minimum at Jubarhatti. The weight of seed collected ranged from 325.14 to 337.68 mg, with Kunihar having the lowest weight and Thunag having the highest. The seed lengths in Thunag and Arki were the longest (10.20 mm) and shortest (11.40 mm), respectively. Thunag had the longest seed length (10.20 mm), while Arki had the shortest (11.40 mm). Arki and Kunihar had the narrowest (8.00mm) seeds, while Thunag (8.10 mm) had the widest (Figure 2).

Germination behaviour (2023)

There were significant differences in the germination percentage for pre-sowing treatments, sites, and their combination suggesting impact of site factors on the response to seed treatments. Out of all the treatments, treating seed with H_2SO_4 at 15% for 20 min (T6) had the highest germination percentage (36.59%). The seeds collected from Bandla (61.33%), Nohradhar (42.67%), Churwadhar (40.00%), and Kunihar (40.00%), this treatment showed remarkable effectiveness in breaking seed dormancy. The other effective treatment were treating seeds with cow dung slurry for 2 days (T1), 500 ppm GA_3 for 24 h (T6) and 10% H_2SO_4 for 20 min (T5) with a germination percentage of 35.56%, 34.96% and 33.04%, respectively (Figure 3). In contrast, poor germination (10.37%) was the outcome of prolonged cow dung slurry treatment (T3: 6 days), showing deleterious impact on seed viability due to prolonged exposure. The control treatment (T10) had the lowest germination rate (9.93%), indicating the presence of intrinsic dormancy and the necessity of pre-sowing interventions to enhance germination. The highest germination rate (29.60%) was seen in Bandla (S3). Thunag (S7), on the other hand, had the lowest germination rate (19.60%), suggesting less favourable edaphic or environmental conditions for seed germination. The treatment × site interaction revealed uneven treatment performance; H_2SO_4 at

15% for 20 min (T6) was most successful in Bandla, but cow dung slurry for 2 days (T1), and T7 were more effective at mid-altitude locations like Daroo and Churwadhar. This emphasizes the necessity of choosing pre-sowing treatments according to the site. T3 (cow dung slurry for six days) showed the highest germination energy (0.45), indicating the fastest and most consistent germination response. The lowest germination energy levels (0.27 each) were recorded by Bandla (S3) and Shalana (S4), indicating delayed germination (Figure 4). Strong site reliance was evident in the substantial treatment × site interaction, with best performance in T6 from Arki (0.53) and T8 from Kunihar (0.50), and minimum germination energy values for T1 at Daroo (0.09). The greatest germination index (0.49) was observed by 10% and 15% H_2SO_4 for 20 min (T5, T6) indicating a higher rate of germination. In instance, T6 demonstrated exceptional performance at Bandla (0.77), Nohradhar (0.56) underlining its efficiency in overcoming seed dormancy. The highest germination index (0.40) was recorded by Bandla (S3) and Daroo (S6), suggesting that the conditions at these sites were relatively more favourable (Figure 5). Treatments had a substantial effect on the mean germination time. However, the effect of treatment × site interaction was non-significant, indicating that site-specific environmental variables had no effect on the response pattern of treatments. T6 (H_2SO_4 15% for 20 min) and control had comparatively lower mean germination time values (17.66, 17.04) than the other treatments, indicating that the greater acid concentration causes faster germination (Figure 6). T6 (H_2SO_4 15% for 20 min) was statistically superior to most other treatments and had the highest seed vigour index 1 (402.55) among the treatments. It showed exceptionally high values at Bandla (692.61) and Nohradhar (440.01) confirming its strong effectiveness in enhancing seed vigour through efficient dormancy breaking (Figure 7).

Seedling growth performance (2023)

The tallest seedling length (7.03 cm) was reported by T4 (H_2SO_4 5% for 20 min), which was statistically higher than seedlings germinated with other pre-sowing treatments and found good performance in seedlings raised from seeds collected from Daroo (7.74 cm), Nohradhar (7.71 cm), Churwadhar (7.70 cm), and Thunag (7.07 cm). Seedlings from seed collected from Jubarhatti (S5) recorded the greatest length (6.86), followed by Shalana (S4: 6.60 cm) and Kunihar (S2: 6.58 cm) as shown in (Figure 8). The shoot length recorded after establishment of seedlings showed highest (55.26 cm) in T4 (H_2SO_4 5% for 20 min) achieved the highest shoot length (55.26 cm). In terms of shoot length, Thunag (S7) had the greatest value (39.80 cm), followed by Jubarhatti (38.23 cm), Arki (37.97 cm), and Daroo (37.93 cm) as shown in (Figure 9). The need for location-specific treatment recommendations was highlighted by the significant treatment × site interaction, which showed site-specific responses, with T4 recording maximum performance at Arki and Jubarhatti and T1 at Daroo and Shalana, while T5 was highest at Kunihar but showed minimum performance at Nohradhar and Churwadhar. However, collar diameter and plant survival percentage following the first transplant showed non-significant treatment, site, and treatment × site effects, indicating consistent response across sites and treatments.

Germination behaviour ((2024)

The majority of germination and seed vigour characteristics were significantly impacted by the pre-sowing treatments implemented in 2024. Germination percentage revealed substantial variation among treatments, increasing from a low of 12.67% in the control (T13) to a maximum of 55.33% in seeds treated with 500 ppm GA_3 for 24 h (T7), showing the efficiency of gibberellic acid in promoting germination. With a value of 4.13, the germination index, which measures both

the rate and uniformity of germination, peaked with 10% H_2SO_4 for 20 minutes (T5). Treating seeds with 500 ppm GA_3 (T7), 1500 ppm GA_3 (T9), Beejamrit 10 min (T11), and 15% H_2SO_4 for 20 min (T6) speeded germination.
Germination energy exhibited an inverse pattern, with the control showing the greatest value (0.22), followed by Beejamrit 15 min (T12) (0.11), and the remaining treatments showing somewhat lower values. Growth regulator and acid scarification treatments significantly increased the seed vigour index-I (SVI-I). The greatest SVI-I was obtained with 500 ppm GA_3 (T7) (1246.00), followed by 15%H_2SO_4 for 20 min (T6) (1149.33), suggesting their obvious impact in boosting seedling performance. A similar pattern was visible for seed vigour index-II (SVI-II), where the highest value was reported with 15% H_2SO_4 for 20 min (T6) (8.57), followed by 500 ppm GA_3 (T7) (6.77) and Beejamrit 5 min (T10) (4.61) as depicted in (Figure 10).

Seedling growth performance in (2024)
T7 (GA_3 500 ppm), the maximum seedling length was measured at 22.5cm, demonstrating it's better effectiveness in fostering total seedling growth. In contrast, the smallest seedling length was recorded in T12 (Beejamrit 15 min) (11.7 cm) and the control T13 (12.4 cm), showing comparatively low growth under these treatments. Further supporting the beneficial effects of GA_3 and acid scarification treatments on root growth, root length showed a pattern comparable to that of seedling length. Shoot length also varies significantly among treatments. T6 had the longest shoot (11.0 cm), followed by T7 (9.7 cm), demonstrating how well these treatments worked to increase shoot elongation. On the other hand, T9 (2.6 cm) and T12 (3.0 cm) had the shortest shoot length, suggesting that shoot growth was inhibited in these circumstances as shown in (Figure 11). Seedling fresh weight ranged from 0.18 to 0.30 g, with the maximum value obtained in T6 (0.30 g), closely followed by T7 (0.29 g). These treatments had a robust stimulatory effect on biomass accumulation and were clearly superior to the others. A similar pattern was seen for seedling dry weight, which obtained its greatest value in T6 (0.15 g), followed by T7 (0.12 g) and T12 (0.10 g). T9 had the lowest seedling dry weight (0.06 g), indicating less dry matter accumulation. Root fresh weight varied between 0.06 and 0.13 g, with the greatest value observed in T6 (0.13 g), followed by T7 (0.12 g) and T11 (0.09 g). The least root fresh weight was reported at T12 (0.06 g), indicating slower root development. Root dry weight ranged from 0.03 to 0.10 g, again reaching its highest in T6 (0.10 g), followed by T7 (0.07 g), while the lowest value was reported in T9 (0.03 g), showing minimal root biomass accumulation. Additionally, there was a significant difference in shoot fresh weight between treatments. T7 had the highest shoot fresh weight (0.17 g), while T5 had the lowest (0.11 g), suggesting relatively weaker shoot growth. The trend for shoot dry weight was similar, with T6 and T7 showing the highest values (0.05 g each). Overall, the findings unequivocally show that T6 and T7 were the best treatments for increasing seedling development, biomass accumulation, and general vigour in *Prunus cerasoides*, while T9, T12, and the control showed relatively poor performance as shown in (Figure 12).

Hierarchical cluster analysis of pre-sowing treatments, based on pooled data of 2023–2024, indicated a distinct grouping pattern that represents their relative closeness in impacting germination and seedling growth parameters of *Prunus cerasoides*. When compared to untreated seeds, the control treatment (T10) produced a clear and isolated cluster, demonstrating the significant beneficial effects of seed pre-treatments on germination behaviour and seedling performance. Two significant clusters were found among the treated seedlings. The first cluster comprised the chemical and growth regulator treatments, where T6 (H_2SO_4 15%) showed close

affinity with T7 (GA_3 500 ppm), suggesting a comparable effect of both treatments on seedling characteristics. A comparable increase pattern in germination and vigour characteristics is indicated by their grouping at a lower Euclidean distance. Later, T5 (H_2SO_4 10%) joined this sub-cluster, creating a larger group of very successful therapies. The second significant cluster revealed a strong correlation between T2 (cow dung slurry for 4 days) and T1 (cow dung slurry for 2 days), exhibiting comparable responses in seed germination and early growth. T4 (H_2SO_4 5%) also grouped close to these treatments, suggesting that organic priming-like effects were achieved at lower acid concentrations. Likewise, T8 (GA_3 1000 ppm) demonstrated proximity to these treatments, showing moderate efficacy in enhancing seedling performance.
Another distinct sub-group was formed by T3 (cow dung slurry for 6 days) and T9 (GA_3 1500 ppm), which clustered together at a slightly higher distance, suggesting a comparable but somewhat different influence on seedling traits compared to the remaining treatments as depicted in (Figure 13).

Genetic parameters study

Genotypic coefficient of variation was found maximum for germination energy (67.94 %) followed by seed vigour index 1 (43.79%). While, the phenotypic coefficient of variation was found maximum germination energy (73.12%) followed by seed vigour index 1 (50.76%). However, the minimum value of GCV was obtained by seed length (4.5 mm) and for PCV minimum value was obtained by seed width (2.86%). The magnitude of the phenotypic coefficient of variation (PCV) values was found slightly higher for all the traits than the genotypic coefficient of variation (GCV). Heritability was found high in seed weight (65.33%), seed length (53.02%), germination percentage (79.41%), mean germination time (51.845), shoot length (52.03%), germination energy (86.35%), seed vigour index 1(74.43%) and in germination percentage after first transplant (78.71%). Moderate heritability was observed in germination index (38.46%), seedling length (49.62%), shoot length (52.035) and no. of leaves (40.21%) while low heritability was found in seed width (28.75%). The maximum genetic gain was found for seed vigour index 1 (77.84%), germination percentage (72.71%), germination index (35.38%), germination percentage after first transplant (75.11%) while moderate genetic gain was observed in mean germination time (25.39%), seedling length (26.07%), shoot length (18.73%), no. of leaves (28.81%). Low genetic gain was found in seed weight (8.065), seed width (1.69%) and seed length (6.75%) as demonstrated in as depicted in (Figure 14).

Correlation studies

Correlation analysis showed strong interrelationships among germination, seed and seedling growth traits, indicating their combined role in determining seedling performance of *Prunus cerasoides*. Germination percentage was highly and positively correlated with germination index ($r = 1.00$), seed vigour index-I ($r = 0.91$), mean daily germination ($r = 0.82$), seedling length ($r = 0.79$), shoot length ($r = 0.77$), collar diameter ($r = 0.73$), seed weight ($r = 0.73$) and seed width ($r = 0.78$), showing that higher germination is directly associated with better seedling growth and vigour. Shoot length was strongly correlated with collar diameter ($r = 0.87$), seed width ($r = 0.91$) and seed weight ($r = 0.81$), while seedling length showed a very strong association with seed vigour index-I ($r = 0.94$), emphasizing its importance in overall seedling vigour. Seed weight and seed width were also highly correlated ($r = 0.92$) and positively associated with germination and vigour

traits, indicating the advantage of larger and heavier seeds. Germination index was strongly related to seed vigour index-I (r = 0.92), mean daily germination (r = 0.80) and seedling length (r = 0.77), confirming that rapid and uniform germination enhances seedling vigour. Mean daily germination similarly showed positive correlations with major vigour parameters. In contrast, germination energy exhibited strong negative correlations with most traits, including germination percentage (r = –0.94), germination index (r = –0.91) and seed vigour index-I (r = –0.84), indicating an inverse trend with germination capacity and seedling growth. (Figure 15).

Discussion

The substantial differences in tree height and DBH between seed sources are a result of both genetic makeup and environmental factors like altitude, soil quality, and moisture availability. This variation provides opportunities to identify superior provenances for planting and breeding methods. Early seedling establishment and germination potential are similarly impacted by genetic diversity in wild populations [19, 22, 12, 46, 30].

Kobmoo and Hellum [27]; Khasa [25]; Yadav [58]; Teketay [49]; Alamgir and Hossain [3]; Azad et al. [5]; Airi et al. [2]; Azad et al. [6] investigated different pre-sowing treatments to break seed dormancy and enhance germination. Seed dormancy might differ with species, maturation stage, etc. Therefore, pre-treatment should be applied accordingly [4, 40]. In this study, scarification with sulfuric acid (10–15% H_2SO_4 for 20 minutes) enhanced germination, which indicates that the species possesses robust physical dormancy imposed by a stiff seed coat. Acid treatment effectively increases the permeability of seed coats, accelerating water absorption and the onset of metabolic activities. Similar effect of sulphuric acid on seed germination has been documented by numerous study groups [7, 48, 32, 39]. Untreated drupes typically germinate erratically and slowly [20, 18]. Pre-sowing therapies have been shown to restore germination in seeds with a hard, rigid, inflexible seed coat [24, 35, 18]. [5] found that 84% of *Xylia carrii* seeds in Bangladesh germinated after 20 minutes in 80% concentrated H2SO4 as germination of seeds treated with strong sulfuric acid are facilitated by the disintegration of the seed coat and micropylar plug. GA_3 treatments increased biomass, root length, and seedling vigour, suggesting that they stimulate enzyme activity and mobilize stored food stores. However [38], recorded best seedling performance with GA_3 @ 200 mg/l for 10 minutes in Jamun. Due to the diffusion of endogenous auxin and gibberellin-like compounds, GA3-treated seeds may help break seed dormancy, leading to early and improved germination [14]. This may be attributed to active amylase which processed the available carbohydrate into simpler sugar, such that energy and nourishment were freely available to rapidly growing seedling. Plant growth regulators in overcoming the adverse effect on growth may be related to the shift in endogenous growth regulators [52]. It has been confirmed that pre-treatment of seed with GA3 enhances seedling growth [11],[28],[41]. Treatments with cow dung slurry demonstrated a moderate degree of effectiveness in this study, perhaps as a result of microbial activity and mild fermentation effects that soften the seed coat. Treatment seeds with Beejamrit was found less effective showing that organic formulations alone are insufficient where dormancy is largely physical.

Due to the impact of the environment on seed germination, differences in seed germination characteristics were discovered in 2024 and 2023. Similar observation was obtained in *C.australis*[45]. Additionally, a number of researchers found that *Albizia chinensis* [10]), *A. lebbek* [53] and *Dalbergia sissoo*[44] all germinate more quickly at a constant temperature of 25°C.

The estimates of genetic variability parameters for various traits of *Prunus cerasoides* revealed considerable scope for genetic improvement. PCV values were higher than the corresponding GCV for all traits, indicating environmental influence, while the relatively small differences between PCV and GCV for most characters suggest substantial genetic control.

High heritability coupled with high genetic gain was observed for germination percentage, germination energy, seed vigour index, and germination % after first transplanting, indicating the predominance of additive gene action and the effectiveness of direct selection based on phenotypic performance. Seed vigour index and germination energy showed particularly high potential as selection criteria for superior seed lots, vigorous seedlings, and early screening for seed quality and performance. Seedling length, shoot length, and root length exhibited moderate PCV and GCV values with moderate to high heritability and appreciable genetic gain, suggesting that improvement through selection is feasible, although progress may be comparatively slower. In contrast, seed weight and seed width showed relatively low PCV and GCV values, moderate heritability, and lower genetic gain, indicating narrow genetic variability and greater environmental influence, making simple selection less efficient for these traits. High heritability coupled with high genetic gain for germination percentage, germination energy and seed vigour index I indicates that these traits are mainly governed by additive gene action and can be effectively improved through simple selection methods and are therefore ideal targets for tree improvement programmes.

Correlation analysis established within seed vigour index I and germination index are important integrative traits, as improvement in these parameters simultaneously enhances several growth and biomass attributes. This makes them highly valuable selection criteria in nursery and breeding programmes. Correlated quantitative traits are of a major interest in an improvement program, as the improvement of one character may cause simultaneous changes in the other character. Similar results have been reported for *Faidherbia albida* (Del.) A. Chev. [19] and *Tectona grandis* L.f. [22]. This supports earlier findings of [47], [15], [10] and [44].

The clustering of populations according to geographical and altitudinal similarity confirms the strong influence of ecological factors on genetic divergence. Such grouping is important for designing conservation strategies and for selecting genetically diverse parents for breeding.

Conclusion

The present investigation clearly demonstrated the existence of substantial variability among seed sources and response of seeds to different pre-sowing treatments, indicating wide scope for genetic improvement and nursery management optimization. This diversity provides a strong foundation for selection of superior seed sources for plantation and breeding programmes.

Seed characteristics showed a close association with germination and early seedling growth, emphasizing the importance of seed quality in successful regeneration. Provenances Bandla, Churwadhar and Daroo consistently exhibited superior performance for germination percentage, germination index and seed vigour, and therefore can be considered promising seed sources for large-scale nursery production. Among the pre-sowing treatments, scarification with 15% H_2SO_4 for 20 minutes was found to be the most effective in breaking seed dormancy and enhancing germination and seedling vigour, seedling length, shoot length and biomass accumulation in both years of experimentation. Gibberellic acid (500 ppm for 24 hours) was particularly effective in improving seed vigour index and root development. The extremely low performance of untreated seeds confirmed the presence of strong physical dormancy and the necessity of pre-sowing

treatments. Overall, the study concludes that the combined use of superior seed sources (notably Bandla, Churwadhar and Daroo) with appropriate pre-sowing treatments, particularly 10–15% H_2SO_4 for 20 minutes and 500 ppm GA_3 for 24 hours, can substantially improve germination, seedling vigour and nursery success. Traits such as germination percentage, germination energy and seed vigour index I should be prioritized as selection criteria in future improvement and conservation programmes. These findings provide a scientific basis for developing efficient propagation protocols and for initiating systematic breeding strategies for genetic enhancement and sustainable utilization of the species.

Declarations

Plant name - *Prunus cerasoides* D. Don

Common name- Himalayan wild cherry

Collection locations- Arki, Kunihar, Bandla, Shalana, Jubarhatti, Daroo, Thunag, Nohradhar and Churwadhar.

Plant identifier-*Prunus cerasoides* D. Don was formally identified by:- 1. Dr. Prem Prakash (Assistant Professor, Department of Silviculture and Agroforestry), Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Himachal Pradesh, India

2. Dr. Rohit Verma (Assistant Professor, Department of Seed Science and Technology) Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Himachal Pradesh .

3. Dr. Lalit Thakur (Assistant Professor, Department of Tree Improvement and Genetic Resources), Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Himachal Pradesh, India

The plant material used in the study was maintained in the nursery of the department. No voucher specimen was deposited in a publicly available herbarium.

Ethics approval and consent to participate- Not applicable. This study did not involve human participants or animals.

Consent for publication- Not applicable. This manuscript does not contain any individual person's data or images requiring consent for publication.

Availability of data and materials - The data analysed during the current study are available from the corresponding author on reasonable request.

Permissions to collect the plants/plant parts

The plant material was taken from private farmland and land owner permission was taken during seed collection.

Ethics and consent to participate

The plant material used in this study was *Prunus cerasoides* D. Don (Himalayan wild cherry), a wild Himalayan tree species. Seeds were collected from nine locations in Himachal Pradesh, India: Arki, Kunihar, Bandla, Shalana, Jubarhatti, Daroo, Thunag, Nohradhar and Churwadhar. The collected plant material was maintained in the nursery of the Department for experimental purposes. The species was identified by Dr. Prem Prakash (Assistant Professor, Department of Silviculture and Agroforestry), Dr. Rohit Verma (Assistant Professor, Department of Seed Science and Technology), and Dr. Lalit Thakur (Assistant Professor, Department of Tree Improvement and Genetic Resources), Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Himachal Pradesh, India. The plant material was collected from private farmland with permission from the respective landowners. No voucher specimens were deposited in a publicly accessible herbarium or other public collection. The authors will ensure that the

collection and use of plant material comply with applicable local and national guidelines and regulations.
Ethics approval and consent to participate: Not applicable. This study did not involve human participants or animals.

Consent for publication

Not applicable. This manuscript does not contain any individual person's data or images requiring consent for publication.

Availability of data and materials

The data analysed during the current study are available from the corresponding author on reasonable request.

Permissions to collect the plants/plant parts

Seeds of *Prunus cerasoides* D. Don were collected from private farmland at the specified collection locations in Himachal Pradesh, India, after obtaining permission from the respective landowners. No voucher specimens were deposited in a publicly accessible herbarium. The authors will provide any additional information regarding collection permissions, if required.

1. Plant guidelines:

Ethics and consent to participate

Seeds of *Prunus cerasoides* D. Don (Himalayan wild cherry) were collected from naturally growing trees on private farmland at Arki, Kunihar, Bandla, Shalana, Jubarhatti, Daroo, Thunag, Nohradhar and Churwadhar in Himachal Pradesh, India. Permission was obtained from the respective landowners before seed collection. The collected seeds were maintained in the departmental nursery for experimental purposes. The collection and use of plant material were conducted in accordance with applicable local and national guidelines and regulations. No separate government permit or licence was obtained.
Ethics approval and consent to participate: Not applicable. This study did not involve human participants or animals.

B. Herbarium Details:

No voucher specimen was deposited in a publicly accessible herbarium or other public collection

3. Permissions to collect the plants/plant parts

Permission was obtained from the respective landowners before seed collection.

The plant material used in the study was maintained in the nursery of the department. No voucher specimen was deposited in a publicly available herbarium.

Ethics approval and consent to participate- Not applicable. This study did not involve human participants or animals.

Consent for publication- Not applicable. This manuscript does not contain any individual person’s data or images requiring consent for publication.

Availability of data and materials - The data analysed during the current study are available from the corresponding author on reasonable request.

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Conflict of interest
The authors declare no conflict of interest.

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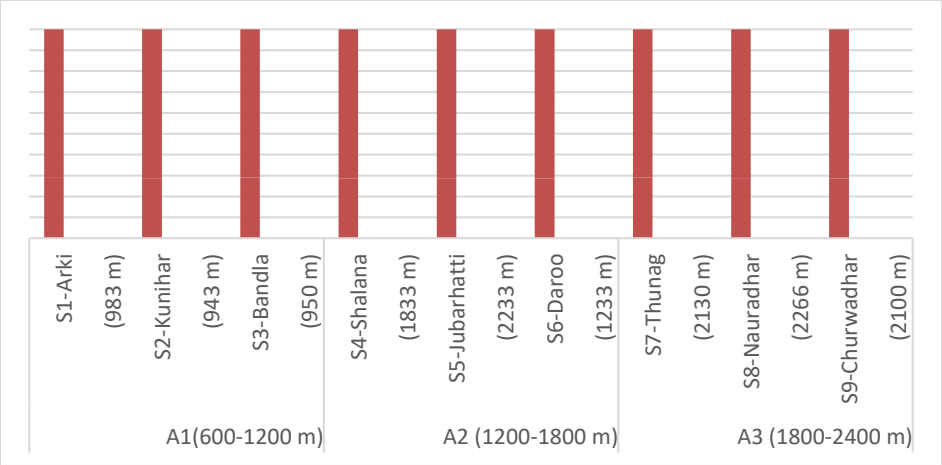


Figure 1 -Sites selected for collection of seeds from different altitudinal ranges

Table 1: pre sowing treatments given in year 2023 and 2024.

Pre sowing treatments in year 2023:-	**Pre sowing treatments in year 2024:-**
T1: Cow dung slurry for 2 days.	T1: Cow dung slurry for 2 days
T2: Cow dung slurry for 4 days.	T2: Cow dung slurry for 4 days
T3: Cow dung slurry for 6 days.	T3: Cow dung slurry for 6 days
T4: Sulfuric acid 5% for 20 min	T4: Sulfuric acid 5% for 20 min
T5: Sulfuric acid 10% for 20 min	T5: Sulfuric acid 10% for 20 min
T6: Sulfuric acid15% for 20 min	T6: Sulfuric acid 15% for 20 min
T7: GA3 acid 500 ppm. for 24h	T7: GA3 acid t00 ppm. for 24h
T8: GA3 acid1000 ppm for 24h	T8: GA3 acid 1000 ppm for 24h
T9: GA3 acid 1500 ppm for 24h	T9: GA3 acid 1500 ppm for 24h
T10: Control	T10: Beejamrit for 5 min
	T11: Beejamrit for 10 min
	T12: Beejamrit for 15 min
	T13: Control

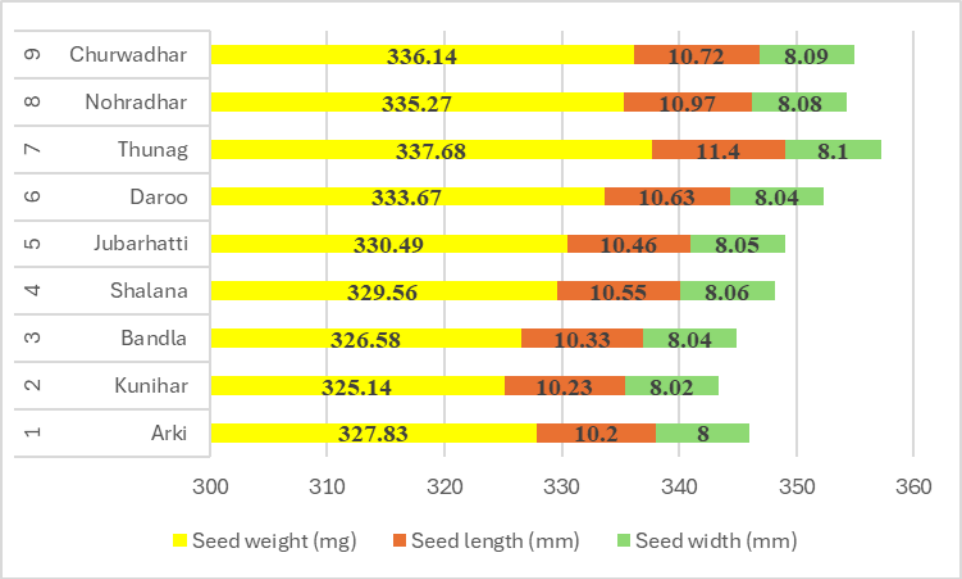


Figure 2- Seed weight, width and length variation with sites and treatments

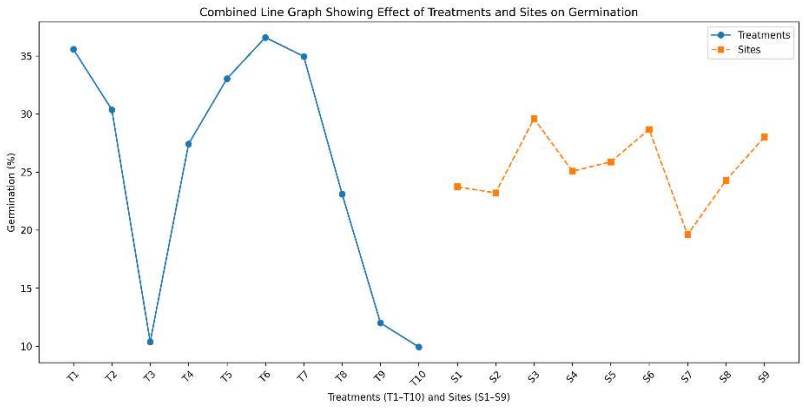


Figure 3- Germination percentage variation with sites and treatments

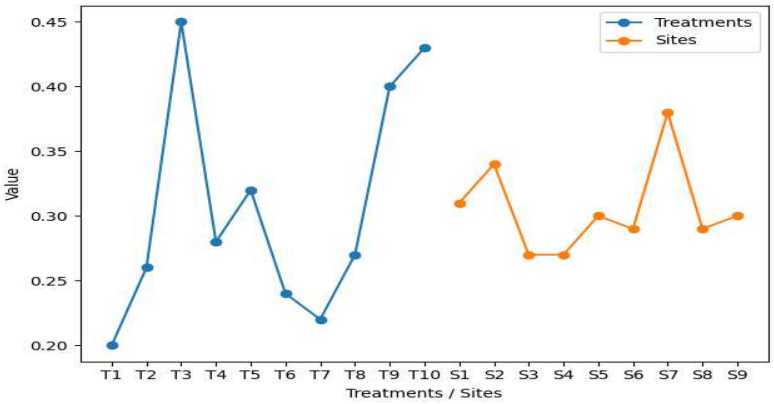


Figure 4 -Effect of pre-sowing treatments on germination energy

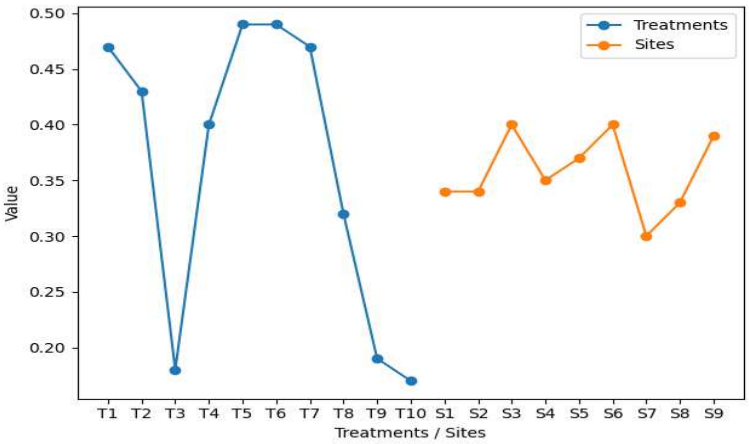


Figure 5 -germination index variation with sites and treatments

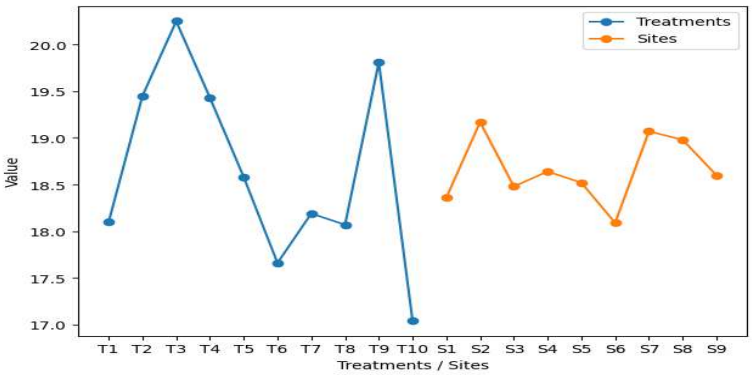


Figure 6- Mean germination time variation with sites and treatments

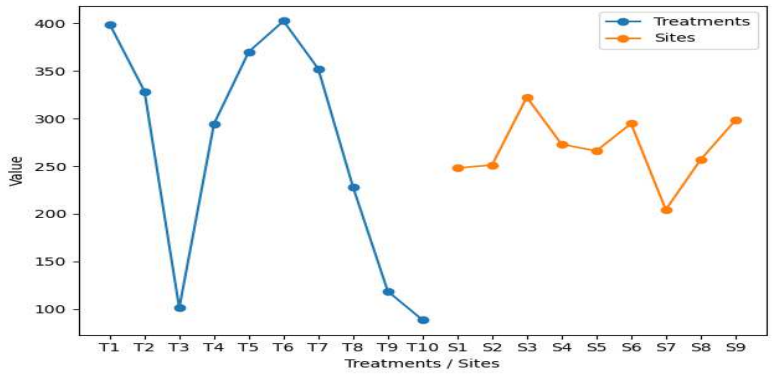


Figure 7- Seed vigour index 1 variation with sites and treatments

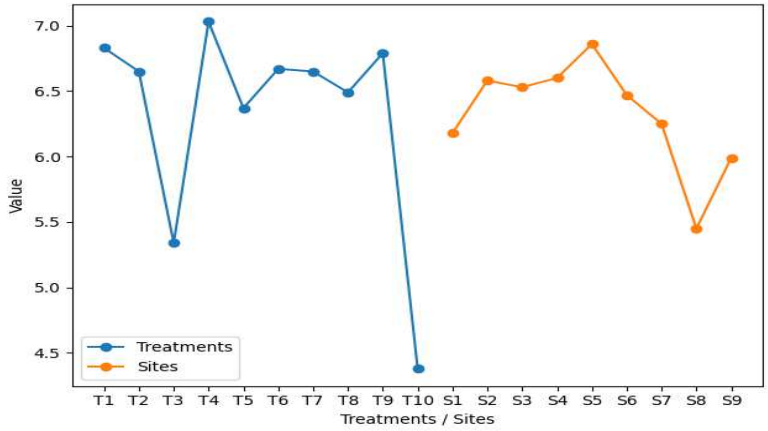


Figure 8- Seedling length variation with sites and treatments

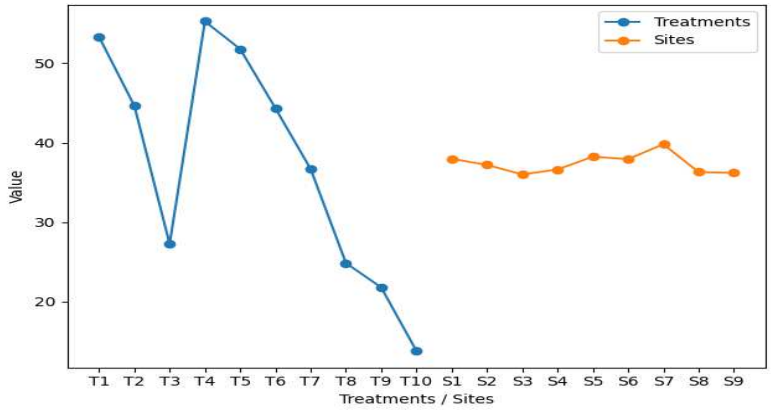


Figure 9- Shoot length variation with sites and treatments

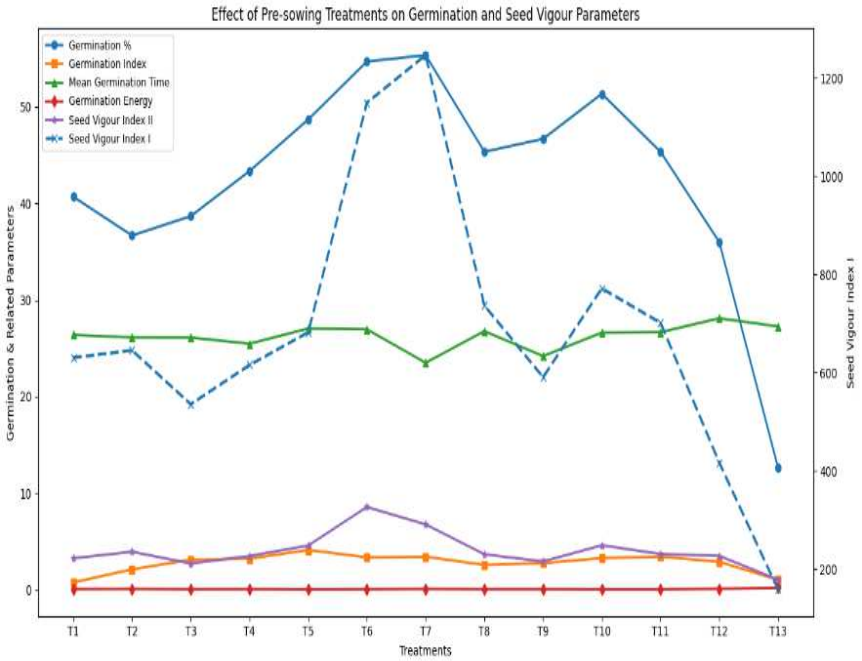


Figure 10- Variation in germination characters in year 2024 with sites and treatments

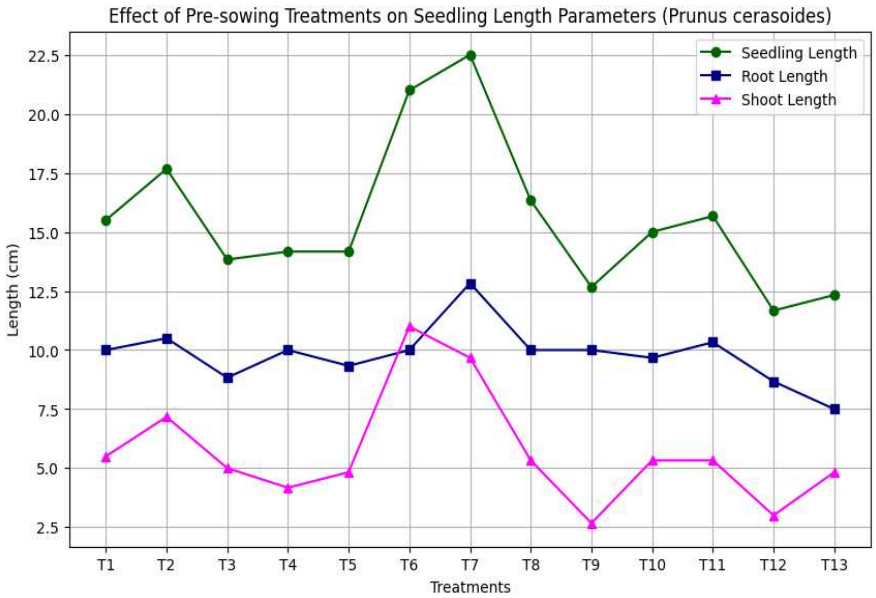


Figure 11- Effect of pre-sowing treatments on seedling length parameters in year 2024

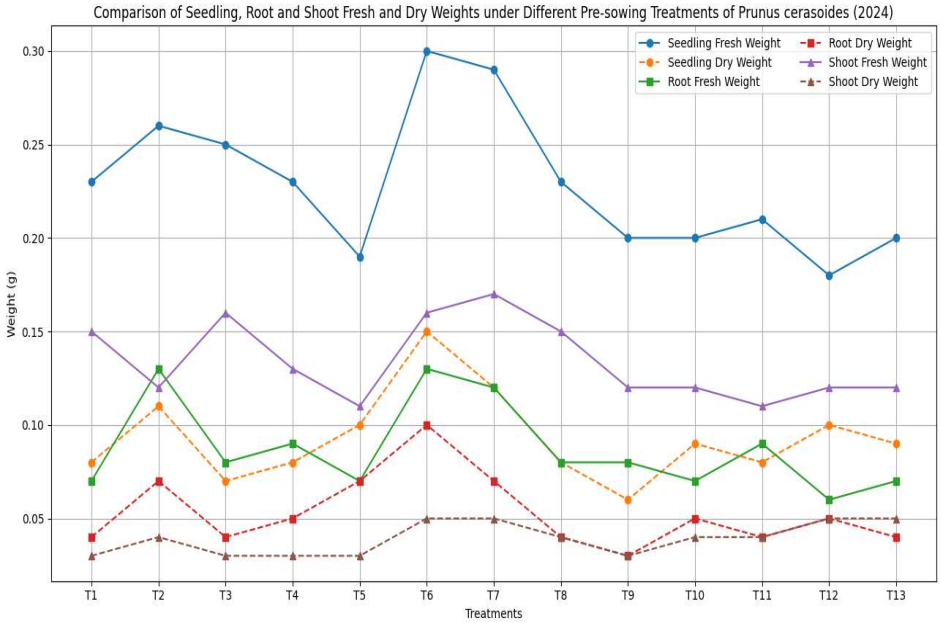


Figure 12- Effect of pre-sowing treatments on seedling biomass parameters in year 2024

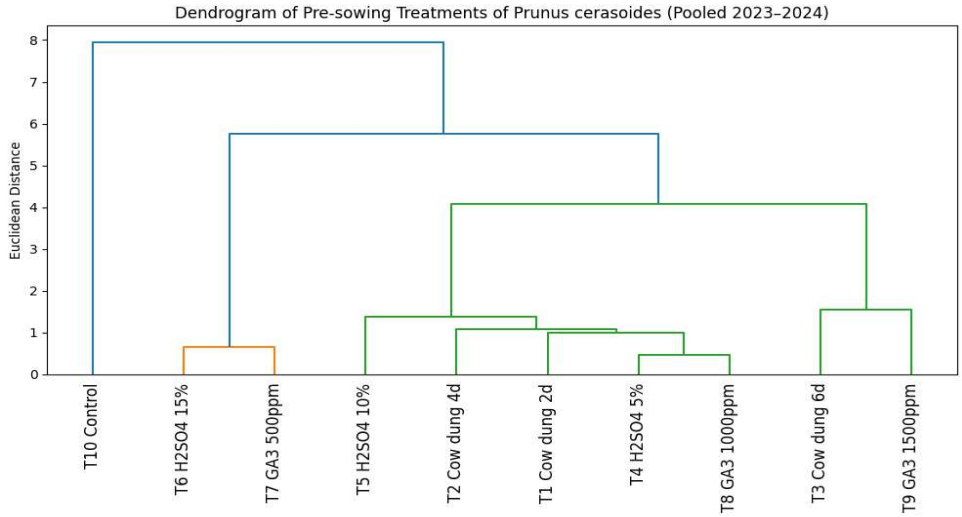


Figure 13- Hierarchical cluster analysis of pre-sowing treatments, based on pooled data of 2023–2024.

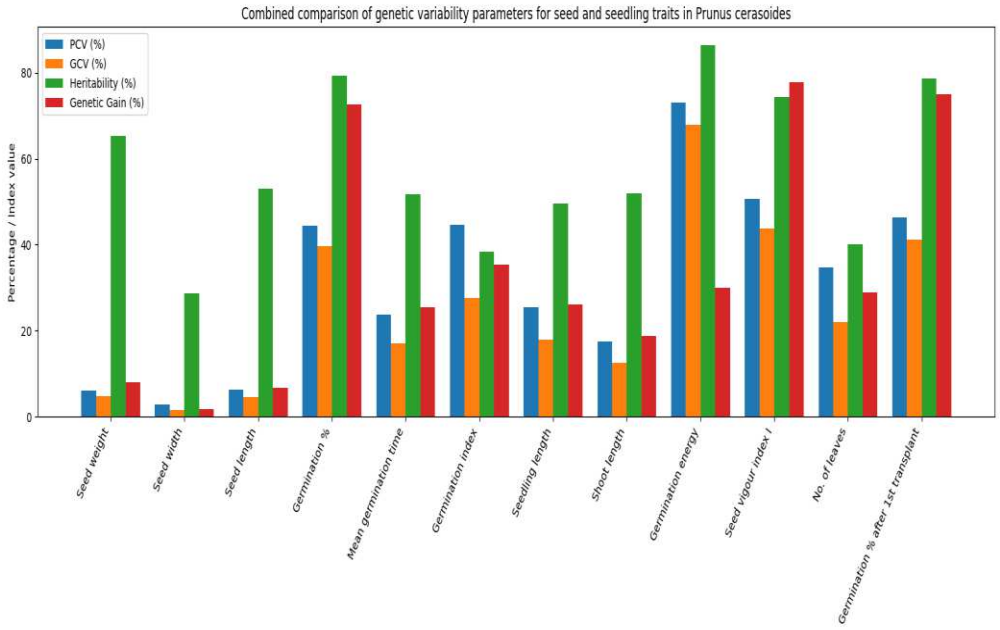


Figure 14- Genetic parameters analysis of seed and seedling traits in *Prunus cerasoides.*

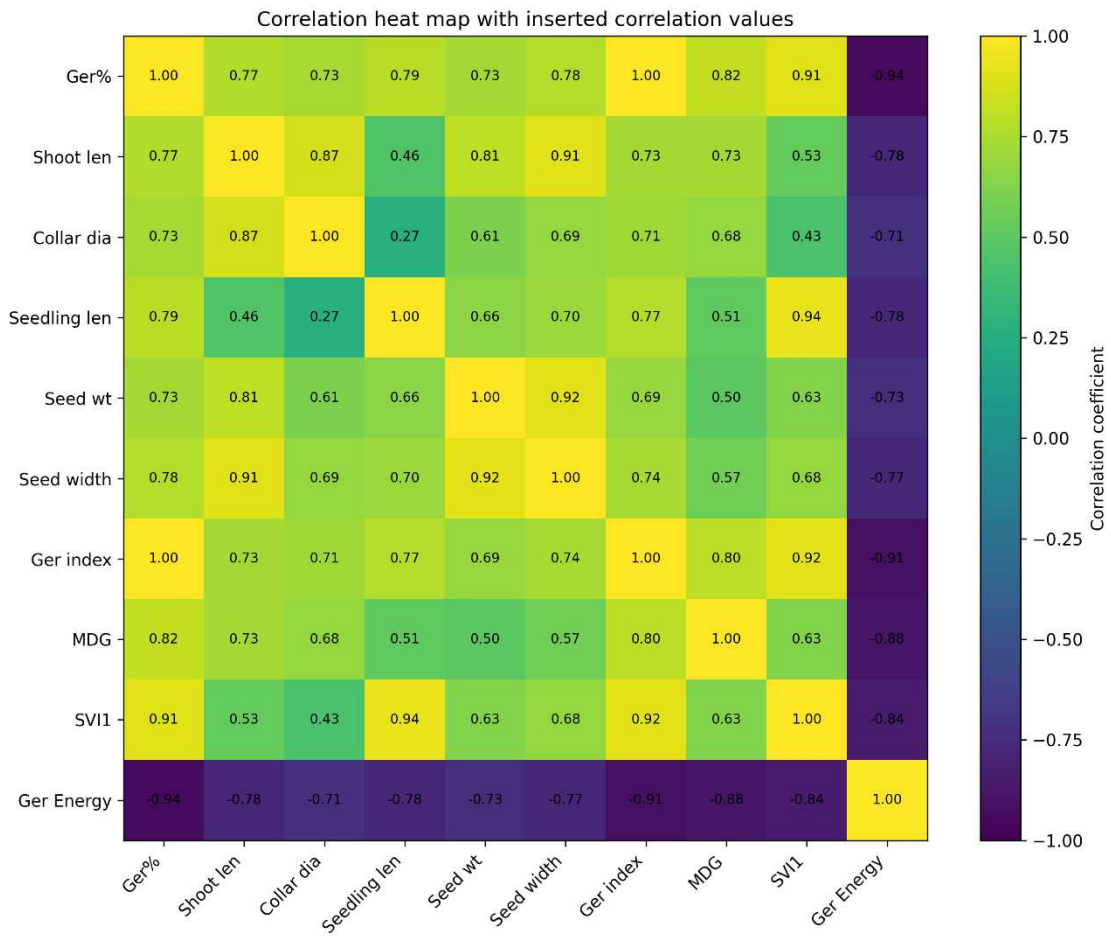


Figure 15- correlation analysis of different parameters