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Evaluation of Ferula Assa-Foetida Accessions for Germination Parameters Under Cold Stratification to Overcome Seed Dormancy and Effect of Media Mixtures on Seedling Growth

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Evaluation of *Ferula assa-foetida* accessions for germination parameters under cold stratification to overcome seed dormancy and effect of media mixtures on seedling growth

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ABSTRACT

Ferula assa-foetida L. is a perennial of Apiaceae family having seed dormancy which inhibits the germination. Oleo-gum resin obtained from the rhizomes of *Ferula assa-foetida* plant has several medicinal properties and used for the treatment of various diseases, pharmaceutical industries and in cooking of food in some countries. In present study, three different temperature treatments (5°, 15° and 25°C) were used to break seed dormancy in six *Ferula assa-foetida* accessions repeatedly for two years. Also the seedling survival rate with other morphological parameters like plant height, no. of leaves, leaf width, leaf length, root length and root diameter were observed on 3 month old seedlings in six different media mixtures during year 2019-20. The germination ranges from 3.63 (EC968466 at 25°C) to 81.88 percent (EC966538 at 5°C) with an average of 31.14 percent over all temperatures and genotypes. The mean germination time was ranged from 19.69 to 42.71 days with an average of 30.14 days. The highest germination (66.9%) and least mean germination time was observed at 5ºC (20.85) which showed that this crop required a 5ºC chilling treatment of about 20 days for breaking seed dormancy. The results pertaining to seedling survival experiment showed that media mixture of sand, soil, FYM and cocopeat (1:2:2:1 and 1:1:1:1 ratio) gave higher seedling survival rate (87.66%) and other morphological traits. It was also observed that the genotype EC966538 and EC968470 were the

best performer for overall germination as well as seedling survival parameters and could be used as base population in future selection and improvement breeding programs.

Keywords: Seed dormancy, chilling, germination, genotypes, Apiaceae and *Ferula assa-foetida*.

Ferula assa-foetida L. is a small perennial monocarpic herb belonging to family Apiaceae (Umbelliferae) and commonly known as "Heeng" in Hindi [1]. The plant height is about 1-1.5 meter in length, large size compound leaves and a large size thick taproot with root hairs is present and has a pungent smell. This plant is native to Iran and Afghanistan[2,3]. About 170 species are found all over the world and 60 species of *Ferula assa-foetida* are geographically distributed around North Africa, Central Asia and Europe[4]. This species is distributed at an elevation between 2000-4000m above mean sea level, with an annual rainfall of 250-350mm [5]. Two species of genus Ferula i.e. *Ferula alliacea* and *Ferula jaeschkeana* are found in India[6]. The oleo-gum resin is present in the fleshy tap roots and this exudate extracted from the roots is called asafoetida. *F. assa-foetida* is one of the most significant plant of Iran and Afghanistan[7] because of high export demand for its oleo-gum resin. It has medicinal use in traditional medicines [8]. Asafoetida plays an important role in cooking food as a condiment and in medicine for the treatment of various diseases in India, China, Nepal, Tibet and Iran. The extract of asafoetida is used to treat various diseases *viz.* Respiratory infections urinary, gastrointestinal and emmenagogues and also used to diagnose for a snake bite, insect bite and worm infection due to bite[9,10]. It is also antispasmodic, carminative and stimulant, diuretic, expectorant, anthelmintic and slightly laxative[11]. The oleo-gum resin of asafoetida contains 25% gum, 62% oleo-resin and approximately 3-7% essential oil[12].

The main problem with the medicinal plants which are native to arid lands is that they germinate and grow well within their habitat or native environment, but fails to show good germination or growth in another environment[13]. For every plant, germination is the most important stage in its life cycle, which controls changes in its population with major useful consequences[14]. Apiaceae family shows very poor germination ability because of seed dormancy [15,16]. Seed dormancy is a state when a viable seed failure to complete germination [17,18]. It is an innate feature of seeds which regulates germination and completion of the plant life cycle[19,20]. But this period of the seed dormancy could be cut short by giving stratified cold treatments to the imbibed seeds. *F.*

assa-foetida seeds are difficult to germinate and take a long time period to germinate seed due to seed dormancy. Two types of seed dormancy have occurred as primary that are internal and external. The internal seed dormancy was belonging to physiological dormancy which can be removed by the chilling, chemical, hormonal and heating treatments but from all the treatments chilling treatment shows the best result to break seed dormancy [21,22,23]. To break seed dormancy there are different methods to germinate dormant seeds[24]. Chilling treatment plays a very important role to break seed dormancy in many species of Apiaceae family[22] and enhances the germination and speed of germination in dormant seeds.

However, germination of *Ferula assa-foetida* does not occur easily because of seed dormancy. Therefore, a systematic study is necessary to standardize germination parameter for a seed propagated plant having dormancy. Keeping this in view, the study was undertaken with the objectives to determine the effect of cold temperature stratification and genotypic variation on seed dormancy and also to identify suitable media mixture for seedling emergence and growth.

Result and Discussion

All the six accessions of *Ferula assa-foetida* were examined for seed viability, germination and seedling survival under various experiments during 2018 and 2019 (Fig. 1a and 1b) The results pertaining to seed viability, germination, germination parameters and seedling survival rate are given as in following headings.

Seed viability

Seed viability was examined in both the years in three tests repetitions. The results of analysis of variance (ANOVA) showed that the accessions were significantly different for seed viability (Table 2). However, the tests repetitions were found non-significant and suggested that the seed viability was not degraded over a time period of 18 months. Overall, 72.22 percent of seed viability (ranged from 56.00 to 92.00%) was observed (Table 3). The average highest seed viability was observed for EC968470 (84%) and EC966538 (84%), while the lowest one was observed for EC968469 (60%).

Seed Germination

The experiment of seed germination of six *Ferula assa-foetida* accessions were examined in 2018-19 and 2019-20 (Fig. 1b). The germination data of both the years 2018-19 and 2019-20 were analyzed individually as well as pooled after conducted Bartlett test to verify the homogeneity assumption for analysis of variance (ANOVA). The different temperature treatments, accessions and their interaction were found highly significant for all the parameters studied (Table 2). The results pertaining to germination, germination parameters and seedling survival rate are given as in following headings.

Effect of various temperature treatments on germination

The pooled and individual analysis of variance (ANOVA) for both the years 2018 and 2019 showed significant difference ($p \leq 0.01$) for temperature treatments (Table 4). The germination ranges from 3.63 percent (EC968466 at 25°C) to 81.88 percent (EC966538 at 5°C) with an average of 31.14 percent over all temperatures and accessions. The average seed germination percentage for all the accessions was highest (Table 5) at 5°C (66.90%) than 15°C (21.23%) and 25°C (5.30%). The highly significant results of temperature treatments showed that seeds of *F. assa-foetida* require chilling treatment of 5°C for their germination. This indicates physiological endogenous dormancy in which factors within embryo inhibits seed germination and require chilling treatment (cold stratification) to initiate germination. This is most common form of seed dormancy in angiosperm plants [26,43,44]. Chilling temperature generally increase the production of germination promoting hormones thereby shifting the balance among promotors and inhibitors towards growth promotors [45,46]. In recent studies, down regulation of ABA and up regulation of GA content in *Hydysarum scoparium* seeds after cold stratification[47]. It is also important to obtain further valid information on plant growth promoting hormones for *F. assa-foetida* seed germination[48]. The significant positive effect of chilling treatment for breaking seed dormancy was also reported in *F. assa-foetida*[49], *Ferula gummosa* and *Ferula ovina*[50]. In *Bunium persicum* species from same Apiaceae family were observed similar results[51].

Effect of genotypes on germination

Genotypes and their interaction with temperature were significantly different ($p \leq 0.01$) for germination percentage in pooled as well as individual analysis of variance (ANOVA) for both the years 2018-19 and 2019-20 (Table 4). The maximum germination (81.88%) was observed in

EC966538 accession at 5°C, while EC968469 accession showed lowest germination (55.50%) at the same (5°C) level of temperature. In case of adverse high temperature of 25°C, the accession EC966538 showed maximum germination (8.13%), while EC968466 had very poor germination (3.63%) at this adverse temperature (Table 5) and (Fig. 2). The results of genotypic effect on germination showed that the accession EC966538 has consistently higher germination percentage among all the accessions in all temperature treatments. Seed dormancy is the major challenge in *F. assa-foetida* and other Ferula species[52,53,54,5] and genetic background of this accession (EC966538) could be used in future germination improvement breeding programs.

Other germination parameters

Seeds of *F. assa-foetida* are not germinated in a single flush, and involvement of multi-level seed dormancy causes continuous germination up to several days even with some favorable environment. Hence, germination percentage should not be only the single parameter to access the germination capability of this crop. Thus, in present study we have observed several other parameters to identify best accession and environment for good germination. These parameters are germination index (GI), mean germination time (MGT), mean daily germination (MDG), coefficient of velocity of germination (CVG), peak value (PV), germination value (GV), days to 25% germination (DG25%), days to 50% germination (DG50%), days to 75% germination (DG75%), radical length (RDL) and seed vigor index (SVI).

The pooled and individual analysis of variance (ANOVA) for both the years 2018 and 2019 showed significant difference ($p \leq 0.01$) for temperature treatments, genotypes and their interaction for all the other germination parameters studied (Table 4). Mean germination time (MGT) measures mean time required by any seed sample to initiate and terminate germination. Lower is the value of MGT, faster a seed lot has germinated [55]. The results pertaining to mean germination time (MGT) showed that germination was achieved very fast (Table 3) and (Fig. 3) at 5°C temperature treatment (20.85 days) than at 15°C (29.88) and 25°C (39.71). MGT ranges from 19.69 days (EC968469 at 5°C) to 42.71 days (EC968466 at 25°C) with an average of 30.14 days over all temperatures and genotypes. The germination rate per day measured as mean daily germination (MDG) was also highest for 5°C (1.48 seedlings/day) than at 15°C (0.47) and 25°C (0.12). Over, all the temperature treatments, the accession EC968469 has lowest mean

germination time (28.80 days) at 0.59 seedlings per day germination rate. Similarly, the days to 25%, 50% and 75% germination were also found lowest at 5°C (15.19, 18.54 and 24.06 days, respectively). The accession EC966538 has highest per day germination rate (0.84 seedlings/day). Whereas, mean germination time decreased significantly by increasing temperature from 15°C to 20°C under constant temperature treatment[56].

Germination index (GI) is a measure of both germination percentage and speed of germination. It gives maximum weightage to early geminating seeds and less weightage to late germination[55]. The GI ranged from 0.09 (EC968466 at 25°C) to 3.94 (EC966538 at 5°C) with an average of 1.44 over all temperatures and genotypes. The average germination index for all the genotypes was highest (Table 5) at 5°C (3.47) than 15°C (0.73) and 25°C (0.14).

Coefficient of velocity of germination (CVG) denotes the rapidity of germination and increases with germination of seeds and time required for their germination is reduced[55]. CVG ranged from 2.35 (EC968466 at 25°C) to 5.08 (EC968469 and EC968470 at 5°C) with an average of 3.59 over all temperatures and genotypes. Recently, the mean germination time, germination index and coefficient variation of germination were studied for *Magnolia grandiflora* plant after cold stratification[40]. The results pertaining to PV, GV and SVI were also in accordance to results of mean germination time and per day germination rate *i.e.* the temperature treatment of 5°C and accession EC966538 followed by EC968470 were found best performer (Table 3). Also, the radical length (Fig. 4 and Fig. 5) and seed vigor index was highest at 5°C (2.55 cm and 1.70, respectively) followed by 15°C (1.71 cm and 0.36,). However, in overall genotypic effects, the highest radical length and seed vigor was found for EC968470 (1.87 cm and 0.84, respectively). These seed germination parameters also studied in some Himalayan leguminous and actinorhizal plants[57,58].

Seedling survival rate

To check the seedling survival rate, all newly germinated plants were examined in the year 2019-20 under six different media mixtures (Table 1) for various morphological traits *viz.* plant height (cm), number of leaves, leaf length (cm), leaf width (cm), root length (cm) and root diameter (mm). The results of analysis of variance for seedling survival traits showed that all the genotypes and media mixtures were highly significant ($p<0.01$) for all traits studied (Table 6). While, genotype × media interaction effect was non-significant for number of leaves, leaf length

and root diameter. It showed that the studied accessions were genetically diverse for seedling survivals and could be utilized as base population for further selection and breeding programs.

The mean performance of different media mixture and genotypes showed that the accession EC968466 in media M4 and EC968467 in M5 have highest seedling survival (91.50%) (Table 5) and (Fig. 6 and Fig. 7). Over all media mixtures, M5 has the highest seedling survival rate (87.66%), followed by M4 (87.08%). Media M4 has highest plant height (21.49 cm), number of leaves (5.27), leaf length (9.22 cm), leaf width (6.58 cm), root length (11.93 cm) and root diameter (5.36 mm). It showed that the combination of soil, sand, FYM and cocopeat in 1:2:2:1 ratio was the best mixture to attained maximum survival of *F. assa-foetida* seedlings. While, media M6, which was only soil has lowest seedling survival rate (46.50) and also found lowest for all other seedling survival parameters, which indicated that this crop needs a survival media at initial stage to get good seedling establishment.

Genotypic effects on seedling establishment showed that accession EC968467 has highest survival rate (75.94%) and number of leaves (5.17) but lowest root diameter (4.16 mm). Accession EC968470 has highest plant height (15.15 cm) and root length (9.67 cm), while accession EC966538 was best for other leaf parameters *i.e.* number of leaves, leaf length and leaf width Table 7. It was observed that the accession EC968469 has lowest seedling survival rate (65.33%), leaf length (6.58 cm), leaf width (4.88 cm) and root length (8.30 cm). vermiculite as suitable media for seed germination of *Jatropha Curcas*[57]. Some Himalayan leguminous and actinorhizal plants shows higher germination on moistened filter paper as compared to mixture of soil and sand[58].

Summary

According to present study, we concluded that the chilling treatment at 5°C is more effective for dormancy breaking of *Ferula asafoetida* seeds. It shows that dormancy is caused by an inhibiting chilling in the interior or exterior surface layers of seeds. Further studies are required to explain the agro-practices to cultivate this endangered plant. Cold treatment at 5°C for about 20 days was appropriate to breaking seed dormancy and maximum seed germination by. Our finding suggests that cold treatments are commercial and effortlessly applicable by poor farmers and nursery manual workers in developing bulk planting material, over costly supplementary technicalities

and plant growth regulators (PGR). The results of germination in Laboratory condition can also be applied to propagation of plants that would help conservation programs within the study area. But maximum germination of *F. asafoetida* through seed is very difficult and almost very low. Media mixture of sand, soil, FYM and cocopeat (1:2:2:1 and 1:1:1:1 ratio) gave higher seedling survival. It was also observed that the accession EC966538 and EC968470 were the best performer for overall germination as well as seedling survival parameters.

Material and methods

Plant material

Six accessions of *Ferula assa-foetida* (Heeng) seeds used in the study were EC966538, EC968466, EC968467, EC968468, EC968469 and EC968470 procured through National Bureau of Plant Genetic Resources, New Delhi. The seeds were cleaned with removing the chaff material and damaged or immature seeds. Healthy and mature seeds separated from the seed lot were stored under ambient laboratory conditions prior to use in the experiments. Weight of 100 seed was 1.82g. The present study was conducted during 2018-2019 and 2019-2020 in the laboratory of Agrotechnology Division, CSIR-Institute of Himalayan Bioresource Technology, Palampur (Himachal Pradesh), India (N 32º6.36546' latitude, E 76º33.52122' longitude at an altitude of 1310.0 m with average annual rainfall 2493mm and average annual temperature is 19.1ºC). All the experiments of germination and seedling survival were conducted in Completely Randomized Design (CRD) design in with four replications.

Seed viability test

Seeds of *F. assa-foetida* were examined for seed viability with the help of Tetrazolium test (2,3,5-tri-phenyltetrazolium chloride). For that, 1% Tetrazolium solution was prepared by adding 1g 2,3,5-tri-phenyl-2H-tetrazolium chloride (TTC) in 100 ml of doubled distilled water in a brown bottle, mixed well and confirmed the pH of the solution at 7. Further, four replicates of 25 seeds from each accessions were dissected using a magnifying lens. Then the dissected seeds with embryo were kept in 1% tetrazolium solution and incubate at room temperature for 24 hours in dark [19]. Seeds were evaluated on their staining pattern and colour intensity as the bright red color stained seeds considered as viable while partially or light stained seeds were considered as

non-viable [25,26]. The seed viability test was repeated three times in every 6 months during the study period *i.e.* in 2018, 2019-I and 2019-II.

Surface sterilization

Healthy seeds of *F. assa-foetida* selected on the basis of their shape and size were surface sterilized by pre-washing with tap water for 1h and then soaking the seeds in 1% sodium hypochlorite solution (NaOCl) with tween-20 (2 drops /100 ml) for 25 min and then washed with sterilized double distilled water to remove traces of sterilizing agents before putting in petri-dishes.

Seed germination

To overcome the seed dormancy in *F. assa-foetida*, an experiment with different temperatures and accessions was conducted during 2018-19 and 2019-20. For the stratification treatments, seeds of six different accessions were kept at three controlled and constant temperature treatments *i.e.* 5°C, 15°C and 25°C for germination. For germination tests, four replicates of 25 seeds were incubated in petri dishes lined with double layer of sterile Whatman no.1 filter paper moistened with 5 ml double distilled water. All the petri plates were sealed with parafilm and seeds were allowed to germinate at 5°C temperature in a cold chamber (Blue star company), 15°C and 25°C temperature in growth room for a time period of 45 days. During the incubation period, filter papers were kept moist with distilled water. The germination counts were observed daily from first to last day of maximum seed germination [27,28,29,30]. Seeds at the time of radicle emergence were considered germinated [31] and an interval 45 days was found enough to get maximum germination and differentiation of non-dormant seeds from dormant ones [22,32,33]**.**

Germination parameters

Seed germination

Seed germination (%) was recorded after 45 days of chilling treatment and under different temperature. The total number of germinated seeds were counted and germination was computed in percentage using following formula:

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

Mean germination time

Mean germination time (MGT) is considered as an indicator of seedling emergence in field and calculated by using formula[34].

$$\text{Mean germination time (Seed per day)} = \frac{\sum F_i n_i}{\text{N}}$$

Where, n_i is the number of germinated seeds on f_i^{th} day of observation during germination time (from 0 to 45 days) and N is the total number of germinated seeds.

Coefficient of velocity of germination

Coefficient of velocity of germination (CVG) was computed using following formula [35].

$$\text{CVG} = \frac{N_1 + N_2 + \ldots + N_i}{100 \text{ x } (N_1 T_1 + \ldots + N_i T_i)}$$

Where N_i is the number of seeds germinated on T_i^{th} day of observation from seeding.

Germination index

Germination index (GI) was calculated by using formula[36].

$$\text{Germination Index (GI)} = (45 \times N_1) + (44 \times N_2) + \ldots + (1 \times N_{45})$$

Where N_1, N_2 ...N_{45} is the number of germinated seeds counted every day till 45^{th} day and the constants (45, 44….1 etc) are the weights provided.

Mean daily germination

Mean daily germination (MDG) was calculated by using formula[37].

$$\text{Mean daily germination (MDG)} = \frac{\text{Total number of germinated seeds}}{\text{Total number of days}}$$

Peak value

Peak value (PV) was calculated by using given formula[37].

$$\text{Peak value (PV)} = \frac{\text{Highest seed germinated}}{\text{number of days}}$$

Germination Value

Germination Value (GV) was calculated by using formula [38,39,40].

$$\text{Germination Value (GV)} = (\sum \text{DGS/N}) \times \text{GP/10}$$

Where DGS is the ratio of cumulative germination percentage to the number of days from seeding, N is days counts from the germination initiation and GP is final germination percentage.

Seed vigor index

The seed vigor index (SVI) was computed using following formula [41].

$$\text{Seed vigor index } (V_i) = \frac{Ls \times \text{Pg}}{100}$$

Where V_i is the vigor index, Ls is the length of seedling and Pg is germination percentage.

Days to 25%, 50% and 75% germination

Days to 25%, 50% and 75% germination were calculated when 25, 50 and 75 percent germination of *Ferula assa-foetida* seeds was achieved.

Radical length

Radical length was measured with the help of meter scale in centimeter (cm).

Media Mixtures

After cold stratification treatment germinating seeds of all the six accessions were transferred to different media mixtures under glasshouse conditions to study the survival and growth of the germinating seedlings Table 1.

Morphological data collection

After three months, data from different media mixtures on seedling survival and other growth parameters *viz.* plant height (cm), number of leaves, leaf length (cm), leaf width (cm), root length (cm) were recorded with the help of geometrical scale and root diameter was measured with the help of Vernier calliper in millimeter (mm).

Seedling survival rate was calculated after three months of transferred seedling in the poly sleeves using following formula.

Survival rate = (Total number of survived seedling / Total number of transferred seedlings) × 100

Statistical analysis

Analysis of variance (ANOVA) for all the three experiments *i.e.* seed viability, germination and seedling survival were computed separately using PROC ANOVA in SAS v9.4 [42] by considering all the variables as fixed effect.

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Figures

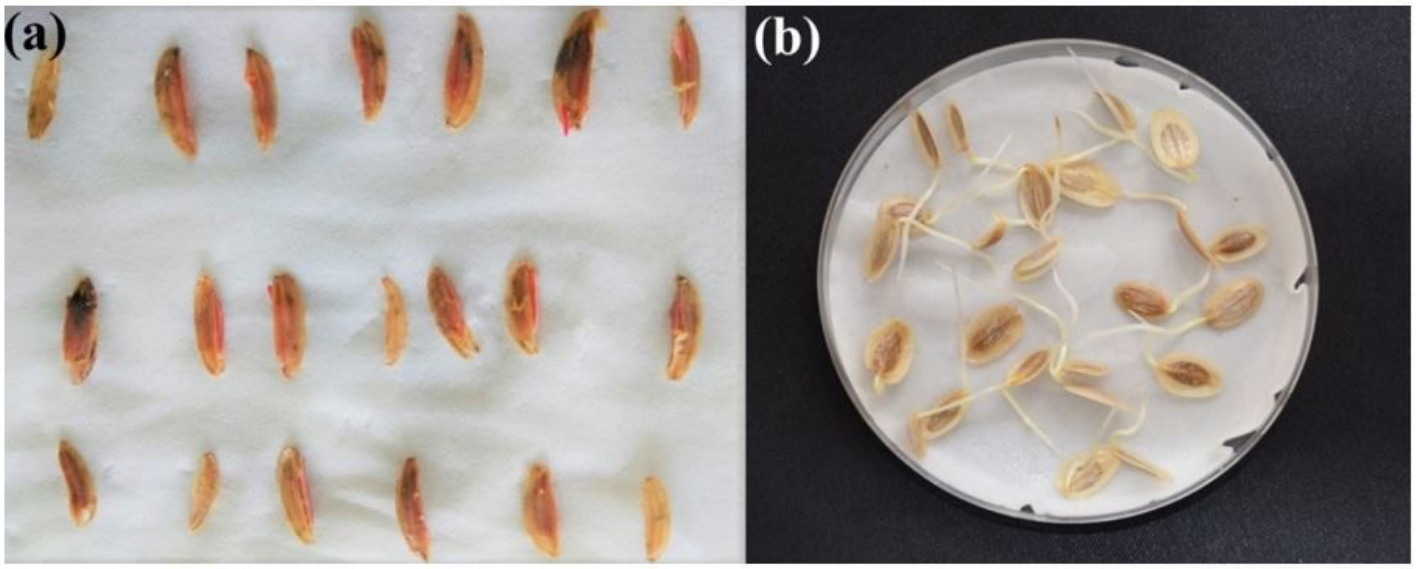

Figure 1

TTC stained seed (a) and germinated seedling (b) of Ferula assa-foetida

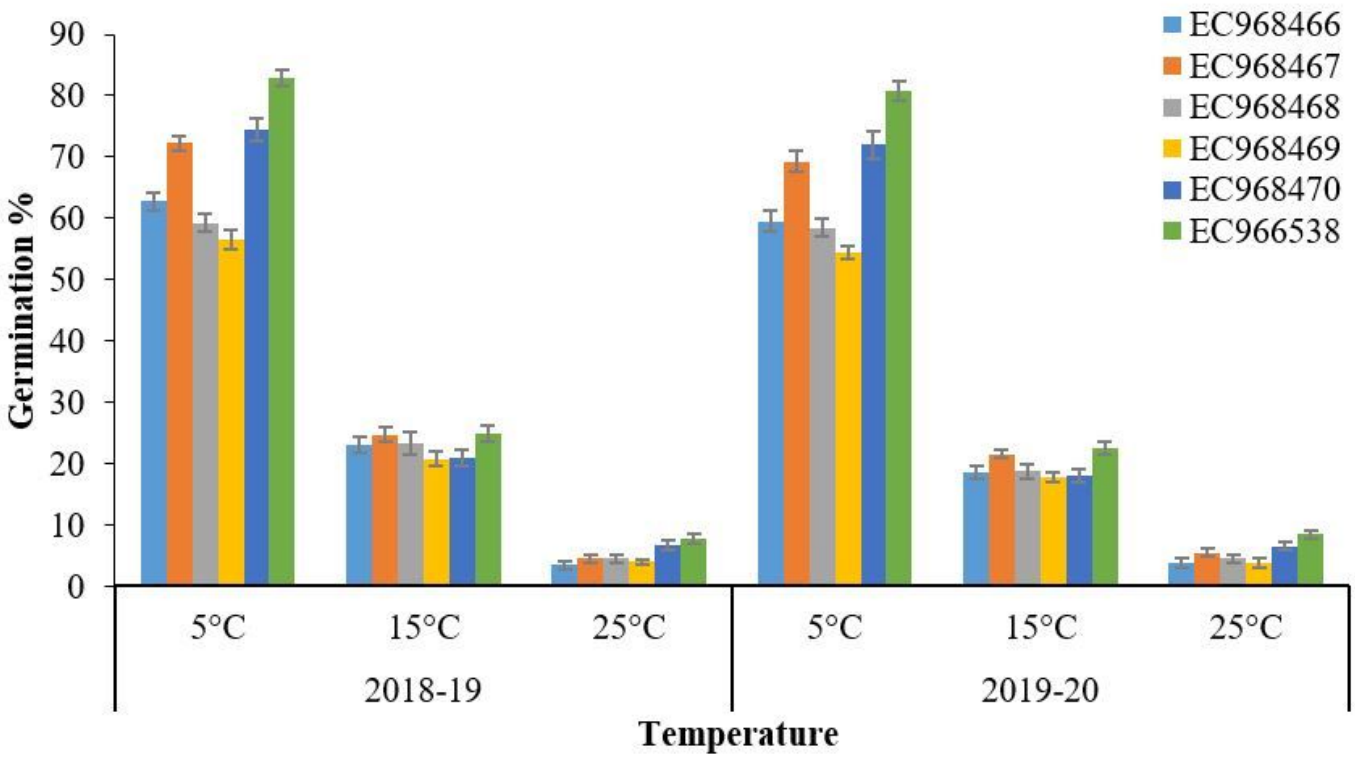

Figure 2

Germination (%) of different accession plotted over different temperature in 2018-19 and 2019-20.

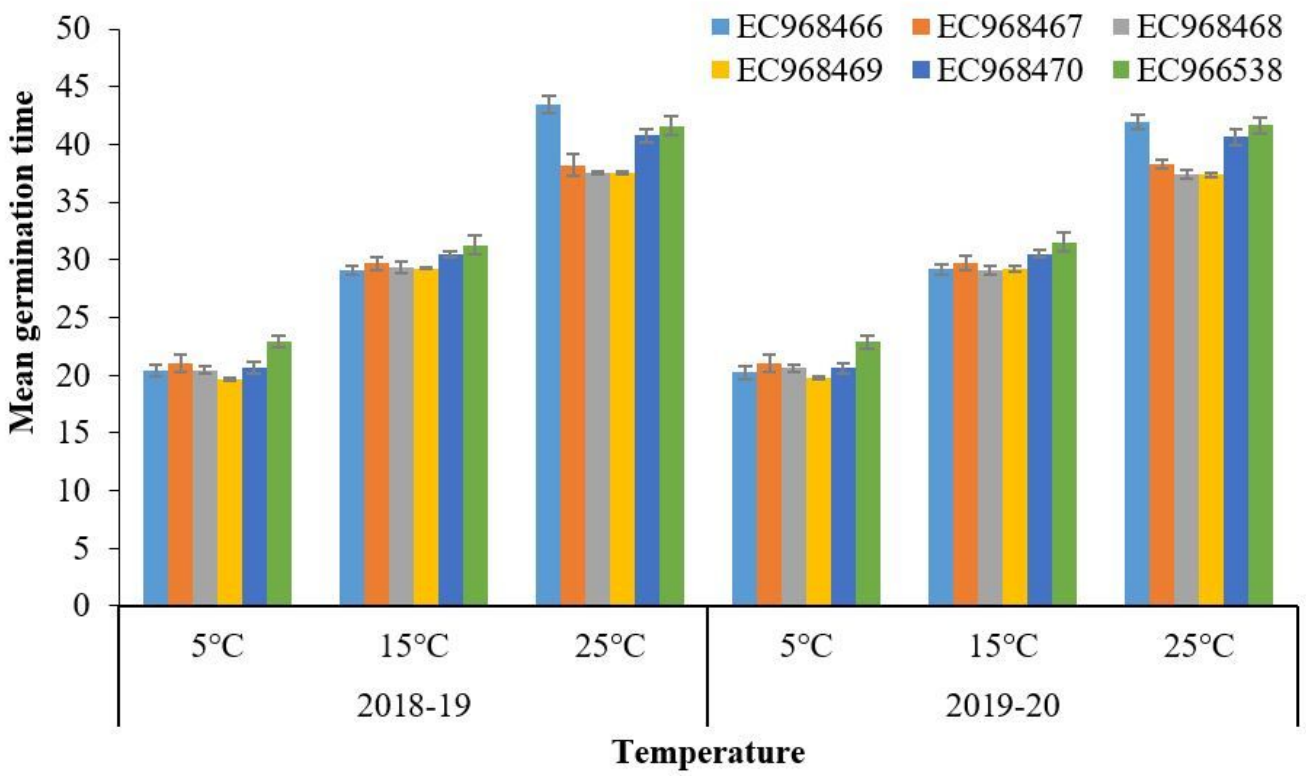

Figure 3

Mean Germination time of different accessions plotted over different temperature in 2018-19 and 2019-20

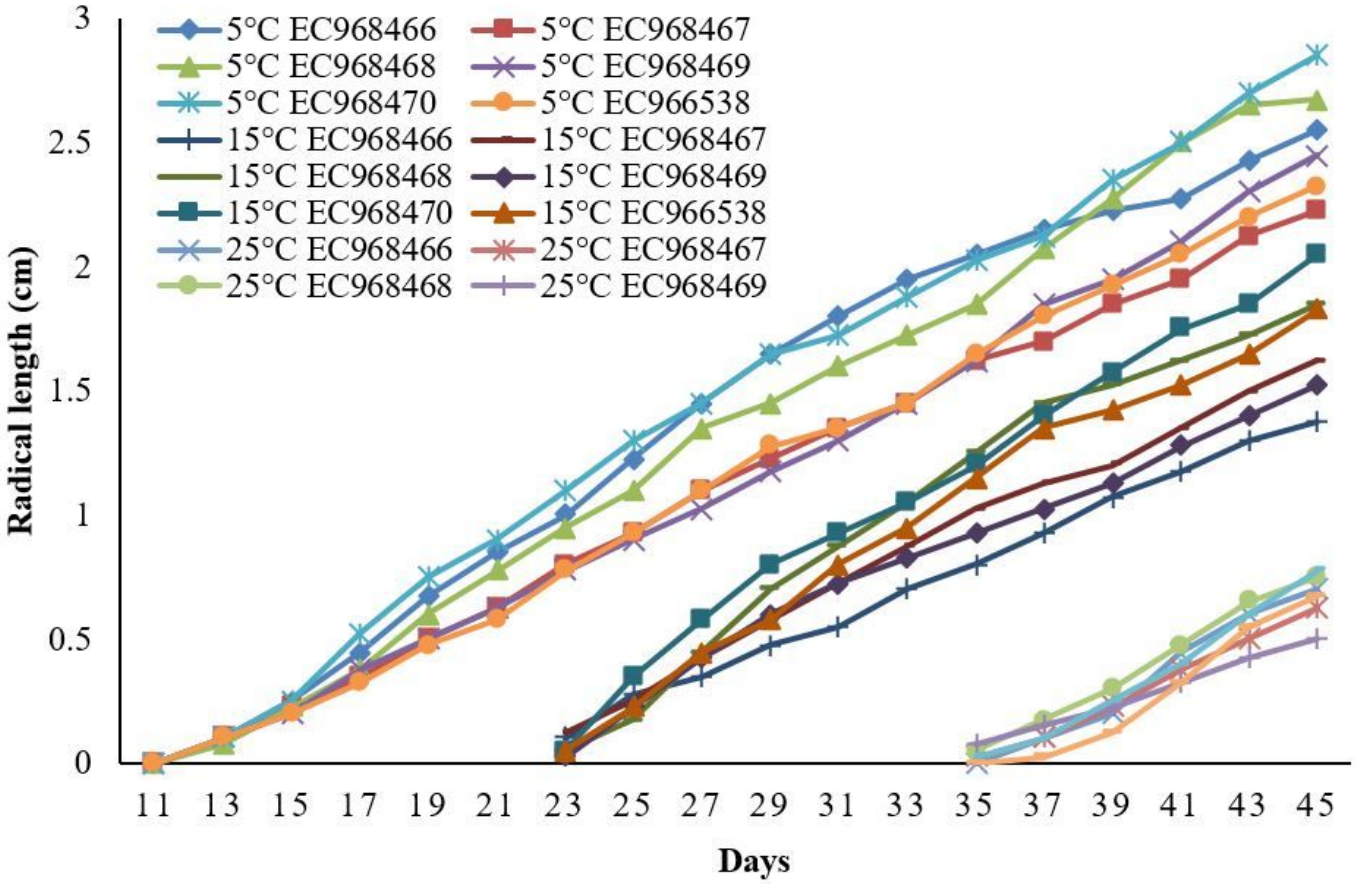

Figure 4

Rate of root emergence per day in year 2018-19.

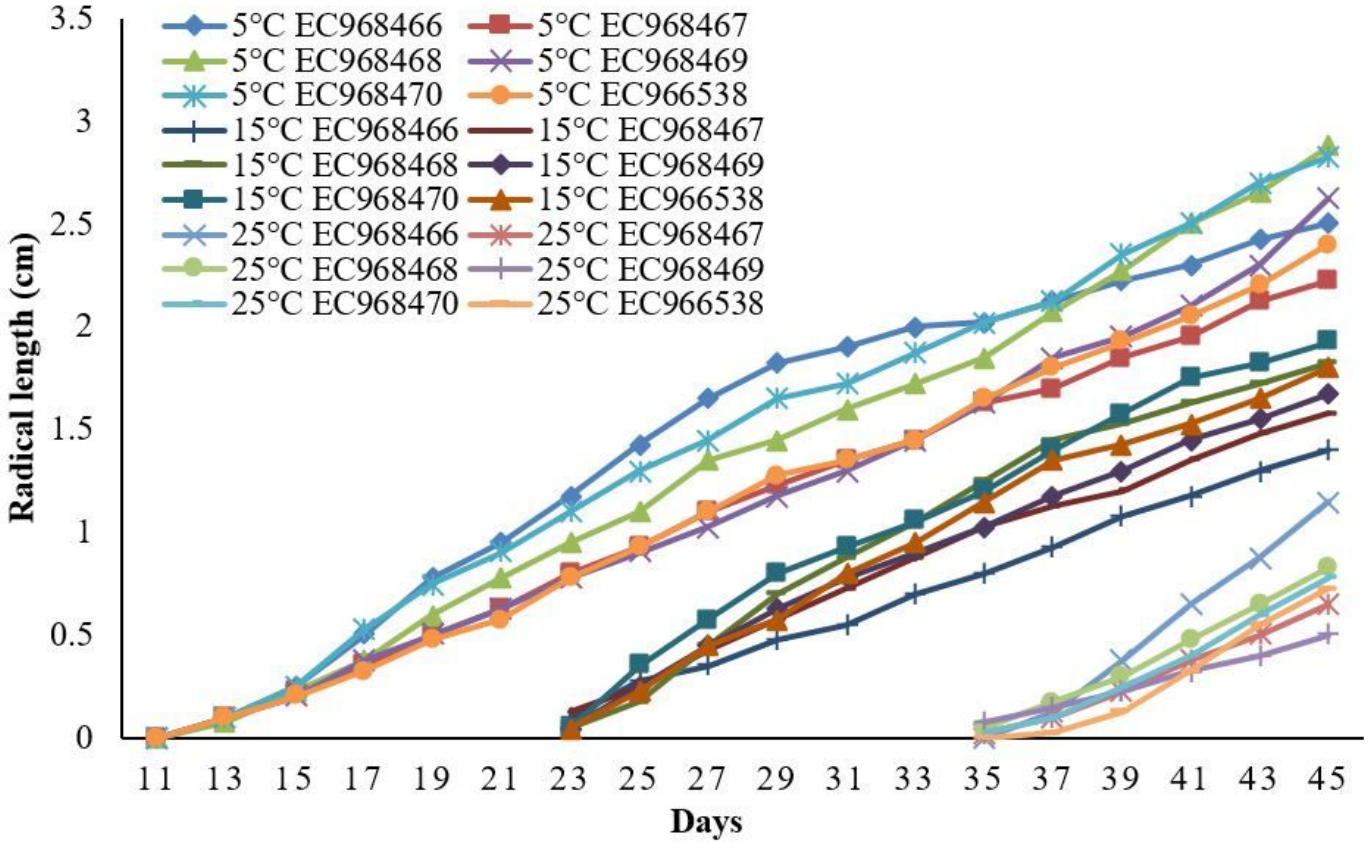

Figure 5

Rate of root emergence per day in year 2019-20.

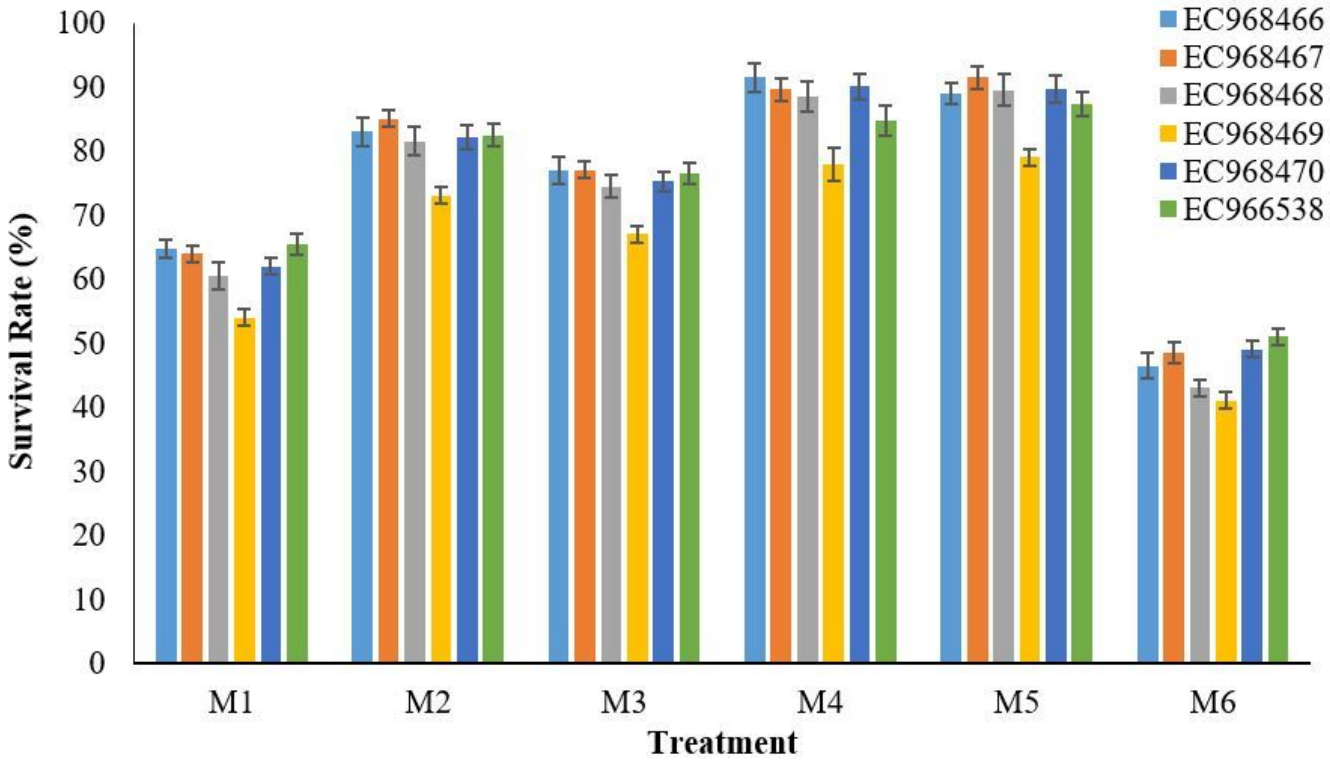

Figure 6

Survival rate of seedling under different media mixture treatments.

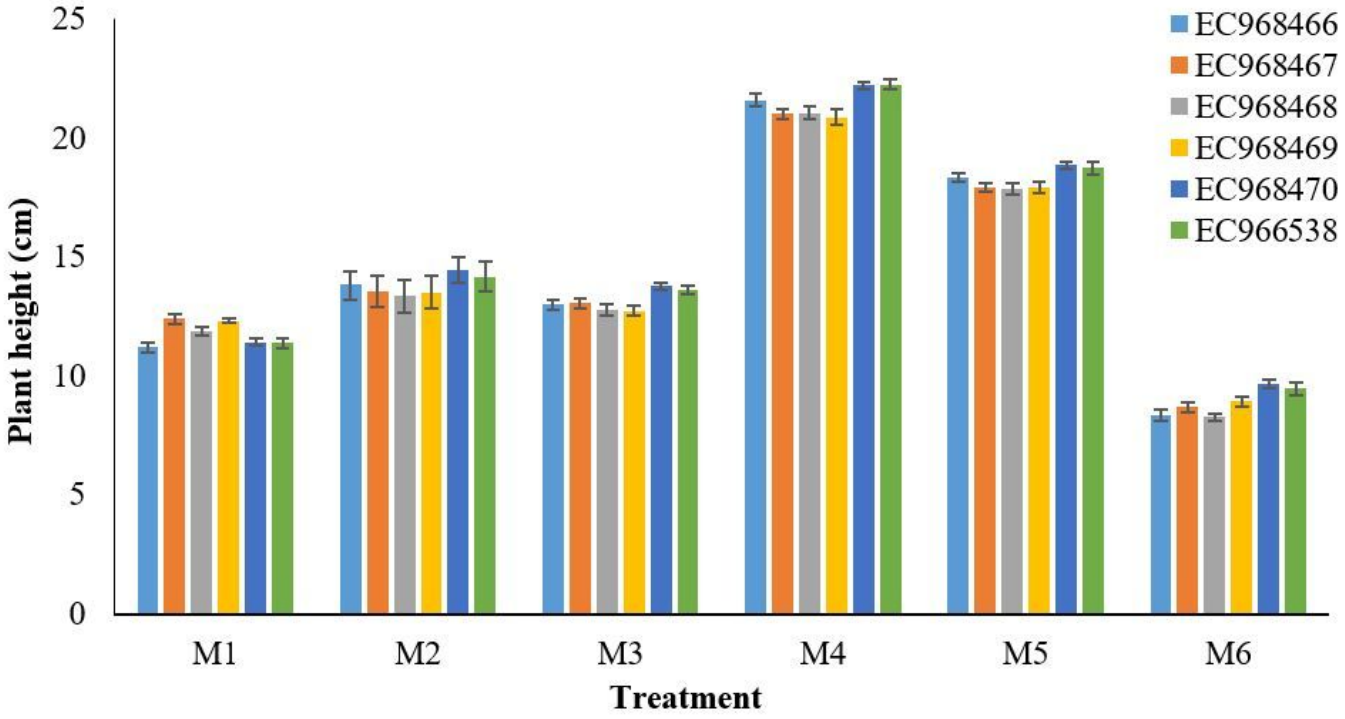

Figure 7

Plant height under different media mixture treatments