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SEED GERMINATION OF IMPORTANT MEDICINAL PLANT *TEPROSIA PURPUREA* (LINN), PERS

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ABSTRACT

T. purpurea (Linn.) pers is one of the important wild plant in Ayurvedic system of medicine which have been on number of liver diseases like viral hepatitis or jaundice in children, diseases of spleen, heart, asthma and healing wounds. Plant with active phytochemicals such as tephrosin, isotephrosin, rutin, quercetin, rotenoids, alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, steroids, chalcones, gums, mucilage, fixed oils, anthracine derivatives. Seed surface, texture and morphology expose that hard and impermeable seed coat is the main barrier for imbibitions and consequently retards germination in T.purpureae. On T.purpurea very meager information has been available on breaking seed coat dormancy, protocol deals with the best pre-sowing seed treatment. Boiling water treatment enhances seed germination percentage compare to normal and hot water. Treatment of 75% H_2SO_4 for 2.5 min and 75% HCl for 5 min for 5 min to be sufficient to hydrolyze the seed coat constituents and improved germination will be very better as compare to control. Seed soaked in IAA and GA_3, 5.0 mM GA_3 for 24 hours treatment gives better germination. This seed germination study would help for sustainable supply of atmospheric nitrogen fixing species and also useful for pharmaceutical industries as medicinally important elite clone for active secondary metabolites.

Keywords: Viral Hepatitis, *Tephrosia purpurea*, medicinal plant, seed germination.

Introduction:

The plant based drugs not only effective but also harmless and inexpensive. More and more people across the globe are turning towards herbal medicines for their day to day health necessity (Sajwan *et al*., 2015).

T. purpurea (Linn.) pers is one of the important wild plant in Ayurvedic system of medicine which have been used as folk medicine for the treatment of large number of diseases like viral hepatitis or jaundice in children, diseases of spleen, heart, diseases of liver asthma and healing wounds (Saad *et al.,* 2013). It has high medicinal value due to the presence of various phytochemicals such as tephrosin, isotephrosin, rutin, quercetin, rotenoids, alkaloids, flavonoids, phenolic compounds, tannins, terpenoids, steroids, chalcones, gums, mucilage, fixed oils, anthracine derivatives etc. which can be isolated from barks, leaves, flowers, roots, fruits, seeds (Muralidhar *et al*., 2014).

Successful establishment of *T.purpurea* may economically beneficial for agriculture due to its nitrogen fixation ability, develop fine vegetation for improving soil fertility, weed suppression, biomass input and avoid soil erosion due to persistence in a given environment (Gathumbi *et al*., 2003). Plant produces large number of seeds but its seed germination and seedling development rate is very poor under normal conditions due to hard seed coat as well as few diseases and pastes have been reported to attack the seeds of this plant (Rangaswami and Rao, 1955).

On *T.purpurea* very meager information has been available on breaking seed coat dormancy. Therefore there is a need of more work to investigate and develop easy methods for enhance germination percentage as well as find out the best pre-sowing seed treatment.

Materials and Methods:

Collection of plant material:

Mature, healthy and disease free pods of *T. purpurea* were collected in month of December 2014 to February 2015, from a number of plants growing in their natural habitat, by hand picking method. Fruits allow drying under normal shade and room temperature to break naturally. At the time of collection number of pods per plants, a seed per pod as well as infected pod was also noticed by a visual observation. Then large, clean seeds were spread upon filter paper and left to dry. Only mature and uniformly-sized seeds were used in various seed dormancy breaking experiments. Dried seeds stored in glass bottles with screw cap until use.

Identification and Authentication of plant material:

Authentication of plant material was carried out from Botanical Survey of India (BSI), Western Circle, Pune. Specimen No. BSI/WRC/T.P /2015/11 and specimen was deposited in Herbarium section of Botanical Survey of India.

Seed Viability:

Flotation method:

Randomly 100 seeds were kept in normal water for 10 minutes and then the seeds that floated on water after 10 minutes of soaking were removed by filtration method. The floated seeds considered as unviable and discarded (Amusa *et al*., 2011). The seeds which settled down at bottom of water used for further experiment after drying.

TTC Test:

Randomly 25 seeds were subjected to 1.0% 2,3,5-tryphnyltetrazolium chloride (TTC; Hi Media, Mumbai, India) test (Hartman *et al*., 1997) for 36 hours in order to calculate their viability characteristics. As well as 25 mechanically scarified seeds for 5 minutes were also soaked in 1.0% TTC solution for 24 hours. For preparing of 1.0% TTC solution phosphate buffer (PH 7) was used. After treatment of TTC solution the seeds were washed with sterilized distilled water (SDW) for ten times to remove traces of TTC solution. Then dissected the embryos by using forceps and needle under simple microscopes and observed for change in color to red.

Surface sterilization of seeds and germination condition:

Large and healthy seeds were surface sterilized using 70% ethanol for 30 seconds followed by 0.5% (w /v) aqueous mercuric chloride ($HgCl_2$; Qualigens, Mumbai, India) solution for 5 minutes. Then seeds were thoroughly washed with sterilized distilled water (SDW) for 10 times to remove the traces of $Hgcl_2$ which avoid fungal and bacterial contamination. Sterilized seeds allow drying on blotting paper under shade condition. Dried seeds were submitted to the following treatments to test the dormancy breaking and germination trials. Seeds without any treatment are considered as control and in treated seeds, germination was observed by using plastic petri plates (90 x 15m Oxygen, India) with double layered germination paper (1 mm thick, Modern paper Ltd, Pune, India). The petri-plates were

arranged in a completely randomized block design with three replications. Treated and untreated (control) total of 25 seeds were kept in each Petri dish. Initially the germination paper was moistened with 3ml SDW and later 1 ml SDW was added at morning and evening of every day. The germination was recorded at morning and evening of every day for 14 day. After completion of the germination experiment lot of germinated seeds from each of the pre-treatment test were transferred into thoroughly washed plastic cups, filled with nursery soil during the period of the experiment. The transparent plastic cups with germinated seeds were maintained at 25 ± 2^0C under an 8 hours photoperiod and light intensity of about 30 umol $m^{-2}s^{-1}$provide by cool white florescent tube lights.

Pre-sowing treatments:

Mechanical scarification (Sand paper scarification):

Surface sterilized seeds, three lots (25 in each) of seeds were placed between the flaps of 0-grade sandpaper and scarified by applying little fraction force for 90,120,150,180 seconds respectively. During scarification if some seeds were damaged, such damaged seed removed and discarded. Only intact, undamaged seeds transfer on germination paper. Seeds without any pretreatment considered as control for this germination test.

Water treatment:

Normal water:

Sterilized seeds soaked in normal water for 12, 24, 36, 48 hours, then seeds allow to dry on filter paper for 5 minutes and dried seeds kept in petri plates with sterilized forceps for germination.

Wet heat treatment:

The three seed lots (each of 25) immersed in separate taste tubes containing hot water (60^0C) and taste tubes kept in water bath for 5, 10, 15 and 20 minutes.

For boiling water treatment seed lot treated with 100 ^{O}C for 30, 60, 90, 120 second. After completion of both treatment seeds were transferred to SDW for 10 minutes till it reaches at normal temperature. Seeds were allowed to dry on blotter paper at the laboratory temperature

(30°C) before being placed in petri dishes. After drying seeds were transferred to Petri dishes for germination trials (Naikawadi *et al*.2012).

Acid scarification:

The healthy and surface sterilized seeds were presoaked in different concentrations of H_2SO_4 and HCL (Two volumes of acid to one volume of seed were used) 25%, 50%, 75%, 98% for 2.5, 5.0, 7.5, 10 minutes. After that treatment seeds were washed thoroughly under tap water (until the pH was neutral) as well with SDW for 5 times to remove any traces of acid. Then seeds transferred in sterilized petriplates on double layered germination paper for germination with the help of pointed sterilized forceps. 5 ml SDW was added to maintain the moisture content. Each treatment was maintained in triplicate along with control.

Growth hormone treatment:

The surface sterilized seeds were soaked in different concentrations GA_3 and IAA (1.0, 1.5, 2.5, 5.0 mM) for different time period 12, 24, 36 and 48 hours. After completion of specific period treatment seeds were placed in sterilized petriplates on double layered Germination paper which were moistened with 5ml SDW (Naikawadi *et al*.2012).

Preparation of stock solution of growth regulator:

Indole -3- acetic acid (IAA): 17.5 mg of IAA was dissolved in 2 ml of 1 N NaOH and then made it to final volume 100 ml with sterilized distilled water using volumetric flask.

Gibberellic acid (GA_3): 34.6 mg GA_3 was dissolved in 50 ml of water (20^O C) and then made it to final volume 100 ml with sterilized distilled water using volumetric flask.

Data collection:

Daily and cumulative germination counts:

The germinated seeds were observed daily and the data of seed germination was collected until the completion of germination (maximum 14 days).The seeds with 0.5 mm or more radical growth occur were counted as germinated seed (Naikawadi *et al*., 2012).

Germination percentage:

The final germination percentage (FGP) was calculated from the total seeds that germinated on the day of completion by using the formula: No.of seeds germinated on day of completion /No of seeds sowed x 100. Daily germination percentage (no. of seeds germinated on a particular day /No of seeds sowed x100) were summed up to obtain cumulative germination percentage (CGP) for each treatment (Naikawadi *et al.*, 2012).

Germination speed and germination value:

The germination value (GV) a composite value that combines germination speed(GS) and total germination which provides an objective mean of the evaluating the result of germination values. It is calculated using formula availed by Djavanshir and pourbeik, Deshpande 1976).

Germination speed = Final germination percentage /Day of completion of germination.

Germination value (GV) = ($\sum$ DGs/N) GP/10.

Where GP = germination percentage at the end of the test, DGs = daily germination speed obtained by dividing the cumulative germination percent by the number or days since sowing, $\sum$ DGs= Total germination obtained by the daily counts, N = The total number of daily counts, starting from the date of first germination, 10 = constant.

Emergence index:

Emergence index were calculated by using the formula as follows:

Emergence index (EI) = dn/n

Where dn = Number of seeds germinated on particular day (emergence) and n=day of emergence (Thakur *et al.*, 2004).

Seedling vigour:

Seedling vigor index (SVI) was calculated as per the recommendation of ISTA (1976):

SVI = germination percentage X shoot length.

For seedling vigor normal seedling from standard germination test were further classified as strong or weak seedling by visual observation and expressed as percentage. The erect and sturdy seedling with well-developed seedling parts were kept in the category and strong seedling (ISTA, 1976). Root length and shoot length were recorded and root to shoot ratio was calculated.

Statistical analysis:

The experiments were set up in a completely randomized design with minimum three replicates per treatment. Data were analyzed by analysis of variance (ANOVA) to detect significant differences between means.

Result and Discussion:

Many species of family Leguminosae shows very low germination rate because of hard, impermeable seed coats which is the major problem towards the success of this species (Zoghi *et al*., 2011). Presence of hard seed coat which prevents the entrance of water, exchange of gases, which causes physical dormancy in several species of the family Leguminosae

Various researchers have been recommended several methods for breaking seed coat dormancy and enhance seed germination include mechanical and chemical scarification, hot water treatment, dry heat, the application of exogenous growth regulators, cold (chilling) stratification. To overcome the seed dormancy of many hard coated seeds these pre-sowing treatments are may be an excellent way to break dormancy. The dormancy period of *T. purpurea* is affected by the hardness of the seed coat. It needed special treatments before sowing for increasing seed germination. Acid treatments and boiling water treatments are often used to break down especially thick impermeable seed coats (Pipinis *et al*., 2005).

Collection of plant material:

The plants of *T. purpurea* were commonly occurring as wasteland weed plant which grows along the road sides, on farm bands, at dry arid, waste places, on dry hills especially in poor rocky soil where competition for water was observed and it can tolerate arid condition. The matured pods were directly collected from large number of plants which grows at different places from villages of Pune and Ahmednagar district from State of Maharashtra, India. Where the climate of area was semi-arid and average annual rainfall is 330 mm. At the time of

collection by visual observation number of pods per plant, number of seeds per pod, seed and pod morphology, any infections were noted. Fruits are legume like pods which are flat, with two valved, slightly curved at tips, variation in size, approximately pods 3 to 5 cm in length, produced in groups of three to seven,159 to 423 pods per plant (depends on edge and size of plant, when immature green in color and brown after ripening. Pods found from rainy to cold season and matured naturally during the month of November to January. The collected pods were dried at normal room temperature and seeds were separated by thrashing, floatation method which removes trash (parts of legumes), empty, broken and insect-damaged seeds. Seed is small, smooth, rectangular to transversely ellipsoid, oblong and very slightly compressed laterally, 5 to 9 seeds per pod, seeds ash or gray to light or dark brown colored. Few seeds are observed infected with insect past. Average weight of the seed is 2.32 mg and hundred seed weight is 1.47gm, 2.5-5.7 mm X 1.8-3 mm in size. Healthy, equal sized seeds used for further germination experiment.

Seed viability:

The viability estimation of *T. purpurea* seeds were carried out through Flotation method and tetrazolium test showed presence of more than (78%) seeds is viable (Fig. a). In Flotation method, about 17 to 24 seeds out of 100 are floated on water surface and observed as empty seeds due to infection of insect paste and abnormal embryo development which was not capable for germination consider as non-viable seeds. Submerged seeds used for further germination treatment.

Seeds without any presoaking treatment used for TTC test required long duration for seed viability detection. Seeds treated with 1.0% TTC solution for 36 hours, only 17 seeds out of 25 were appeared red in color which shows 68% viability without any scarification treatment. Mechanically scarified seeds (for 5 min.) usually give better response i.e. 21 out of 25 seeds become red after 24 hours (84 %) seeds viable. Excise embryos and cotyledons from intact treated seeds with pointed forceps which appear as red colored. The failure of germination of scarified seed may be due to presence of some other type of dormancy in addition to hard seed coat.

Mechanical Scarification:

Sand paper scarification:

Over the period of 14 days, seeds without any treatments failed to germinate due to seed coat barrier. In the present study, the mechanical scarification was not efficient in dormancy breaking in *T. purpurea* seeds. Treatment of sandpaper scarification for 150 seconds, results indicated that germination of seeds significantly increase up to 10.67±1.53%. It also shows the greatest imbibition, increase germination percentage, seedling establishment and also the highest values of seedling growth characteristics compared with untreated (control) seeds (Table1). Mechanical scarification cracks the seed coat in some extent and also distinctly improved other germination parameters such as GS (0.76±2.55), GV (0.24±0.76), EI (0.92±0.60) and VI (9.33±1.49).

For breaking hard seed coat dormancy mechanical scarification is done by nicking; filling with needles or knife and abrasion in sand paper (Karaguzel *et al*., 2002; Mackay *et. al.,*1996) which will enhancing seed germination. Mechanical (sand paper) scarification is the most common and highly recommended method for overcoming impermeability of seed coat because it use the abrasion by rough surfaces so seed coat is softened, increase the water absorption, seed imbibition which boosting germination but do not reduce the average time of emergence (Mitra *et.al*.,2013; Irwin 1982). However, in *T.purpurea* seeds, mechanical (sand paper) scarification gives very poor result because seeds are small in size, very smooth and hard impermeable seed coat which did not cracked properly so it does not undergoes imbibition and germination (Table-1). It indicates that seeds have the typical hard, impermeable seed coat which develops physical dormancy of the leguminous species. It is also possible that the smooth seed coat contains certain inhibitory chemicals that do not leach out by this method. This results shows agreement with the results of sandpaper scarified seeds of *C. siliquastrum* did not exhibit any imbibition or germination due to the hard seed coat (Gebre and Karam, 2004). In *L. varius* seeds also shows very negligible seed germination by sandpaper scarification was found (Karaguzel *et al.*, 2002, Osman *et al*., 2004). In *T. purpurea* seeds mechanical scarification did not help to increase significant germination. Similar observations are found in a number of different *Acacia* species (Demel 1996); in *Uraria picta* seeds (Ahire *et al.*,2009) and in *Evolvulus alsinoids* (Naikawadi *et al*.,2012). However, mechanical scarification treatments have not been found more effective therefore it is necessary to investigate more effective seed coat treatments for the improvement

of germination of *T. purpurea* seeds. Because of unsatisfactory results, the mechanical scarification was not suggested.

Water treatments:

Normal water treatment:

Seeds without any pre-treatment showed nil germination over the period of germination (14 days) which indicating the presence of seed dormancy. In the normal water seed soaked for 12, 24, 36 and 48 hours duration. Seeds does not show any sign of imbibitions up to 12 hours water treatment. Seeds were starting too imbibed slowly after 24 hours soaking. Seeds soaked for 36 hours in normal water show better germination as compare to control. In normal water seed germination percentage is increases up to 7.5 % (Table: 2), GS (0.11±0.54), GV (0.35±0.40), EI (0.18±0.05), VI (0.18±0.23) and root shoot ratio is (0.02±0.01) (Table: 2). Germination percentage declines after 36 hours of normal water treatment.

Wet heat treatment:

Generally all hot water treatments enhanced seed germination rate as compared to control and normal water treatment with imbibitions of seeds. Germination was improved when the seeds were soaked in hot water (60 ^{0}C) for 5, 10, 15, 20 minutes. Seeds exposed to 5 minutes hot water pre-treatment gave a very low germination percentage (Table: 2). Seeds soaked for 10 minutes in hot water recorded the highest germination percentage. Results also indicated that seed germination increased with increasing water temperature and soaking period but low survival percentage were noticed. Further increase in the temperature and duration of the treatment resulted in decreased germination percentage. About 24.66±1.33% germination was observed on HW for 10 minutes. Other germination parameters such as GS (1.04±0.09), GV (0.91±0.24), VI (24.53±5.07), EI (0.84±0.18) and root /shoot ratio (0.46±0.16) (Table: 2).

In the boiling water treatment, low duration of boiling water i.e. 90 Sec (100^{o}C) show better result as compare with hot water treatment. About 43.33±1.33% germination was perceived in 90 Sec BW treatments. Other germination parameters such as GS (3.71±0.32), GV (4.93±0.57), VI (76.67±0.49), EI (1.50±0.00) and root /shoot ratio (0.43±0.15) (Table: 2). It was

recorded that BW decreases germination time. If duration of boiling water (100^{o}C) treatment increases more than 3 minutes the water become green to yellow colored due to chemicals present in seed coat may leach out by high temperature. It was also noticed that germination percentage and germination parameters and survival of seedlings also decreases by BW treatment for more than 3 minutes.

In present work, unscarified seeds soaked in normal water for 12, 24, 36 and 48 hours shows very poor germination, indicating that *T. purpurea* seeds have the typical hard water impermeable seed coat which is common character of the many leguminous species.

In many species with hard seed coat, soaking in hot and boiling water is useful techniques for improve the seed germination but the efficiency of such treatment depends on the species, water temperature, the durations of soaking time etc. (Baskin, 2003; Basbag *et al.*, 2010). Hot and boiling water treatments improve seeds germination due to water soluble inhibitors present in the seed coat were leached out by hot water, rapture of the seed coat wall which increases water and O_2 permeability causing physiological changes required for germination (Wang *et al.,* 2007). Also seeds treated with hot water showed some loss of cuticular material like cutin, a waxy, fatty hemi-cellulose present on outer layer of the seed testa (Gunn 1990).

In present investigation hot water and boiling water are effective for improvement of seed germination in *T. purpurea* but any duration of boiling time did not completely overcome the seed coat impermeability. About 24.66% germination was observed seeds soaked in HW for 10minutes.Further increase in temperature and duration of treatment decreased germination (Table-2). Treatment of boiling water (100^{o}C) for 90 sec shows 43.33% germination and further increase reduced germination (Table-2). Because of prolonged contact with hot water embryo may killed and seed germination was decreased. However, much lower imbibition and slower germination rate of hot water pretreatment is a limitation for many hard seeds (Wang *et al*., 2007). In seeds of *Lupinus prennis* soaking in hot water for 24 hours increase germination up to the 32% (Mackay *et al*., 1996). Same results had been reported in black locust seeds (Basbag *et al*., 2010) and in *T.indica seeds* when soaked in hot water (Abubakar and Muhammad, 2013).

Acid Scarification:

In the present study, acid scarified seeds with H_2SO_4 and HCl with different concentration and duration significantly increases seed germination as compared with non-treated seeds. About 48% germination was observed in the seed treated with 75% H_2SO_4 for 2.5 min. The GS (2.48±0.50), GV (2.86±0.68), EI (1.22±0.33) and VI (134.00±15.77) respectively (Table 3). Further increase in concentration and treatment duration, H_2SO_4 become yellowish colored and emits bitter odor of chemicals leached out from seed coat and germination percentage also decreased. Treatment of H_2SO_4 and HCl shows germination completed during early period i.e. within 4-5 days.

Pre-treatment of HCl of different concentration also enhances germination in *T. purpurea* seeds**.** About 24% germination was observed in the seed treated with 75% HCl for 5 minutes, other germination parameters such as GS (2.6±0.3), GV (1.71±0.26), VI (65.83±12.19), EI (0.93±0.14) and root /shoot ratio (0.24±0.01) as like this (Table:4).If concentration and duration of treatment increases germination percentage was also decreases.

In the present investigation, treatment of 75% H_2SO_4 for 2.5 min and 75% HCl for 5 min for 5 min might be sufficient to hydrolyze the seed coat constituents and improved germination will be very better as compare to control. Different acids (HCl, and H_2SO_4) had been widely used for breaking dormancy of many hard seed coat species like blackdisk medick (*Medicago orbicularis* (Linn.) Bartal. and *Albizia* sps.(Tigabu and Oden, 2001). Acid scarification is extremely effective for improving germination of seeds with thick impermeable, hard seed coats by softening the seed coat in the epidermal layer, removal of the cuticular layer and destroys the plugged natural opening which helps in entry of water and oxygen diffusion required for germination (Baskin and Baskin, 1998; Malavasi and Malavasi, 2004). Acid scarification results into rapid and highest imbibition, germination percentage, seedling establishment and seedling growth. The concentrated sulfuric acid treatment has been widely used to improve seed germination of several hard seed coat species (Tigabu and Oden, 2001).

Our experiment showed that all acid scarification significantly increased seed germination rate as compared to control treatment. Treatment of 75% H_2SO_4 for 2.5 min shows 48% germination and other germination parameters also, which the best results are as compare to other acid treatments or control (Table-4). For breaking the dormancy of *T. purpurea* seeds by

H_2SO_4 was the best treatment because it has capability to hydrolyzes the chemicals in seed coat, rupture seed coat more rapidly and increases the permeability of seed coat for water absorption which leading to imbibition and germination of seeds. It may cause physiological changes and biochemical activities needed for seed germination. Similar results are found several hard coated species such as *Acacia* species, *Uraria picta* (Rana and Nuatiyal, 1989; Ahire *et.al*., 2009). *A. auriculiformis* (Olatunji *et al*., 2012) in which treatment of sulphuric acid enhanced germination percentage and other germination parameters also. Seeds of *A. africana* seeds (Family Fabaceae) acid pre-treatments gave an impressive, more uniform, regular and highest germination within shortest time. *A. africana* seeds 50% H_2SO_4 acid treatment shows 83.33% germination and 98% H_2SO_4 gives 76.67% germination (Amusa 2011). In addition, this is also similar to the report by Olatunji *et al*., 2012) that seeds *A. auriculiformis* that were soaked in H_2SO_4 for 10 minutes recorded the best germination percentage of 96%. In *Cassia fistula seeds also* maximum germination percentages (91%) was observed by seeds treated with 50% H_2SO_4 for 5 minutes (Amira *et al.,2013)*. Abubakar and Muhammad, 2013 also demonstrated that seeds of tamarind treated with sulphuric acid of 50% concentration gave a 92% germination. reported a similar result of 98% germination when *Tamarind* (Jabbe) seeds were treated with 50% sulphuric acid for 60 minutes.

A similar effect of concentrated HCl on germination was observed but it is less effective than H_2SO_4. Nitric acid is well documented as a compound, which increases the germination and release dormancy in seeds of many species may be due to sensing soil N_2 availability in soil (Bethke *et al.*, 2007). Seeds of *Rhynchosia capitata* (Roth DC) treated with HCl for 30 min germination significantly increased over control.

But in our study, if concentration and duration of acid scarification(H_2SO_4 and HCL) increases it will shows negative effects because embryo may get destroyed on contact with acid for a prolonged period. Longer period of time for acid treatment resulted in damaged embryos and seeds (Wang *et al*., 2007). The mechanism of seed germination influenced by H_2SO_4 and HCl are alone not completely sufficient to rupture the seed coat or overcome dormancy of *T. purpurea* seeds.

Plant growth regulators:

Seed germination is influenced by internal Growth hormones controlling dormancy. Seeds treated with different concentrations and duration of growth regulators, increases seed

germination up to 59 to 82 %. Growth regulators like GA_3 and IAA of 1.0 mM, 2.5 mM, 5.0 mM and 7.5 mM concentration used for treatment of seeds for 12, 24, 36 and 48 hours show better germination percentage as compare with control or few other treatments.

The better germination percentage was 81.30% detected under 5.0 mM GA_3 for 24 hours treatment. Other germination parameters such as GS (2.61±0.13), GV (2.57±0.15), EI (1.39±0.05) and VI (265.73±12.19) (Table: 5).

The 37.33% germination percentage was observed by treatment of 5.0 mM IAA for 24 hours. Other germination parameters such as GS (1.17±0.15), GV (0.50±0.13), EI (0.78±0.12) and VI (50.00±2.73) (Table: 5).

In the present study, pre-soaking in GA_3 resulted in the greatest germination percentage, seedling establishment, the highest values of seedling growth characteristics, seedling survival compared with untreated or other treatments. Gibberellic acid stimulate germination percentage by inducing hydrolytic enzymes which weaken the barrier tissues such as the endosperm or seed coat, storage reserves nutrients hydrolysis of seed and trigger expansion of the embryo (Karssen *et al.,*1989). Similar germination was increased by GA_3 in *Fagus sylvatica* (Nicolas *et al.,* 1996). About 60-67% grmination was observed in *Morus nigra* Linn.,seeds (Koyuncu, 2005) and in *Sapindus trifoliatus* germination enhanced up to the 70-89% (Naidu *et al.,* 2000). Also 5.0 mM GA_3 increases germination in *E.alsinoids* Linn (Naikawadi *et al*.,2012).

Similar pattern of germination result was found in IAA but is was less effective than GA_3. GA_3 and IAA suppress the inhibitory action of ethylene when they act as a promoter (Banerji U.K.,1998). No doubt all growth hormones gave better result than control, But at higher concentration of GA_3 the germination percentage decreased which indicate that higher concentrations of gibberellic acid have inhibitory effect on germination of *T. purpuria* seeds (Dhoran and Gudadhe, 2012).

Results indicates that boiling water and growth hormone treatments are required for enhance germination percentage and seedlings formation in *T. purpurea*. This seed germination study would help for sustainable supply of atmospheric nitrogen fixing species and also useful for pharmaceutical industries as medicinally important elite clone for active secondary metabolites.

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Table 1: -Effect of Mechanical scarification on seed germination of *Tephrosia purpurea* (Linn.) Pers

Treatment (Hrs)	GP (%)	GS	GV	VI	EI
12	10.67±1.53	0.76±2.55	0.24±0.76	9.33±1.49	0.92±0.60
24	2.67±0.78	0.19±0.87	0.27±0.13	3.93±0.18	0.16±0.76
36	8.05±0.88	0.57±1.13	0.16±0.68	7.20±0.90	0.48±0.86
48	5.33±0.91	0.28±1.01	0.13±0.43	6.07±0.97	0.52±0.53

Table 2: -Effect of water treatment on seed germination of *Tephrosia purpurea* (Linn.)Pers

Treatment (Hrs)	GP (%)	GS	GV	VI	EI	Root/Shoot ratio
Control	1.20±3.33	0.01±0.18	0.01±0.04	0.02±0.01	0.01±0.01	0.08±0.06
Normal water (Hours)						
12	5.61±1.02	0.05±0.24	0.16±0.23	0.09±0.15	0.12±0.30	0.06±0.04
24	6.98±1.53	0.08±0.31	0.24±0.32	0.12±0.21	0.15±0.33	0.07±0.08
36	7.50±1.24	0.11±0.54	0.35±0.40	0.18±0.23	0.18±0.05	0.02±0.01
48	6.44±1.09	0.09±0.65	0.21±0.13	0.13±0.19	0.13±0.04	0.03±0.02
Hot water (Minutes)						
5	20.00±2.31	0.97±0.48	0.51±0.12	22.00±4.80	0.36±0.06	0.16±0.01
10	24.66±1.33	1.04±0.09	0.91±0.24	24.53±5.07	0.84±0.18	0.46±0.16
15	19.16±2.66	0.76±0.19	0.70±0.21	20.33±7.93	0.78±0.18	0.15±0.01
20	18.04±1.98	0.54±0.49	0.41±0.18	19.05±8.45	0.72±0.12	0.13±0.02
Boiling water (Second)						
30	29.33±3.52	2.09±0.25	1.17±0.23	45.07±0.57	0.94±0.28	0.13±0.71
60	32.02±4.61	2.5±0.64	2.94±0.65	64.80±0.02	1.02±0.36	0.20±0.01
90	43.33±1.33	3.71±0.32	4.93±0.57	76.67±0.49	1.50±0.00	0.43±0.15
120	30.19±1.03	2.70±0.49	2.52±0.74	56.01±0.54	1.10±0.17	0.30±0.28

Table 3: Effect of various concentration of H_2So_4 treatment on seed germination of *Tephrosia purpurea* (Linn.)Pers.

Treatment (Min)	GP (%)	GS	GV	VI	EI	Root/Shoot ratio
H_2So_4 25%						
2.5	12.67±3.53	0.67±0.81	0.93±0.43	88.3±6.50	0.08±0.01	0.12±0.01
5	31.90±1.31	1.66±0.40	1.37±0.38	67.4±5.71	0.89±0.08	0.30±0.19
7.5	39.33±2.67	2.55±0.73	1.89±0.60	79.9±8.46	1.75±0.67	0.46±0.21
10	20.33±1.4	1.15±1.07	1.28±0.05	57.04±4.6	0.57±0.09	0.29±0.11
H_2So_4 50%						
2.5	34.63±1.81	2.42±0.66	1.29±0.31	67.46±12.39	0.89±0.09	0.16±0.01
5	43.32±2.55	3.43±0.75	1.99±0.77	101.66±19.98	0.94±0.14	0.43±0.46
7.5	33.13±1.16	2.28±0.48	1.20±0.30	53.99±12.45	0.83±0.28	0.38±0.21
10	14.06±1.02	0.75±0.23	0.28±0.12	42.56±10.22	0.22±0.15	0.23±0.09
H_2So_4 75%						
2.5	48.00±2.31	2.48±0.50	2.86±0.68	134.00±15.77	1.22±0.33	0.32±0.07
5	34.00±4.05	1.38±0.41	1.24±0.46	98.4±24.93	0.83±0.14	0.25±0.02
7.5	18.00±0.09	0.96±0.69	0.66±0.73	64.4±24.80	0.60±0.00	0.11±0.16
10	09.07±0.07	0.19±0.02	0.13±0.25	15.00±12.05	0.03±.02	0.02±0.07
H_2So_4 98%						
2.5	23.66±3.53	1.61±0.62	1.13±0.83	65.86±6.89	1.33±0.27	0.30±0.01
5	12.00±4.0	0.26±0.52	0.21±0.71	40.80±2.05	0.72±0.12	0.26±0.03
7.5	05.00±2.30	0.18±0.10	0.18±0.06	24.00±1.32	0.22±0.33	0.21±0.02
10	01.05±0.5	0.01±0.07	0.08±0.01	13.08±0.33	0.14±0.5	0.8±0.01

Table 4: Effect of various concentration of HCL treatment on seed germination of *Tephrosia purpurea* (Linn.)Pers

Treatment (Min)	GP (%)	GS	GV	VI	EI	Root/Shoot ratio
HCl 25 %						
2.5	15.7±4.8	0.91±0.21	0.09±0.08	40.00±7.17	0.53±10	0.14±0.01
5	17.3±2.7	1.2±0.32	0.12±0.14	58.82±11.07	0.67±0.16	0.12±0.01
7.5	20.0±2.3	1.4±0.64	0.13±0.07	36.00±6.42	0.56±0.05	0.15±0.01
10	13.3±1.3	0.4±0.14	0.9±0.03	34.21±3.14	0.22±0.12	0.9±0.02
HCl 50 %						
2.5	20.0±2.3	1.4±0.2	0.19±0.17	52.00±9.39	0.53±0.24	0.16±0.02
5	21.7±3.5	1.5±0.3	0.21±0.26	60.37±13.15	0.67±0.14	0.10±0.01
7.5	22.32±2.7	1.6±0.2	0.25±0.32	76.21±6.57	1.67±0.14	0.23±0.02
10	18.26±0.9	1.4±0.08	0.03±0.04	21.02±6.15	0.45±0.9	0.18±0.1
HCl 75 %						
2.5	20.7±2.7	1.3±0.2	0.44±0.04	54.23±9.95	0.64±0.20	0.18±0.02
5	23.7±3.5	2.6±0.3	1.71±0.26	65.83±12.19	0.93±0.14	0.24±0.01
7.5	21.0±2.3	1.0±0.2	0.61±0.10	39.20±7.42	0.72±0.12	0.13±0.03
10	13.0±0.7	0.8±0.00	0.06±0.16	23.73±7.11	0.29±0.27	0.11±0.05
HCl 98 %						
2.5	20.3±3.5	1.5±0.3	0.38±0.36	59.64±11.04	1.00±0.24	0.20±0.09
5	18.0±2.3	1.0±0.4	0.28±0.29	44.8±11.80	0.83±0.14	0.11±0.02
7.5	16.3±1.3	0.9±0.1	0.12±0.26	39.81±8.90	0.70±0.24	0.05±0.03
10	13.29±0.9	0.9±0.06	0.11±0.09	27.93±4.05	0.09±0.11	0.03±0.06

Table 5: Effect of Growth hormones on seed germination of *Tephrosia purpurea* (Linn.)Pers.

Treatment (Hrs)	GP (%)	GS	GV	VI	EI	Root/Shoot ratio
GA_3 1.0 mM						
12	28.00±1.88	0.42±0.13	0.86±0.19	55.80±10.19	0.67±0.14	0.31±0.08
24	42.66±1.92	0.81±3.15	1.12±0.23	69.60±12.80	0.74±0.43	0.49±0.09
36	68 ±1.85	2.42±0.13	1.86±0.59	100.00±13.21	0.91±0.16	0.60±0.07
48	40±1.88	1.00±0.33	0.66±0.27	73.60±19.83	0.83±0.24	0.51±0.05
GA_3 2.5mM						
12	22.66±108	0.95±0.07	0.37±0.19	77.60±8.38	0.50±0.08	0.36±0.01
24	64.66±1.08	1.01±0.08	0.73±0.29	107.07±14.46	0.67±0.16	0.41±0.03
36	72.66±2.17	2.91±0.15	1.90±0.10	126.60±15.52	0.97±0.14	0.47±0.04
48	46.66±2.88	1.00±0.20	0.40±0.21	109.33±21.32	0.87±0.16	0.39±0.05
GA_3 5.0 mM						
12	64.33±1.08	1.09±0.07	0.97±0.22	105.07±15.65	0.83±0.14	0.24±0.07
24	81.30±3.46	2.61±0.13	2.57±0.15	265.73±12.19	1.39±0.05	0.31±0.04
36	62.02±1.88	1.71±0.13	1.43±0.32	153.60±18.02	0.78±0.18	0.22±0.01
48	33.43±1.08	0.38±0.07	0.53±0.03	70.00±27.37	0.33±0.14	0.13±0.02
GA_3 7.5mM						
12	59.33±1.08	2.09±0.07	1.97±0.22	207.47±12.04	1.17±0.14	0.08±0.02
24	44.88±3.15	1.49±0.07	1.37±0.15	152.73±12.19	0.83±0.27	0.12±0.04
36	31.43±4.74	1.15±0.33	0.73±0.32	92.93±29.62	0.50±0.24	0.24±0.14
48	23.23±1.08	0.80±0.07	0.53±0.03	48.00±40.44	0.47±0.12	0.31±0.02
IAA 1.0 mM						
12	8.00±0.88	0.28±0.13	0.06±0.04	4.80±1.63	0.19±0.08	0.11±0.03
24	15.33±2.21	0.47±1.78	0.12±0.09	8.00±2.18	0.26±0.03	0.18±0.04
36	30.00±4.99	0.86±0.36	0.96±0.24	36.47±6.28	0.33±0.16	0.19±0.43
48	22.00±2.33	0.16±0.40	0.19±0.58	21.00±7.35	0.28±0.01	0.20±0.24
IAA 2.5mM						
12	9.49±3.26	0.57±0.23	0.20±0.10	7.20±3.02	0.28±0.12	0.20±0.02
24	18.67±2.91	0.38±0.00	0.30±0.40	11.53±2.57	0.18±0.08	0.34±0.05
36	36.00±1.89	1.64±0.13	0.97±0.06	45.60±4.67	0.67±0.14	0.27±0.08
48	23.67±2.18	0.62±0.16	0.37±0.30	49.07±8.68	0.44±0.09	0.22±0.09
IAA 5.0 Mm						
12	12.00±1.88	0.57±0.13	0.23±0.51	12.40±2.30	0.36±0.06	0.18±0.44
24	37.33±3.46	1.17±0.15	0.50±0.13	50.00±2.73	0.78±0.12	0.14±0.23
36	24.67±4.75	0.75±0.34	0.40±0.06	27.60±4.99	0.28±0.30	0.28±0.57
48	13.67±2.18	0.60±0.16	0.37±0.30	10.67±9.06	0.17±0.36	0.24±0.14
IAA 7.5mM						
12	31.66±2.17	1.04±0.15	0.43±0.61	40.00±4.87	0.56±0.18	0.20±0.03
24	21.44±1.78	0.95±0.20	0.37±0.23	25.33±4.36	0.39±0.05	0.22±0.05
36	14.00±3.77	0.71±0.27	0.24±0.56	21.60±6.10	0.12±0.24	0.32±0.02
48	09.33±3.93	0.10±0.28	0.10±0.40	16.47±12.39	0.06±0.20	0.30±0.09

GS: Germination speed, GV: Germination value, VI: Vigor index EI: Emergence index, Values are mean ± SE of three independent experiment each with three replicates.

REFERANCES

1. Abubakar, Z.A. and Muhammad, A., 2013. Breaking Seed Dormancy in Tamarind (*Tamarindus Indica*) A Case Study of Gombe Local Government Area, *Journal of Appled Science Environment Manageger* **17** (1), 83-87.
2. Ahire, M.L., Ghane, S.G., and Nikam, T.D., 2009. Seed viability and influence of presowing treatments on germination and seedling development of *Uraria picta* (Jacq) DC., *Seed Science and Biotechology*, **3**. 48-53.
3. Amusa, T.O., 2011. Effects of three pre-treatment techniques on dormancy and germination of seeds of *Afzelia africana* (Sm. Ex pers). *Journal of Horticulture and Forestry* **3** (4). 96-103.
4. Basbag, M., Aydin, A. and Ayzit, D., 2010. The effect of different temperatures and durations on the dormancy breaking of black Locust (*Robinia pseudoacacia* L.) and Honey Locust (*Gleditsia triacanthos* L.) Seeds. *National Science Biology*. **2** (4). 125-128.
5. Baskin, C.C. and Baskin, J.M., 1998. Seeds, ecology, biogeography, and evolution of dormancy and germination. Academic Press, New York.
6. Baskin, J.M., Baskin, C.C. and Li, X., 2000. Taxonomy, anatomy and evolution of physical dormancy in seeds. *Plant Specific Biology*. **15**. 139-152.
7. Bethke, P.C., Libourel, I.G. and Jones, R.L., 2007. Nitric oxide in seed dormancy and germination. *Seed Science Technology*. **20**.153–175.
8. Demel, T., 1996. Germination ecology of twelve indigenous and eight exotic multipurpose leguminous species from Ethiopia. *Forest Ecology and Management*. **80**. 209-223.
9. Dhoran, V.S. and Gudadhe. S.P., 2012. Effect of Plant Growth Regulators on Seed Germination and Seedling Vigour in *Asparagus sprengeri* Regelin. *International Research Journal Biological Science.* **1** (7). 6-10.
10. Gathumbi, S.M., Cadisch, G. and Giller, K.E., 2003. Improved fallows: effects of species interaction on growth and productivity in monoculture and mixed stands. *Forestry Ecology and Management.* **187**. 267–280.
11. Gebre, G.H. and Karam, N.S., 2004. Germination of *Cercis siliquastrum* seeds in response to gibberellic acid and stratification. *Seed Science and Technology.* **32**. 255-260.

12. Gunn, B.V., 1990. Germination pretreatments for selected acacia species from the Pilbara region of Weston Australia. *ICIAR Proceedings series.* **28**. 46-50.

13. Irwin, P.T., 1982. Plant Physiology Addision-Wesley Pub. Co. Inc. U.S.A. 501-540.

14. ISTA., 1976. International rules for seed testing. *Proceedings of the International Seed Testing Association.* **31**. 1-52.

15. Karaguzel, O., Baktir, I., Cakmakci, S., Ortacesme, V., Aydınoglu, B. and Atik, M., 2002. Effects of scarification methods, temperature and sowing date on some germination characteristics of *Lupinus varius* L. *2nd National Congress on Ornamental Plants, Citrus and Greenhouse Research Institute, Antalya, Turkey*. 40-47.

16. Karssen, C.M., Zagorsi, S., Kepczynski, J. and Groot, S.P.C., 1989. Key role for endogenous gibberellinsin the control of seed germination. *Annals of Botany.* **63**. 71-80.

17. Koyuncu, F., 2005. Breaking seed dormancy in black mulberry (*Morus alba* Linn.) by cold stratification and exogenous application of gibberellic acid. *Acta Bio-logica Cracoviensia Series Botaniva*. **47**:23-26.

18. Mackay, W.A., Davis, T.D., Sankhla, D., Reinmenschneider, D.E., 1996. Factors influencing seed germination of *Lupinus prennis* Linn., **Journal of Environmental Horticulture**. **14** (4). 167-169.

19. Malavasi, U.C. and Malavasi, M., 2004. Dormancy breaking and germination of *Enterolobium contortisiliquum* (Vell.) Morong seeds. **Brazilian Archives of Biology and Technology 47**. 851-854.

20. Mitra, M., Ali, L.M. and Zahra, O.A., 2013. The induction of seed germination using sulfuric acid, gibberellic acid and hot water in *Robini pseudoacacia* Linn., *International Research Journal of Applied and Basic Sciences*. **4** (1). 96-98.

21. Muralidhar, A., Somasekhar, A., Sumanjali, B., Praveen, K., Lakshmi, P., Shahdul, V. and Bhargavi, D., 2014. Anticancer activity of *Tephrosia purpurea* root extracts against Ehrlich Ascites Carcinoma (EAC) cells in swiss albino mice, Pelagia *Research Library Der Pharmacia Sinica.* **5** (2). 81-87.

22. Naidu, C.V., Rajendrudu, G. and Swamy, P.M., 2000. Effect of plant growth regulators on seed germination of *Sapindus trifoliatus* Vahl. *Seed Science and Technology.* **28**. 249-252.

23. Naikawadi, V.B., Ahire, M.L. and Nikam, T.D., 2012. Seeds characterization, Viability and Promotion of Seed Germination in Nervine Tonic Plant *Evolvulus alsinoids* Linn., *The Asia and Australian Journal of Plant Science and Biotechnology*. **6** (1). 5-11.

24. Nicolas, C., Nicolus, G. and Rodriguez, D., 1996. Antagonistic effects of abscisic acid and gibberellic acid on the breaking of dormancy of *Fagus sylvatica* seeds. *Physiology Plantarum.* **96**. 244-250.

25. Olatunji, D., Maku, J.O. and Odumefun, O.P., 2012. Effect of pre-treatments on the germination and early seedlings growth of *Acacia auriculiformis* Cunn. Ex. Benth. *African Journal of Plant Science*. **6** (14). 364-369.

26. Osman, K., Sadik, C., Veli, O. and Bilal. A., 2004. Influence of seed coat treatments on germination and early seedling growth of *Lupinus varius* Linn. *Pakistan Journal Botany*. **36** (1). 65-74.

27. Pipinis, E., Aslanidou, M., Milios, E., Gkioumousides, C. and Karakosta, C., 2005. Germination study on three leguminous species *Calycotome villosa, Spartium junceum* and *Medicago arborea*), *Scientific Annals of the Department of Forestry and Natural Environment.* **43**. 253-263.

28. Rana, U. and Nuatiyal, A.R., 1989. Coat emposeed dormancy in *Acacia farnesiana* seeds, *Seed Research*, **17**. 122-127.

29. Rangaswami, S. and Rao, G.R., 1955. Occurrence of rutin in the leaves of *Tephrosia* species. *Indian Journal Pharmacy.* **17**. 136-137.

30. Saad, T., Muhammad, A.S. and Muhammad, A., 2013. A Review on the Phytochemistry and Pharmacology of Genus *Tephrosia*. *Phytopharmacology.* **4** (3). 598-637.

31. Sajwan, S., Sajwan, K., Chandra, M., Alvi, A.I., Asim, S.M. and Sahar, N., 2015. Studies on Unani Herbal Medicinal plant. *International Research Journal of Natural and Applied Sciences*. **2** (7). 148-179.

32. Thakur, A., Mehta, R. and Thakur, P.S., 2004. Germination, viability and vigour of fresh and aged seeds of some endangered plant species of Western Himmalayas. *Indian Journal of Plant Physiology.* **9**. 247-254.

33. Tigabu, M. and Oden, P.C., 2001. Effect of seed scarification, gibberellic acid and temperature on seed germination of two multipurpose *Albizia* species from Ethiopia. *Seed Science Technology*. **29**. 11-20.

34. Wang, Y.R., Hanson, J. and Mariam, Y.W., 2007. Effect of sulphuric acid pretreatment on breaking hard seed dormancy in diverse accessions of five wild Vigna species. *Seed Science Technology*. **35**. 550-559.

35. Zoghi, Z., Azadfar, D. and Kooch, Y., 2011. The effect of different treatments on seeds dormancy breaking and germination of caspian locust (*Gleditschia caspica*) tree. *Annals of Biological Research.* **2** (5). 400-406.