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STUDIES ON *TEPHROSIA PURPUREA* L. WITH SPECIAL REFERENCE TO SEED GERMINATION PHYSIOLOGY

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ABSTRACT

The nature of much germination regulating mechanism in seeds lends itself to an interpretation in terms of survival value of species. There are always consistent efforts from farmers, researchers and scientists all over the world to produce good quality seeds. Earlier studies showed that seeds of *Tephrosia purpurea* are small oblong and very slightly compressed laterally. Each pod of the plant is 4.67 cm long and contains 4-6 such seeds. Present investigation was carried out to study seed dormancy and seed germination physiology of *Tephrosia purpurea*.

KEY WORDS: *Tephrosia purpurea*, seed germination etc.

INTRODUCTION

Tephrosia purpurea is a commonly occurring weedy plant in the ecosystem, where there is competition for water. It grows only in the rainy season and passes the dry season in the form of speed. The plant *Tephrosia purpurea* (L) Pers is an important source of medicinal drugs and has a well recognized place in the indigenous system of medicine. It has been mentioned in both the Ayurvedic and Unani systems of medicine [1, 2]. It is used as a cure for poisonous snake bite. The root of the mature plant is grounded to make a paste. Slaked lime soaked in water is then mixed in the paste in the ratio of 2:1. Half teaspoon full of turmeric powder is then added in to the mixture. This mixture is then heated over a low flame for a few minutes, then applied over the injured part and bandaged. The patient is not allowed to sleep. The drug is said to have the sucking effect on the poison which is sucked out of the system [3, 4, 5].

The occurrence of dormancy is studied in a large number of seeds coat [6, 7]. There are many causes of dormancy. The causes can be due to impermeability of seeds coat, presence of endogenous inhibitors in the seed [8], immature embryo, imposed dormancy or specific requirement of seeds [9]. Seed germination has been variously defined by different workers. Most of them call it 'the sprouting of seeds' or 'resumption of growth by dormant embryo' [10]. Mayer has defined germination as 'that group processes which causes the sudden transformation of dry seed into the young seedling' [11].

The morphological studies of seeds of *Tephrosia purpurea* show that the seed is small oblong and very slightly compressed laterally. Each pod of the plant is 4.67 cm long and contains 4-6 such seeds. The average volume of the seeds is 0.028 cc. and average weight of the seed is 2.54 mg [12]. The seeds of *Tephrosia purpurea* showed strong dormancy. The reason of the dormancy in the seeds is due to the yellowish pigment with a strong odour given out by the seed coat when the seed comes in contact with moisture. Mechanically it was impossible to remove the seed coat when they are dry. But when the seeds were treated with concentrated H_2SO_4 and the seed coat was dissolved in the germination response was quite good [13, 14, 15].

In present investigation seed germination studies have been done from a variety of angles. A number of factors appear to influence the process of germination. Different types of dormancies, occurrences of ecotypes, presence of germination and growth inhibitory factors, permeability level of the seed coat, drought, resistance mechanism, polymorphism, different perceptions of light and temperature have attracted the attention to carry out this research for germination studies.

MATERIALS AND METHODS

Physiology of seed germination

Dormancy studies

Freshly harvested seeds were used for the purpose of dormancy studies. Under favorable external conditions freshly harvested seeds were unable to germinate. This showed the dormancy phenomena in the seeds.

Methods of breaking dormancy

Various physical treatments were given to break dormancy. The following methods were employed to break the dormancy of seeds.

1. **Treatment of varying light periods**

Thoroughly washed seeds were subjected to normal diurnal cycle along with continuous light and continuous darkness for fifty days to study the effect on the germination response of *Tephrosia purpurea* seeds to varying light periods.

2. Treatment of different action spectra

Thoroughly washed seeds were placed for germination under different cellophane double filters viz. blue, green, yellow, red and blue and red together to obtain germination responses for different action spectra.

3. Treatment with hot water

Seeds were treated with different temperatures in hot water for five minutes. The germination response for different water temperature was calculated.

4. Treatment with cold water for different time period

Seeds were soaked in the room temperature for different time periods in cold water, for calculation of germination response.

5. Treatment with concentrated H_2SO_4

Thoroughly washed seeds were treated with concentrated H_2SO_4 for different time periods. Then they were again thoroughly washed under tap water, before placing them in petridishes which were set for germinated. The concentrated acid treated for different time periods was calculated.

6. Treatment with lime

Thoroughly washed seeds were placed in petridishes set for germination with different ratio's of lime and sand. The germination responses were calculated to know the effect on germination for different concentrations of lime.

7. Treatment with growth hormones

synthetic auxin (NAA)

Thoroughly washed seeds were placed in petridishes, set with different concentrations of auxin. The germination response for synthetic auxin was obtained.

Gibberellins (GA3)

Thoroughly washed seeds were placed in petridishes set with different concentrations of gibberellins to obtain germination responses.

8. Treatment with morphactin

Thoroughly washed seeds were placed under different concentrations of morphactin in petridishes set for germination. Thus the germination response was obtained.

9. Treatment with ethylene

Thoroughly washed seeds were placed in petridishes which were set for germination with different concentrations of ethylene. Germination responses of seeds of *Tephrosia purpurea* were obtained.

RESULTS AND DISCUSSIONS

Seed germination response of *Tephrosia purpurea* to varying light periods

Seed germination in many species is inhibited by continuous white light. Well-Known examples are Nemophila insignis, Phacelia tanacetifolia and Amaranthus caudatus etc. Such seeds are normally dark germinators. They are frequently referred to as light-inhibited or negatively photoblastic seeds. Some seeds whose dormancy is broken by light can also be inhibited by prolonged exposure. So it can be said that the phenomena is time dependent. In some cases inhibition brought about by intermittent light of a few hours each day. Since the proportion of inhibited seeds increases with the duration of each light period, a quasiphoto periodic effect results, when most of the seed can germinate under short days but few can do so in long days. Photo-inhibition is also fluence rate dependent, the degree of inhibition generally increasing linearly with the logarithm of the fluence rate.

The degree of inhibition also may depend on temperature, but no general can be formulated for the seed germination responses of varying light periods three petridishes with 100 seeds each were set for the test. Moistened pads were placed in the petridishes before placing the seeds. Seeds which are exposed for continuous illumination are placed under 40 watt, white light bulb, for 50 days at a distance of one metter. A glass jar filled with water is placed between petridish and bulb to eliminate the heating effect. The petridishes with seeds set for continuous darkness was placed inside a black painted wooden chamber for fifty days. The third lot of seeds within the petridish was subjected to normal diurnal cycle for period of 50 days. The moisture content in each petridish was maintained by putting adequate water. The three petridishes were set under normal room temperature (table-1).

Seed germination response of *Tephrosia purpurea* in different action spectra

Light is an extremely important factor for releasing seeds from dormancy. Almost all light requiring seeds have coat-imposed dormancy. Seeds of many species are affected by

exposure to white light for many just a few minutes or seconds. Some require intermittent illumination (eg. kalanchoe blossfeldiana). Photoperiodic effect also exists for some species require exposure to long days and other too short days. The light requirement frequently depends on the temperature.

In nature white light (i.e. sunlight) breaks dormancy, but we know that the wavelengths in the orange/red region of the spectrum are responsible. Action spectrum for the breaking of dormancy in the Grand Rapids cultivars of lettuce, obtained by Borthwick and Hendricks (1954) and their colleagues revealed that major activity is 660nm. The action spectrum showed that 730 nm is the wavelength of maximum activity [16]. The action spectrum discovered by red light is mutually antagonistic. This was done by exposing lettuce seeds to a sequence of red and far-red irradiations. Only when last exposure in the sequence was to red light was dormancy terminated. This established the fact of photo- reversibility, i.e. the two wavelengths 660 nm and 730nm are able to reverse each other's effect. Light is of course absorbed by molecules of a pigment, and the one participating in the breaking of dormancy is known as phytochrome. Phytochrome exists in two forms: One is present in unirradiated dormant seeds; it absorbs red light (peak at 660 nm). This form of the phytochrome cannot break dormancy (if it could, the seeds would not be dormant); but when activated by 660 nm light, it is changed into an active, dormancy breaking form. This active form absorbs far light (730 nm).

Seeds of *Tephrosia purpurea* were collected and washed in running water. Germination tests were carried out in six petridishes containing filter paper pads moistened with demineralized water. Each petridish contained 100 seeds for different experiments of action spectra. one was kept as control, exposed to normal day light, while the remaining five were subjected to different action for obtaining various isolated spectra, the dishes were covered with a double layer of cellophane filters of desired colors i.e., blue, green, yellow, and red. Far-red condition was obtained by covering the dish with one blue and one red filter. All the petridishes were kept exposed to the natural diurnal cycle. Adequate moisture was maintained by adding water. The total germination percentage was recorded when the seeds ceased to germinate. Mean maximum and mean minimum temperatures during experiments were 28^0c and 12.2^0c respectively(table-2).

Seed germination response of *Tephrosia purpurea* to hot water treatments

The environment is important in softening hard coats that are impermeable to water. Impermeability of seed can be due to the wax found on the seed coat. Softening of hard seed coat in nature is thought to be done by microbial attack or abrasion by soil particles. Of interest is the effect of high temperatures on some leguminous seeds. During exposure to heat, cracks appear in the seed coat of some species, especially in the region of the micropyle. The response is seen in some seeds, such as Albiia lophantha in which the strophiolar plug is audibly ejected from the seed as high temperature is reached, leaving a strophiolar crater through which water, can enter, Such effect of high temperature is thought to be important in pyric species whose seedlings emerge as a consequence of forest fires.

Hot water treatment given to seeds of *Tephrosia purpurea* which were previously soaked for 24 hrs prior to their treatment germination responses was nil. So germination treatment for hot water treatment was conducted on the seeds of *Tephrosia purpurea*_which were not soaked earlier. Eight groups of healthy 100 seeds, each were given hot water treatment by soaking them in hot water of different temperature for 5 minutes which were then taken out from hot water. The seeds were set for germination in different petridishes which contained which contained moistened pads. The germination responses are given in table-3.

Seed germination response of *Tephrosia purpurea* to water soaking treatment

Seed of *Tephrosia purpurea* were soaked in water for varied time periods for the test of germination. For each time period 100 seeds were set for the test. After the seeds were soaked for a definite time they were set for germination in the moisture laden petridishes. The germination response is given in the table-4.

Seed germination response of *Tephrosia purpurea* to H_2SO_4 treatments

Seven lots of 100 seeds of *Tephrosia purpurea* were soaked in concentrated H_2SO_4 for different time periods. After the treatment of H_2SO_4 the seeds were taken out from the acid and washed thoroughly in tap water till there was no trace of acid left on the seed. Then the different lots of seeds were placed in different petridishes which were well padded with moisture laden cotton. The seeds were set for germination. The germination response is given in the table-5.

The untreated seeds of *Tephrosia purpurea* when soaked in water gave out a yellow pigment of strong odour. The pigment was enough to make the water light in color, in which they

were soaked. To confirm that the yellow pigment which was given out by seed coat was the inhibitory factor for germination the seeds were treated with concentrate $H_2SO_4$and soaked in water containing in yellow pigment showed a strong tendency of dormancy.

Seed germination response of *Tephrosia purpurea* to limestone and sand

Eight lots of 100 healthy seeds each of *Tephrosia purpurea* were taken. Different petridishes which were set for the test were half filled with different ratios of powered limestone and sand. Then adequate water was poured in each Petridis to maintain the moisture level. When the petridishes were ready for the seeds were placed in the petridishes for germination. The germination responses are given in the following table-6.

Treatment with growth hormones

The hormones as growth regulators, gibberellins (usually gibberellic acid GA_3, GA_4 and GA_7), cytokinin (usually kinetin, benzyladenine), and ethylene variously affect seed dormancy. Seeds that normally requires chilling or light or after ripening exhibit striking responses to these substances, which often also accelerate germination of nondormant seeds. However, the success of these regulators is mixed, and many species of seed do not respond at all.

The growth regulators with the widest spectrum of activity are the gibberellins. Cytokinins are less widely effective, and even when they do act, they often induce abnormal germination in lettuce for example, the cotyledons tend to emerge from the seed before the radical. Ethylene is also only limitedly effective, and many species remain unaffected. Frequently, the growth regulators interact with other factors or among themselves. Kinetin, for example, promotes normal germination of dormant lettuce in combination with low levels of light and ethylene stimulates Chenopodium album most effectively in the presence of light and gibberellins. Numerous studies have demonstrated interactions among the growth regulators.

According to hormonal theory of dormancy, dormancy maintained (and possibly even induced) by inhibitors, and it can end only when the inhibitor is removed or when promoters overcome it. The theory owes it inception primarily to know effects of applied growth regulators on dormancy some of which, cause a dormant seed to germinate whereas others inhibit germination of a nondormant seed. A second concept is that important metabolic changes occur as a consequence of the action of the dormancy breaking factor. One such change is thought to involve the synthesis of RNA and protein and another, the operation of the pentose phosphate pathway.

synthetic auxin (NAA)

For the experiment six petridishes were taken, thoroughly washed sets of seeds (a set of 100 seeds) were placed in sterilized petridishes on Whatmann No.2 filter paper which were moistened with different concentrations of synthetic auxin (NAA). The germination responses are given in the following table-7.

Gibberellins (GA_3)

Seeds of *Tephrosia purpurea* were thoroughly washed under tap water. Seven sterilized petridishes containing Whatmann No. 2 filter paper were moistened with different concentrations of gibberellins. 100 seeds were in each Petridis for the test of gibberellins' action on seed germination. The germination responses are given in the following table-8.

Seed germination response of *Tephrosia purpurea* with treatment of morphactin

Thoroughly washed seeds of *Tephrosia purpurea* of four lots of 100 seeds were placed in the petridishes which were set for experiment. Four petridishes were taken, each containing Whatmann No. 2 filter paper which was moistened with different concentrations of morphactin. Then 100 seeds each were placed in the petridishes to observe the germination response affected with morphactin. The germination responses are given in table-9.

Table-1 Seed germination response of *Tephrosia purpurea* to varying light periods.

S. No.	Light periods	Germination percentage/50 days
1	Control	10
2	Continuous light	48
3	Continuous darkness	09
4	Diurnal cycle	10

Table-2 Seed germination response of *Tephrosia purpurea* in different action spectra.

S. No.	Different light spectra	Germination percentage
1	Control (Normal day light)	10
2	Blue	
3	Green	
4	Yellow	
5	Red	
6	Far red	

Table-3 Seed germination response of *Tephrosia purpurea* to hot water treatments.

S. No.	Water temperature	Percentage of seed germination
-	Control	10
1	30°C	7
2	40 °C	10
3	50 °C	10

4	60 °C	20
5	70 °C	22
6	80 °C	40
7	90 °C	19
8	100 °C	10

Table-4 Seed germination response of *Tephrosia purpurea* to water soaking treatment.

S. No.	Duration (in hours) for seeds soaked	Germination Percentage
1	Control	10
2	6 hours	0
3	12 hours	0
4	18 hours	0
5	24 hours	0
6	30 hours	0
7	36 hours	0
8	42 hours	0
9	46 hours	0
10	48 hours	0

Table-5 Treatment of concentrate H_2SO_4 on the seed of *Tephrosia purpurea*.

S. No.	Duration of treatment	Percentage of seed germination
-	Control	10
1	05 min.	33
2	10 min.	40
3	15 min.	43
4	20 min.	30
5	25 min.	20
6	30 min.	15

Table-6 Treatment of lime on the seed of *Tephrosia purpurea* for germination.

S. No.	Lime/sand ratio	Percentage of seed germination
-	Control	10
1	3: 100	10
2	4: 100	15
3	5: 100	15
4	6: 100	20
5	7: 100	50
6	8: 100	40
7	9: 100	10
8	10: 100	2

Table-7 Germination response of *Tephrosia purpurea* to Auxins (NAA) concentrations.

S. No.	Concentration of Auxin (NAA)	Percentage of seed germination
-	Control	10
1	010 ppm	36
2	020 ppm	20
3	050 ppm	15

4	070 ppm	05
5	100 ppm	00
6	200 ppm	00

Table-8 Seed germination response of *Tephrosia purpurea* to Gibberellins.

S. No.	Concentrations of Gibberellins	Germination percentage
-	Control	10
1-	010 ppm.	49
2-	020 ppm.	50
3-	050 ppm.	56
4-	070 ppm.	31
5-	100 ppm.	30
6-	150 ppm.	28
7-	200 ppm.	25

Table- 9 Seed germination response of *Tephrosia purpurea* to morphactin.

S. No.	Concentration of morphactin	Germination percentage
-	Control	10
1	001 ppm.	25
2	010 ppm.	52
3	020 ppm.	24
4	050 ppm.	13

CONCLUSION

Seed germination is a crucial stage in the life history of plants and salt tolerance during germination is critical for the stand establishment of plants growing in saline soils. Several investigations have indicated that seeds of most species attain their maximum germination in distilled water and are very sensitive to elevated salinity at the germination and seedling phases of development. This study demonstrated that seed germination in *Tephrosia purpurea* varied accordingly concentrations of different factors which affect seed dormancy. 100% seed germination was broken when seed dispersed on barren rock. They lie on the barren rock for whole of the summer i.e. 3-4 months and as soon as the rains started in rainy season the seeds started germinating from the crevices.

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