**MATERIAL AND METHODS**

The experiment was conducted at ICAR-Indian Institute of soybean research farm, Indore during rainy season (June-October) of 2022 (22.78° N, 75.88° E), India. The experimental site has black (Vertisols) soils with high to moderate depth, high water holding capacity and medium fertility. Recommended dose of fertilizers was applied at the time of planting. Standard agronomic practices for weed and insect control were uniformly followed.

The recommended dose of fertilizer: N:P:K and S is 20:60:40 & 20 kg per hectare. In this experiment N,P,K and S was applied in the form of NPK : 12:32:16 at the rate of 2 quintal per hectare and bentonite Sulphur 20 kg/ha.

In this practice land preparation was done with summer plough using reversible mould board plough following 2 crisscross harrowing or cultivation for breaking soil clods. Harrowing was followed after arrival of monsoon and planking to level the field for proper sowing. In this process the preceding wheat crop stubble are broken and mixed in soil. The wheat stubbles are seen on the field which get decomposed with the onset of monsoon and soil moisture. The wheat stubble if not broken and mixed with soil, cause disturbance in proper sowing. The fertilizers were broadcasted and mixed before sowing while doing harrowing and planking or sowing was followed with ferti-seed drill where fertilizer and seeds were placed side by side at 3inch gap. Soybean genotype NRC130 and NRC 86 was planted in two different field.

**Herbicides spray apply at seed crop maturity**

|  |  |  |
| --- | --- | --- |
| **S. No.** | **Treatment** | **Dose** |
| 1 | T1 Imazethapyr 35% + Imazamox 35 % WG | 100 g/ha |
| 2 | T2 Glyphosate 41% S.L. | 2 L/ha |
| 3 | T3 Paraquat dichloride 24% S.L. | 2 L/ha |
| 4 | Control |  |

**Seed Index (g)**

Hundred seed weight was recorded as per the procedure given by ISTA rules (Anon., 1993). The average weight was recorded in grams.

**Seed yield (q/ha)**

Seed yield per plot was recorded by taking the weight of the seeds obtained from the net plot after rejecting out the border rows. The seed yield of net plot was expressed in quintal per hectare.

**Germination testing**

Germination of a seed lot in a laboratory is the emergence and development of the seedling to a stage where the aspect of its essential structures indicates whether or not it is able to develop further into a satisfactory plant under favorable conditions in soil. These essential structures are a well-developed and intact root system, hypocotyl, plumule and one or two cotyledons Seedlings cannot be evaluated in a germination test until these essential structures are clearly identifiable and the reported percentage germination expresses the proportion of seeds which have produced normal seedlings within the period specified for each species.

**General Principles**

Germination tests shall be made with seeds from the pure seed fraction of a purity test. A minimum of four hundred seeds are required in four replicates of 100 seeds each or eight replicates of 50 seeds each depending on the size of seeds and size of containers of substrate.

**General Requirements for Germination.**

Seeds require certain conditions for normal germination. The most important requirements are substrata, moisture, temperature and light.

**1. Suitable substratum**

The substrata serve as a moisture reservoir and provide a surface or medium for which the seeds can germinate and the seedlings grow. The commonly used substrates are paper, sand and soil.

**a. Paper substratum**

Most widely used paper substrates are filter paper, blotter or towel (kraft paper), these are easy to handle versatile and comparatively cheap.

**Specification of Germination paper:**

Germination paper should preferably possess a creaped surface. The paper should have an open, porous formation and he free from impurities or toxic substances that may affect seed germination. It should be free of fungi or bacteria which might interfere with the growth or evaluation of seedlings. It should hold sufficient moisture during the period of test and should possess sufficient strength to resist wear and tear during handling. The texture should be such that the roots of germinating seedlings will grow on and not into the paper.

**The paper shall meet the following requirements.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Type of Paper | Basis Mass  (g/m2) | Bursting Strength(kg/em') | Capillary rise (in mm). Min | PH | Ash, % by mass (Max.) |
| Filter paper | 130-135 | 1.0 | 30 | 6.0 to 7.5 | 1.20 |
| Towel paper | 95-100 | 2.0 | 30 | 6.0 to 7.5 | 1.50 |

**Biological Test for Toxic Materials:**

In this, test, comparison shall be made between germination papers of unknown quality and known acceptable quality. Pieces of paper should be cut to size and placed in petri dishes or plastic boxes. Petri dishes or plastic boxes should be lined with two thickness of such paper. The papers should be saturated with tap water and seeds of Brassica species or onion should be germinated. Evaluation may be done by comparing the development of the seedlings grown on unknown quality of paper and those grown on the known quality of paper. The evaluation of seedlings shall be made after 3 days in case of Brassica and after 6 days in case of onion. If paper of unknown quality contains toxic substances, the root tips will be shortened and sometimes discolored, root hairs bunched and sometimes plumules shortened.

**Determination of Capillary Rise**

Cut ten strips of paper each 10 mm wide, five in the machine direction of the paper and five in the cross-machine direction. Immense each strip in distilled water at 27 12° C to a depth of 20 mm at the end of the strip. After 2 min measure the height to which the water has risen in the strip, to the nearest 1 mm. Calculate the average of the 5 strips in the cross machine direction. The lower of these two averages shall be taken as the result for the test.

**2. Adequate Moisture of Water**

High concentration of water at cellular level is necessary for the seed to start germination. Moisture is supplied to the seeds through the substratum. Generally, the moistened substrata is sufficient to rehydrate to 30-80 per cent. However, the moment the radicle emerges, additional moisture contributes better seedling growth. In the case of vegetable seeds, care is necessary in moistening the substrata. Too much water would allow fungal growth and decay of seeds.

**The general specifications for water are:** It should be free from organic or inorganic impurities. The pH value should be within the range of 6.0 to 7.5. If the usual water supply in the laboratory is not satisfactory, distilled, de-ionized water may be used. To ensure the quality of water being used, an analysis should be obtained from time to time.

**3. Favorable Temperature**

Seeds of most of agricultural and horticultural crops germinate in the temperature range of 10° C to 35° C Some seeds germinate better at constant temperature Others require an alternating temperature.

Temperature control is also necessary to overcome dormancy wherever it occurs. Exposure of seeds to the temperature at 40° C or higher, alternation of temperature, low temperature applications are the easiest and safest method to overcome seed dormancy although methods to overcome dormancy by chemical treatments do exist.

Therefore, the temperatures prescribed in the above Table should be determined at the level of the seeds on the substrate.

Temperatures should be as uniform as possible throughout the germination apparatus and care should be taken that the temperature of tests does not exceed the level prescribed in the Table and should not be more than ± 1° C.

Where alternating temperatures are indicated, the lower temperature should usually be maintained for 16 hours and the higher for 8 hours. If alternation of temperatures cannot be controlled over week-ends or public holidays, the test should be kept at lower temperature.

**4. Light:**

There are crops for which light is not required during germination test. However, presence of light is desirable to enable the evaluation of seedlings easier and with greater certainty. Other crops like lettuce and tobacco require light during germination on the test. Seeds of most of the species in the Table will germinate either in light or in darkness. However, illumination of the substrate from artificial source or by day light is generally recommended for better seedling development to avoid etiolation and also to detect seedlings having chlorophyll deficiency.

**Specifications for soybean germination testing**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Crop** | **Media** | **Temperature** | **1st count** | **Final count** | **Addl. directions** |
| Soybean | BP, S | 20-30° C, 25° C | 5 | 8 | **-** |

**Procedures of germination testing**

**Working sample**

Four hundred seeds are counted at random from the well-mixed pure seed. Replicates of 100 seeds are normally used, spaced sufficiently far apart on the seed bed to minimize the effect of adjacent seeds on seedling development. To ensure adequate spacing, split replicates of 50 or even 25 seeds may be necessary, particularly where there is seed-borne disease.

Testing four hundred seeds is recommended on seed law enforcement, seed certification and service samples.

**Methods using paper:**

Paper substrates are used for the following methods:

1. **BP (Between paper)**:

The seeds are germinated between two layers of paper. This may be achieved by loosely covering the seeds with an additional layer of paper or by placing the seeds in rolled towels. The rolled towels are to be placed inside the germinator in an upright position.

**Methods using sand**

The seeds are planted on a leveled layer of moist sand and covered with 10-20 mm of uncompressed sand depending on the size of the seed. To ensure good aeration it is recommended that the bottom layer of sand be loosened by raking before sowing Sand may be used instead of paper, even if not prescribed in Table when the evaluation of a diseased sample proved impracticable because of the contamination of the paper substrate.

**Seedlings Evaluation**

Seedling which have reached a stage when all essential structures can be accurately assessed, shall be removed from the test at the first or any other intermediate counts. Badly decayed seedlings should be removed in order to reduce the risk of secondary infection, but abnormal seedlings with other defects should be left on the substrate until the final count.

**Categories of seedlings**

**Normal Seedlings**

Normal seedling is one which shows the capacity for continued development into mature plant when grown in good quality soil and under favorable conditions of water supply. temperature and light.

According to the International Seed Testing Association (1985) seedlings to be classified as normal seedling, must conform with one of the following categories:

**a) Intact seedlings:** Seedlings with all their essential structures, well developed complete in all proportion and healthy.

**b) Seedlings with slight defects:** Seedlings showing certain slight defects of their essential structures provided they show an otherwise satisfactory and balanced development comparable to that of intact seedlings of the same test

**c) Seedlings with secondary infections:** Seedlings which are seriously infected by fungi or bacteria are classified as normal, if it is evident that the parent seed is not the source of infection, and if it can be determined that all the essential structures were present

**Abnormal seedlings**

An abnormal seedling is one which does not have the capacity to develop into a normal plant when grown in the soil under favorable conditions because one of more of the essential

structures is irreparably defective.

Three major classes of abnormal seedlings are:

**a) Damaged Seedlings** Seedlings with any of the essential structures missing or so body damaged that balanced development does not occur. The damage to the embryo in the seed usually results from external cause i.e., mechanical handing.

**b) Deformed or unbalanced seedlings** Seedlings with weak and unbalanced development which may be caused by internal disturbances of physiological biochemical character. Such internal disturbances, however, are often due to the earlier external disturbances such as unfavorable growing conditions of the parent plants, poor ripening conditions for the seed, premature harvesting, effect of herbicides or pesticides and inappropriate storage conditions or ageing of the seed.

**c) Decayed seedlings:** Seedlings with any of their essential structures se diseased or decayed as a result of primary infection that normal development is prevented. These may result from the external or internal seed borne disease.

**Staring of Samples**

The official samples after testing should be stored in controlled storage (3° C and 50% RH) for a minimum period of two years from the date of grant/extension of the certificate, unless required for longer period. Every care should be taken to protect the samples from insects and rodents.

**Determination of seedling vigour**

Seedling vigour is in important quality parameter which needs to be assessed to supplement germination and viability test to gain an insight into the performance of a seed lot in the field or in storage. Vigour index was calculated by using the formula suggested by Abdul Baki and Anderson (1973).

1. Vigour index I-germination percentage x seedling length (cm).

(ii) Vigour index II-germination percentage x seedling dry weight (mg).

**Shoot length (cm)**

From the germination test, ten normal seedlings were selected randomly in each treatment from all the replications on eighth day. The shoot length was measured from the base of the primary leaf to the base of the hypocotyls and the mean shoot length was expressed in centimeter.

**Root length (cm)**

Ten normal seedlings used for shoot length measurement, were also used for the measurement of root length. The root length was measured from the tip of the primary root to base of hypocotyls and the moan root length was expressed in centimeter.

**Seedling dry weight estimation**

After standard germination test 10 normal seedlings were taken at random and their shot and root length were recorded by linear scale. For dry weight these seedlings were kept in an oven at 80° C for 17 hours and then dry weight has been taken with the help of electronic balance.

**Accelerated ageing Studies**

The test was developed by Deloche and Baskin (1972) at Mississippi State University. Seeds are held at high humidity (usually near 100%), with the result that their moisture content increases, and at temperatures of 40-45° C for varying length of time depending on the type of seeds. This method of rapid ageing is then followed by a germination test. This test was initially developed as a test of seed storage potential where by seeds that retained high germination after accelerated ageing would store well whereas rapid loss of germination was indicative of poor storage potential. Subsequently, the test came into use as a vigour test so that seeds with higher germination were considered high vigour seeds and those with poor germination, low vigour seeds. The application test has however been mainly to predict seed storage potential.

**Methods:**

The 50 g seeds were treated with carbendazim fungicide powder to avoid any fungal growth interference to artificial ageing of seeds packed in packet of nylon mesh. Nylon mesh (or net) packets are used because these packets does not hold or absorb moisture.

Then the seed packets were placed in desiccators, containing saturated solution of barium chloride to create the internal relative humidity of 90%.

The desiccators containing seed packets were then kept in BOD incubator, at high temperature of 40°C and relative humidity of 90% for different duration of 3 days and 7 days.

After this specific duration of artificial ageing the seeds are removed from the nylon packet and tested for seed germination following standard protocol.

The germination test result is interpreted for high vigour and low vigour seed lots as already mentioned high vigour of seeds will result in higher germination after accelerated ageing than the low vigour seeds.