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Seed Vigour Tests on seven Cultivars of *Phaseolus vulgaris* Grown in Chamoli District of Uttarakhand, India

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ABSTRACT

Rajmash (*Phaseolus vulgaris* L. family; fabaceae) is also known as kidney bean/ French bean. It is an annual plant and cultivated throughout the world for their edible beans. The present study was conducted to investigate the effect of seed vigour by germination test of different cultivars of *Phaseolus vulgaris* grown in Chamoli district of Uttarakhand. The experimental material constituted a collection of 7 cultivars (Red small, Red big, Grey, Pink, Yellow, Brown, White) of *Phaseolus vulgaris*. The seed samples of seven cultivars of *Phaseolus vulgaris* were taken from Joshimath, District Chamoli Garhwal Uttarakhand. Morphological aspects viz; seed weight, seed moisture content, seed density and seed diameter were recorded prior to germination test of *Phaseolus vulgaris* at Seed Testing Laboratory, Department of Seed Science and Technology, H.N.B Garhwal University. Seeds were sterilized with 0.2% sodium hypochlorite solution for germination test. Two replicates of each variety and 25 seeds in each replicates were kept in petriplates on whatman filter paper. Petriplates were kept in seed germinator at 25 ± 2°C until final count. The number of normal seedling was counted at the final count, higher the number of normal seedlings greater is the seed vigour. The germination percentage was found 100% in five cultivars, and the other two cultivars showed 96.66% in yellow seeds and 68.33% in Grey cultivar of *Phaseolus vulgaris*. Brown cultivars represented maximum dry weight of root (0.029 g) while minimum dry weight of root has been recorded in yellow cultivar (0.01353 g). Maximum vigour index were recorded in brown cultivar and minimum was recorded in pink cultivar. The findings from the study indicate that brown cultivar of *Phaseolus vulgaris* is highly vigours.

Key words : *Phaseolus vulgaris, Germination, Morphological, Physiological, Variations.*

Introduction

Phaseolus vulgaris L, Beans are consumed as mature grain, as immature seed, as well as a vegetable in the form of raw leaves. As fruit (pods) can be obtained in as little as two months, rotations are possible with other crops during short growing seasons. Short bush growth habits offer minimal competition and permit inter-planting with other species, for example, in reforestation projects or among fruit trees or coffee plantations during the early years until the main crop can be exploited. At the other extreme are aggressive climbers found at higher altitudes on subsistence farms where a few plants are main-

tained as a sort of insurance and are continually harvested for about six months. Legumes are vital in agriculture as they form associations with bacteria that "fix-nitrogen" from the air. Effectively this amounts to internal fertilization and is the main reason that legumes are richer in proteins than all other plants.

Additionally, it is reported that some green bean genotypes are cultivated in the Gevaþ and Erciþ areas of Van province from the Eastern Anatoli region. In India the major French bean growing states are Tamilnadu, Andhra Pradesh, Haryana, Punjab, Karnataka, Madhya Pradesh. They are also grown in some places of Uttarakhand. The common bean is a highly variable species with a long history. Bush varieties from erect bushes 20-60 centimeters (7.9 - 24) tall, while pole or running varieties from vines 2 -3 meters (6 ft 7 in -9 ft 10 in) long. The beans are smooth, pulp, kidney – shaped, up to 1.5 cm long, range widely in color, and are often mottled in two or more colors.

Seeds are fundamental to agriculture and natural ecosystem. Fundamental knowledge about mechanisms pertaining to seed development, germinability, dormancy and storability is required for improving seed performance. The crop production relies heavily on high quality planting material. The seed quality parameters (known as Seed Standards) have been notified for more than 95 crops viz. cereals, pulses, vegetables etc. However, no such standards of seed quality parameters are available for many medicinal plants. Seed testing protocols are also not available, which is a pre requisite for testing the seeds and also for recommending minimum limits of germination (Parihar, 2006). Seed testing protocols are regularly updated by ISTA (International Seed Testing Association) on the basis of research work done globally through publication of research papers. The latest ISTA rules (ISTA, 2008) contain seed testing protocols of a large number of species cultivated all over the world and it forms the basic reference book for all kinds of seed testing activities and also for the international seed trade. Many well –known beans varieties belong to this species, their colors and shapes and size of pods and seeds vary tremendously. Most of the French bean varieties are day neutrals except some semi pole varieties which are short day types. It is a cool weather crop but thrives well in the optimum temperature ranging between 20°C to 25°C. French bean is sensitive to frost; high temperature and high rain fall. High rain fall destroy the young growing seedlings of *Phaseolus vulgaris*.

Materials and Method

Seeds of *phaseolus vulgaris* were collected from Joshimath, District Chamoli of Uttarakhand. The collections were carried out in the ripening seasons of *Phaseolus* in October last week to November. Different parameters were taken to find out one of the best cultivar among the seven.

Like morphology, seed weight, moisture determination, seed density, seed diameter, rate of imbibitions, viability and seed germination of *Phaseolus vulgaris.* The experimental material constituted a collection of 7 cultivars of *Phaseolus vulgaris*. The experiments were performed in the seed testing laboratory of Department of Seed Science and Technology at HNB Garhwal University. The observation recorded on various morphological features of the seed such as seed colour as well as the texture. The morphological variations shown by the shape and size of the seeds were determined by obtaining the photographs. On the basis of their morphological studies we find so many variations in those cultivars and that's why they are concluded as different from each other.

Moisture Determinations

The moisture content of seeds is determined by "air oven method". In this method the moisture present in the seed is expelled in the form of water vapors by application of heat under control conditions, which permits the loss of moisture to be measured quantitatively. One of the widely used methods to measure seed moisture is "air oven method" for drying. In which we took two replicates of each cultivars in the sample pan weighed with its lid in electronic balance that weight is noted as "W_1". Then these samples are kept in oven for 1 hour at 130 (Recommended by the rules for seed testing by ISTA). After one hour with draw the pans from the oven, and then they are kept in desiccators for 30 -45 minutes for cooling. After cooling they are again weight with its lid and this weight is (w_2) and moisture percentage is calculated by given formula

$$\text{Moisture percentage} = \frac{\text{fresh wt } (w_1) - \text{dry wt}}{(w_2) \text{ fresh wt } (w_1)} \times 100$$

Seed diameter

Seed diameter of each cultivar was observed with the help of graph paper. For calculating the values of seed diameter some seeds were put in a line by which seeds were attached with one another on a graph e no on graph paper was measured and then divided by the total no. of seeds.

Seed Density

The density of each cultivar of *Phaseolus vulgaris* is observed with the help of kerosene oil. Some seed of each cultivar are put into the kerosene oil one by one. To find out density of seed we take 5ml of kerosene in a measuring cylinder and seeds are put into it up to a certain fixed marked on the measuring cylinder. The total increase in volume is calculated as seed density.

Density = weight / volume

Viability of Seed

To ensure that the seeds used for the experiment were viable and high quality, the samples were subjected to viability by using the tetrazolium test. 25 seeds from each variety were subjected to 2, 3, 5-triphenyltetrazolium chloride (TTC) test was done just before the germination of seeds. In this method the seed were removed and the seeds were immersed in 0.1% aqueous solution of TTC (pH-6.5) for 24 hours at room temperature (25 °C) under dark conditions. The TTC solution was drained and seeds were rinsed 2-3 times with refine water. The topographical staining pattern of the embryo (plumaged and radical) and cotyledons were studies under a dissection microscope.

Standard Germination Test

The germination percentage was calculated using the formula:

Germination (%) = (germinated seed / Total no. of seed) × 100

Evaluation of seedlings: Seedling length (root and shoot: The root and shoot length was taken separately after completion of germination period in randomly selected five seedlings from each replication.

Seedling biomass (dry weight): For biomass percentage, fresh (wet) weight of five individual from each replicate of all the cultivars were taken and oven dried at 80 °C for 24 hours.

After oven dried, the dry weights of all the seedlings were measured:

$$\text{Formula for biomass percentage} = \frac{\text{Fresh weight-dry weight}}{\text{Fresh weigh}} \times 100$$

Statistical analysis: The data obtained from experiment were subjected to the statistical variance analysis and mean was calculated by the following formula:

$$\text{Mean} = \Sigma FX / N$$

Where, X = sum of all observations; N = number of observation

Results and Discussion

Morphological variation

The morphological variation is assessed on the basis of physical appearance and the colour of the seed coat, shape size etc. These are the first parameters on the basis of which seeds are categorized and named as different cultivars. The names are assigned to the cultivars on the basis of their seed coat colour. Grey, Pink, Yellow Brown, White Red small Red big.

A seed is a mature ovule containing an embryo that is usually the result of sexual fertilization. Seeds and fruit of different species vary greatly in appearance, size, shape and taxation and structure of the embryo in relation to storage tissues. These points are not useful for the identification but affects germination requirements. The main aim of conducting the experiment was to find the best cultivars in respect to their morphological characters. If they are morphologically locking well then posses all the qualities of good cultivars or not in respect to the nutritional values of a pulse.

Moisture content of the seed is one of the most important factor influencing the seed vigor and viability. In an organized seed programme, the measurement of moisture content determines the decision-making policies pertaining to the harvesting, threshing, processing and marketing of seeds. It is therefore, essential that moisture content should be in accordance to the requirement of seed storage behavior and desiccation and chilling sensitivity/tolerance of a species (Parihar *et al.,* 2013). Many seeds fail to germinate after processing and placement in favorable growing conditions such seeds are said to be dormant.

Seed dormancy is one of important limiting factor in exploitation economically product of valuable specious (Gupta *et al.,* 2011). Uniform seed dor-

mancy is created by different treatments of seed dormancy (Yucel and Yilmaz, 2009). Two major forms of physiological seed dormancy have been described, namely embryo and seed coat dormancy (sometimes termed coat-enhanced dormancy). Germination commences with the uptake of water by imbibitions of the dry seeds, followed by embryo expansion (Kucera *et al.*, 2005).

The moisture content is a major factor which influences the quality and quantity of the seed. Greater the moisture content in the seed half the life of the seed or deterioration of the seed life. Seed moisture content is a major factor influencing seed longevity. Moisture content should receive periodic checking during the time a seed lot remains in storage. This may be done either by testing moisture content or by measuring relative humidity around the seed. The seed moisture percent was calculated before testing as it affects the result of other tests. The air oven method is used to determine the moisture content. The moisture content of different cultivars is different the higher moisture found in grey cultivar and the lowest moisture content is found in red small. This moisture influences the longevity of these cultivars. The moisture content of grey is highest and the lowest moisture is of red small. Moisture determination is a factor which influences the longevity of seed life.

Germination is a crucial stage in the life cycle of plants and tends to be highly unpredictable over space and time. Successful growth and establishment of plants considerably depends on optimum germination (Gorai and Neffati, 2007). Several environmental factors such as temperature, salinity, light, water availability, soil nutrient, and soil moisture simultaneously influence the germination (Ungar, 1995;; Huang *et al.*, 2003; Gorai and Neffati, 2007; Socolowski *et al.*, 2008 and Farji-Brener *et al.*, 2009).

The major event occurring in the seed germination is imbibitions of water. Early seed germination begins with imbibitions of water by the seed through natural opening in the seed coat and diffused through the seed tissues. The water causes the cell become turgid, the entire seeds grow in volume and the seed more permeable to O_2 and CO_2 as swelling occurs, the seed coat often ruptures facilitating both water and gas uptake and emergence of the growing points. The rate of imbibitions is different in different cultivars of *Phaseolus vulgaris*. The imbibition period is varies from each other. The variation may be due to their shape and size or may be due to some unnatural process occurring in the seed.

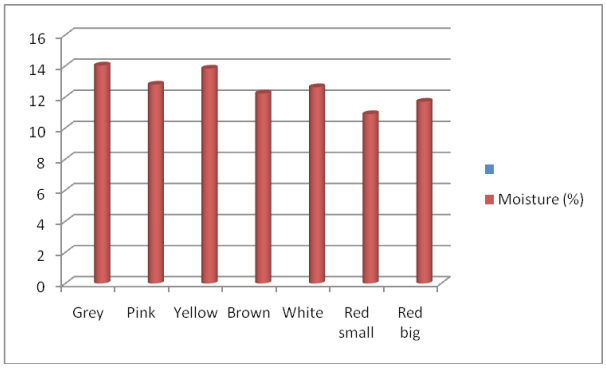


Fig. 1. Presence of seed moisture content in various cultivars.

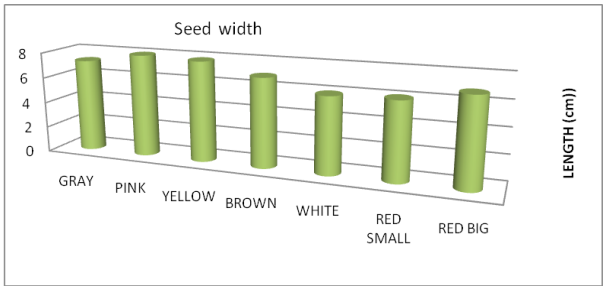


Fig. 2. Seed width of different cultivars.

Table 1. Morphological variations in different parameters of phaselous vulgaris seeds.

Cultivars	Seed length (mm)	Moisture (%)	Width (mm)	Density	Thousand seed weight
Grey	10.88	14.03	7.28	0.252	288.66
Pink	12.4	12.8	8	0.38	476.46
Yellow	11.25	13.83	7.85	0.27	281.94
Brown	15.66	12.23	6.96	0.364	431.5
White	8.872	12.63	5.98	0.144	181.74
Red small	9.22	10.9	6.08	0.134	196.58
Red big	14.62	11.7	6.86	0.35	420.5

Imbibition is a problem in the grey cultivars of *Phaseolus vulgaris*, the seed coat is hard and it is not able to imbibe the water easily. So we provide a treatment to break the seed coat, because seed coat is hard and it is not permitting the seed to imbibe the water. By scratching the seed coat water is imbibed by the seed and then easily imbibitions are occurred in these seeds.

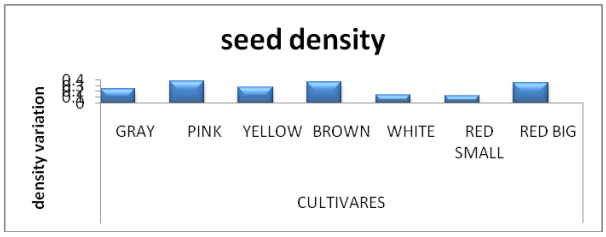
Seed density, and seed weight is based on the reserver accumulated in the seed. The shape and size of seed is a major cause of seed weight. The larger the amount of food reserves in the seed the greater the weight of the seed. Reserve accumulation is the main cause of seed weight, size and density. More the accumulated material in the seed means the good quality of the seed. It means it posses all the qualities of a good seed and posses the maximum capability of germination and grown up into a good seedling and into a healthy plant.

To ensure that the seed used for the experiment were viable and of high quality, to check the viability of seed tetrazolium technique is used to find the viable seed. And we find the 100% viability in all the cultivars. But the germination is not 100 % it may be due to some other reason of other factor related with seed germination. By checking the viability it is known that the seeds are able to germinate and there is not any problem in germination.

The rate of imbibitions of different cultivars is recorded for a long duration. At the starting hours of imbibing period the rate of imbibitions is very slow. After 16 hours measurement there is a large variation is found among the cultivars. If the seed imbibe extraordinarily there may be some problem is there which may caused by the excessive hard seededness or due to some unnatural phenomenon of seed physiology. The maximum period of imbibitions is found in red big and the imbibitions are stopped at a certain period in seeds of white colours.

Dimension of seed plays an important role in the reserve accumulation of seeds. Length and width play important role in seed morphology. The maximum seed length was recorded in the brown coloured seeds (15.66 cm) while the minimum length was recorded in Grey colored seed (10.88 cm).

As for as seed width is concerned, the maximum was observed in pink cultivar (8 cm) and the minimum width is observed on white seeds of *Phaseolus vulgaris* (5.98 cm).



Fg. 3. Variation in seed density of different cultivars.

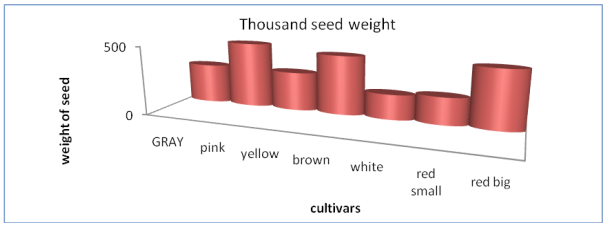


Fig. 4. Thousand seed weight.

Table 2. Variation among apicotyl, hypocotyls and root root length (cm).

Cultivars	Apicotyl	Hypocotyls	Root
Grey	0.72	4.7	9.12
Pink	1.013	4.46	10.5
Yellow	0.98	5.03	6.73
Brown	0.973	5.713	10.27
White	1.906	6.83	12.96
Red small	0.963	6.953	6.56
Red big	1.146	5.673	

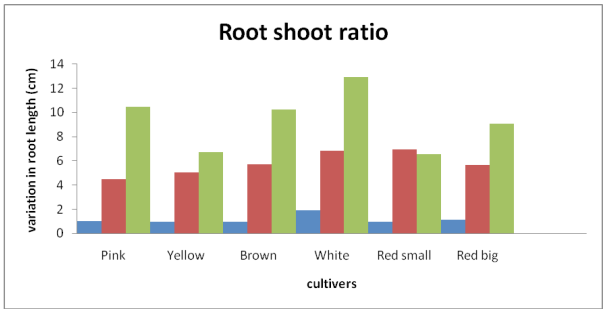


Fig. 5. Root shoot Ratio.

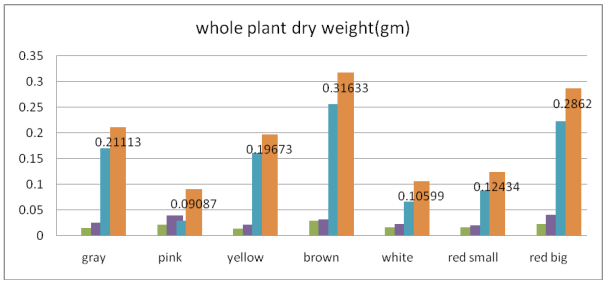


Fig. 6. Whole plant dry weight.

The density plays a role in the vigour of the seed. How much a seed is dense it shows the physical strength of a seed. The maximum density has been reported in pink colored seed (0.38 ml) and the minimum density was recorded in red small (0.134 ml) seeds.

The maximum seed weight has been recorded in the seeds of pink cultivar (476.46 g) whereas the minimum was observed in white small seeds (181.74 g). This weight is measured according to thousand seed weight. Weight shows the quantity of seeds, less in number and maximum in weight is a quality of good seed because it accumulates the maximum amount of food reserve in it, according to the seed morphology and its physiological process. Medicinal plants are rich source of secondary metabolites, biosynthetically derived from primary metabolites but restricted to specific taxonomic genera of plant kingdom and specific parts of plant body. Secondary plant products are of major interest because of their biological activities ranging from antibacterial, antibiotic, insecticidal, hormonal, pharmacological and pharmaceutical (Tarija *et al.,* 2012). Many traditional healing herbs and their parts have been shown to have medicinal value and can be used to prevent cure several human diseases (Dhar *et al.,* 1999; Savithramma and Sudrasanamma, 2006). Consumption of herbal medicines is widespread and increasing in recent years and nearly 80% population of developing countries relies on traditional system of medicine (WHO, 2005).

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